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HEAD AND NECK CANCERS (EY HANNA, SECTION EDITOR)

How Each Component of Betel Quid Is Involved in Oral Carcinogenesis: Mutual Interactions and Synergistic Effects with Other Carcinogens—a Review Article

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Abstract

Purpose of Review The roles of the components of betel quid in oral carcinogenesis remain unclear. The purpose of the present review is to highlight the effect of each component of betel quid and to discuss the synergistic effects of other carcinogens along with betel quid in the development of oral cancer in habitual betel quid chewers.

Recent Findings Betel quid may synergistically participate in carcinogenesis by disrupting the compositions of oral microbiota, accompanied by endotoxins secretion and reactive oxygen species (ROS) production. Microbiome dysbiosis mediated by synergistic effects of betel quid chewing, smoking, and alcohol drinking is possibly linked to oral carcinogenesis, which is firstly discussed in this report.

Summary Betel quid and other carcinogenic components, mainly contribute to downregulate the antioxidant proteins and lead to the induction of ROS. The elimination of ROS may prove most effective chemoprevention for betel quid-mediated oral carcinogenesis.

Keywords Betel quid · Areca nut · Slaked lime · Tobacco · Oral cancer · Oral microbiomes · Reactive oxygen species · Chemoprevention

Introduction

Oral cancer is the fifteenth most frequent and major cause of death from cancer around the world [1]. The highest prevalence rate of oral cancer has been reported in South Asian countries, including India, Pakistan, Sri Lanka, and Bangladesh, where it is the third most common and fifth leading cause of cancer death, followed by breast, lung, stomach, and cervical cancer [1]. The mean age of occurrence of this disease in different parts of the oral cavity ranges between 51 and 55 years or above [1, 2]. Globally, this condition is more predominant in males than in females; in South Asian countries, it is the most common malignancy and third major cause of deaths in males, followed by lung and stomach cancer [1]. The site of occurrence of this disease depends on region-specific epidemiological risk factors. In South Asian countries, the cheek (buccal mucosa) and gingiva are the leading sites of involvement, whereas, in western societies, the tongue is most commonly affected [2]. The high prevalence of oral cancer at characteristic sites in the South Asian population may be attributed to the habit of betel quid chewing, which is one of the main etiological factors for the development of this cancer, and has been a significant threat to public health in many parts of South Asia [2, 3]. Several epidemiological studies have estimated that 10% of the world's population

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consumes betel quid, and 20–40% of the population in South Asian countries have been associated with this habit [2]. The term "quid" is defined as "a substance, or mixture of substances, placed in the mouth or chewed and remaining in contact with the mucosa, usually containing one or both of the two basic ingredients, tobacco and/or areca nut, in a raw or any manufactured or processed form" [4]. Thus, betel quid may be considered as any quid comprising of betel leaf and a combination of areca nut and slaked lime, with or without tobacco. The principal components of betel quid and the prevalence of betel quid chewing habits are shown in Fig. 1. Additionally, Table 1 briefly describes the variations in betel quid ingredients according to different geographical regions [2, 5–22]. Intriguingly, in contrast to most other South Asian countries, tobacco is usually not added to the betel quid mixture during any of the stages of preparation in Taiwan, China, and Papua New Guinea [5, 11, 17]. However, the carcinogenicity of betel quid despite the absence of tobacco is a well-recognized fact [2, 3, 23]. It has been described that different components of betel quid act on the oral epithelium and induce molecular alterations. Subsequently, these alterations lead to the initiation and promotion of oral carcinogenesis in the oral mucosa [2, 3, 23]. They are sometimes preceded by oral potentially malignant disorders (OPMDs) such as oral leukoplakia (OLP), erythroplakia, and oral submucous fibrosis (OSF) [24]. However, the association between betel quid components and development of oral carcinogenesis remains unclear. The objectives of this review are to highlight the following: the impact of betel quid components on the development of oral cancer, the synergistic effects of other carcinogens with betel quid, the possible molecular pathways involved in betel quid-induced oral carcinogenesis, and the chemopreventive methods used for betel quid-induced OPMDs that may turn to oral cancer.

Components of Betel Quid with Carcinogenic Potential

Areca Nut

Areca nut is one of the basic ingredients wrapped in a betel leaf along with the other components of the betel quid. The chemical composition of areca nut varies, and may include carbohydrates, fats, proteins, fiber, polyphenols, tannins, alkaloids, and various trace elements as the major constituents [25]. Alkaloids, polyphenols, and tannins may be responsible for the areca nut-associated effects on oral mucosa. The major areca nut alkaloids are arecoline, arecaidine, guvacaine, and guvacoline, wherein, arecoline is the most abundant alkaloid and considered to be the most important carcinogen present in areca nut [26]. This carcinogen undergoes a nitrosation reaction and gives rise to a variety of betel quid-specific nitrosamines (BSNAs) within the acidic environment of the oral cavity and stomach. Likewise, bacterial nitric oxide or other trace elements present in areca nut may facilitate the formation of nitrosamines [27–29]. The formation of these nitrosamines induces oxidative stress by interacting with DNA, proteins, or other macromolecules, and contributes to carcinogenesis in the oral mucosa, including epithelium and sub-mucosal connective tissues [23, 28–30]. Nevertheless, the mechanisms by which nitrosamines interact with DNA or other macromolecules and exert their carcinogenic activities remain unclear. Arecoline, the main alkaloid present in the areca nut, induces chromatin relaxation by inhibiting poly (ADP-ribose) polymerase (PARP), a family of proteins involved in DNA repair and maintenance of genomic stability [31]. The relaxation of the chromatin structure by arecoline allows for the interaction of nitrosamines with DNA resulting in the formation of adducts, followed by the epigenetic silencing of tumor suppressor genes (TSGs) [32, 33]. Previous studies have demonstrated the arecoline-induced downregulated expression of genes involved in preventing tumor development [34, 35]. Furthermore, arecoline N-oxide, an end product of nitrosamines, may be involved in the cytochrome p450 system [36]. Arecoline increases the formation of arecoline N-oxide by downregulating xenobiotic enzymes [37]. Additionally, recent reports have suggested that the pathogenesis of OSF is closely related to arecoline in areca nuts [38•, 39•]. Arecoline activates the production of transforming growth factor-beta (TGF-β) that acts on fibroblasts leading to the accumulation of collagen by inducing the expression of tissue inhibitors of matrix metalloproteinases (TIMPs), heat shock protein 47 (HSP47), and cyclooxygenase-2 (Cox-2), and decreasing the expression levels of matrix metalloproteinases (MMPs; Figs. 2 and 3) [38•, 39•]. Approximately, 80% of oral cancers originating from OSF have been reported to overexpress TGF-β and its downstream molecules [38•, 39•]. Arecoline may lead to the development of OSF via the pathways involved in TGF-β production [38•, 39•]. Moreover, along with the other components of betel quid (for instance, slaked lime), arecoline induces the generation of reactive oxygen species (ROS), such as superoxide anion radicals (O_2–), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2). These ROS abrogate the epithelial antioxidant defense mechanisms thereby indicating their involvement in epithelial aging by the inhibition of their auto-renewal abilities [23, 28]. Figure 3 illustrates the processes that may be involved in ROS-mediated oral carcinogenesis induced by betel quid chewing. Besides arecoline, catechin, flavonoids, and leucoanthocyanidin (flavan 3:4-diols) are the major polyphenols in areca nut that have exhibited pathological alterations after epithelial inflammation [28]. Taken together, these findings indicate that areca nut alone or in combination with other components of betel quid may play pivotal roles in carcinogenesis in the oral mucosa.

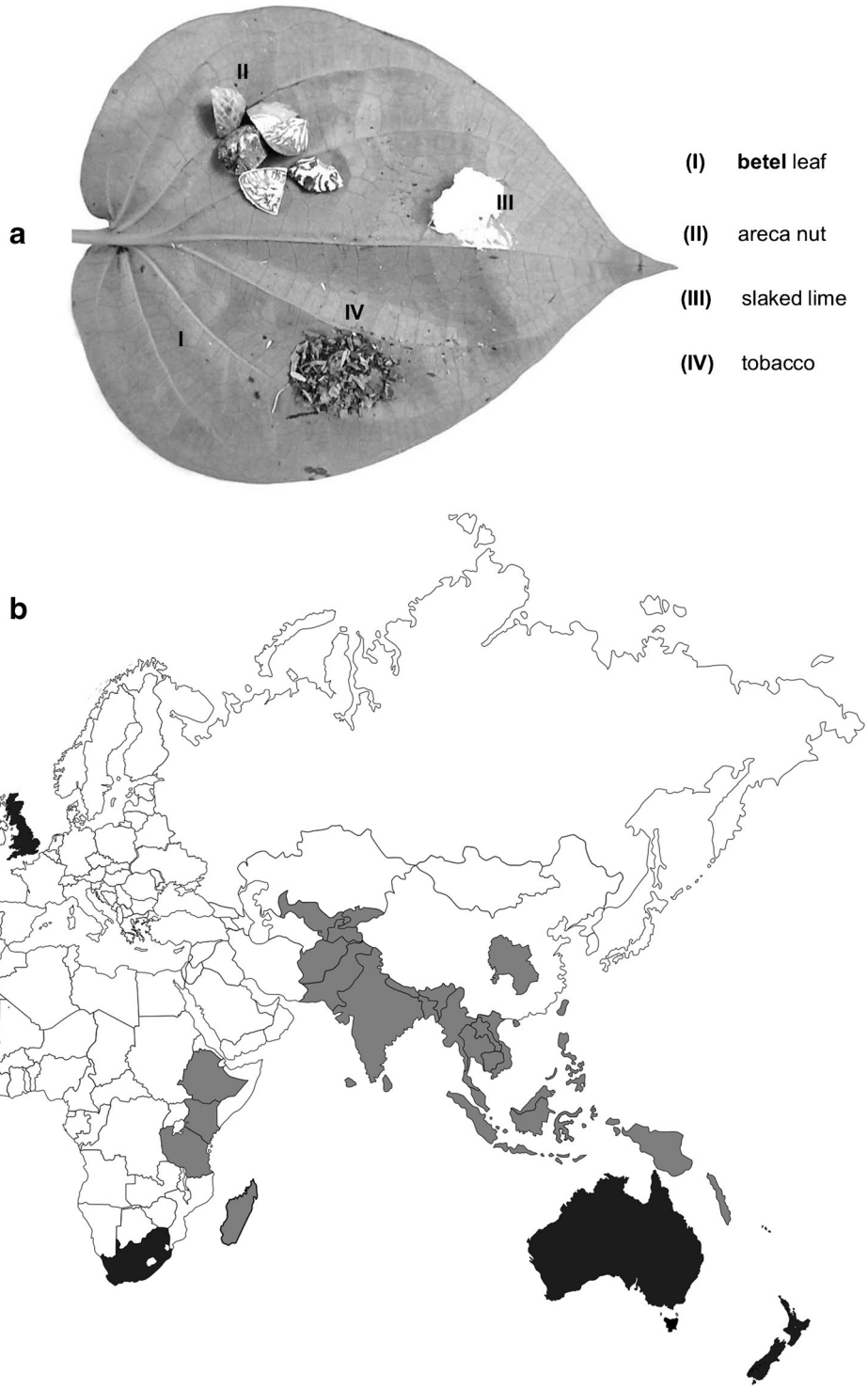


Fig. 1 The major ingredients of betel quid and regions where betel quid chewing is prevalent. **a** The major ingredients of betel quid include the following: (I) betel leaf; (II) areca nut; (II) slaked lime, most commonly prepared in paste form; and (IV) tobacco components (sun-dried or fermented). **b** The gray areas on the map depict regions where betel quid chewing is prevalent in South Asia and the South Pacific Islands. The black-colored areas represent the countries comprising habitual betel quid chewers originating from the Indian subcontinent; the countries include UK (mainly at Yorkshire, Birmingham, Leicester, East and West London), South Africa (Durban and Johannesburg), Australia, and New Zealand

Betel Leaf and Betel Inflorescence

Betel leaf is one of the basic ingredients in betel quid. Hydroxychavicol, a phenolic component present in betel leaf, has been detected in saliva at 4.6 mM concentration after betel quid chewing [40]. An ortho-quinone (quinones, quinone methide, and imide methide) produced during hydroxychavicol metabolism induces the production of ROS leading to the development of oxidative stress [41]. Furthermore, hydroxychavicol has been shown to induce Cox-2 and prostaglandin E2 (PGE2) production in oral keratinocytes, which may contribute to cellular transformation [42]. Additionally, hydroxychavicol can modulate cigarette carcinogen benzo[a]pyrene-mediated toxic effects by

Table 1 Betel quid composition according to different geographical regions [5–22]

Countries	Betel quid compositions					Reference
	BL	AN	SL	Add.	smokeless tobacco and its types	
India Nepal	+	+	+	+	+ −; Pan masala, gutka, khaini, gul, etc.	Lee et al.; Wazir et al. [5, 6]
Pakistan Bangladesh	+	+	+	NA	+ −; Nasar, gutka, etc. (Pakistan), Zarda, gul, khaini, etc. (Bangladesh)	Bile et al.; Huque et al. [7, 8]
Sri Lanka	+	+	+	NA	+ −; Pan masala, mawa, red tooth powder, khaini, zarda etc.	Lee et al.; Chiba et al. [5, 9]
[a]Thailand	+	+	+	+	+ −; NA	Tangjaturonrasme et al. [10]
[a]Taiwan	++	+	+	+	–	Lee et al.; Yang et al. [5, 11]
China (Hunan Province and Hainan Island)	+	+	+	+	–	Lee et al.; Pindborg et al. [5, 12]
[a]Vietnam	+	+	+	–	+ −; NA	Reichart et al. [13]
[a]Cambodia	+	+	+	+	+ −; NA	Ikeda et al. [14]
Lao People's Republic Myanmar	+	+	+	+	+ −; Zarda, gul, etc.	Kyaing et al. [15]
Papua New Guinea	++	+	+	+	–	MacLennan et al. [16]
Malaysia	+	+	+	+ −	+ −; Pan masala, zarda, gutka etc.	Lee et al.; Gan et al. [5, 17]
[a]Indonesia	+	+	–	NA	+ −; Kretek	Lee et al.; Amtha et al. [5, 18]
Guam	+	+	+	NA	+ −; Snuff	Annette et al. [19]
UK, South Africa, New Zealand, Australia (migrant communities)	+	+	+	+ −	+ −; Pan masala, zarda, gutka etc.	Bedi et al.; Cox et al.; Yoganatham et al. [20–22]

BL, betel leaf; *AN*, areca nut; *SL*, slaked lime; *Add.*, additives, including cloves, cardamom, aniseed, dried dates, menthol etc.; +, present; −, absent; ++, betel leaf and betel inflorescence, + −, may or may not; *NA*, data unavailable

[a] Betel quid chewing habit declining

Betel quid chewers, having a prolonged history of chewing, often smoke tobacco and/or consume alcoholic beverage (Lee et al.; Chiba et al.; Reichart et al)

induction of dihydrodiol dehydrogenase (DDH) and hypoxanthine phosphoribosyltransferase (HPRT) [43]. Apart from the leaves, other parts including the stem and inflorescence have been consumed along with betel quid. Chewers consuming betel inflorescence and betel leaves in the quid are at highest and low risk for carcinogenesis, respectively [44]. A high concentration of safrole is present in betel inflorescence resulting in the formation of safrole-DNA adducts, which play an important role in the pathogenesis of oral cancer [44]. A high frequency of safrole-like DNA adducts were detected in betel quid-associated oral cancer and their surrounding tissues, but absent in non-betel-associated oral cancers [45]. These data indicate that safrole-DNA adducts may be associated with oral carcinogenesis in betel quid chewers.

Slaked Lime

Slaked lime in the form of calcium hydroxide and calcium oxide is often added to betel quid. It is usually obtained in powder or paste form; in coastal areas, it is obtained by the burning of shells or harvesting of corals [9]. Slaked lime has been implicated in the development of inflammation and generation of ROS, which exerts harmful effects on DNA, proteins, lipids, and other macromolecules [46]. Slaked lime may alter intracellular calcium homeostasis, promote ATP synthesis, and modulate different mitochondrial protein functions [47]. Excess calcium has been shown to induce keratinocyte differentiation resulting in the downregulation of *p63*, a member of the p53 family of proteins [48, 49]. Nevertheless, elevation in calcium concentration is thought to be responsible for mutations or deletions in the extracellular calcium-sensing receptor, which may induce various pathways, such as phosphoinositide 3-kinase (PI3K/AKT/mTOR) and mitogen-activated protein kinase (MAPK) among others [50]. Thus, calcium-mediated aberrant activation of transduction cascades by slaked lime appears to be involved in oral carcinogenesis.

Synergistic Effects of Other Carcinogens with Betel Quid

Betel Quid Chewing and Tobacco

Betel quid and tobacco may act synergistically as carcinogens [44]. Betel quid chewing with tobacco may result in higher exposure to BSNAs and tobacco-specific nitrosamines (TSNAs) [51, 52]. 3-(Methylnitrosamino) propionitrile (MNPN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1butanone (NNK) are the two major carcinogenic nitrosamines present in betel quid and tobacco, respectively, and have been detected in the saliva of betel quid chewers [52]. Activation of these nitrosamines promotes the DNA methylation of genes whose expression are lost during carcinogenesis [53]. Furthermore, activation of nitrosamines leads to the formation of bulky pyridyloxobutyl DNA adducts via induction of the formation

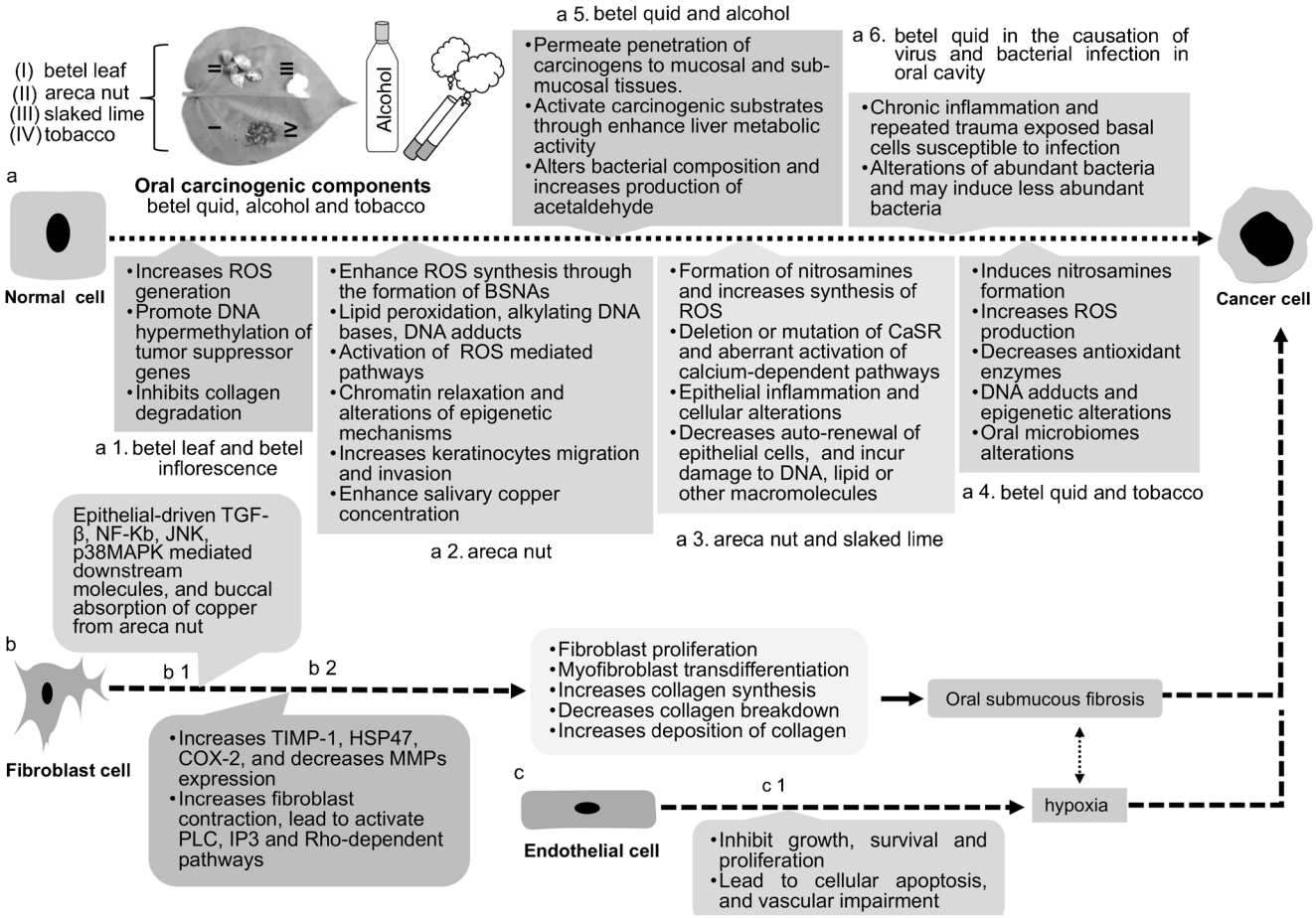


Fig. 2 Possible mechanisms involved in betel quid-induced oral carcinogenesis. (a, a 1) Betel leaf and betel inflorescence increase ROS generation and contribute to DNA adducts. (a, a 2) Areca nut alkaloids increase ROS generation, alkylate the DNA bases, and form DNA adducts. ROS can apparently activate TGF-β, NF-kB, JNK, and p38MAPK pathways. Areca nut induces αvβ6 integrin and mediates the migration and invasion of keratinocytes. (a, a 3) Areca nut and slaked lime induce the generation of ROS through formation of nitrosamines leading to epithelial inflammation. Excess calcium can alter macromolecules and contribute to oral mucosal inflammation. (a, a 4) Tobacco and betel quid can form BSNAs and TSNAs resulting in the induction of epigenetic silencing of tumor suppressor genes. Tobacco may alter oral bacterial microbiomes involved in carcinogenesis. (a, a 5) Alcohol may damage epithelial cells and facilitate the penetration of carcinogens by increasing the epithelial permeability of cell membranes. Additionally, alcohol might activate carcinogenic substrates by increasing its metabolism. It can also induce the production of acetaldehyde, an oral carcinogen involved in oral cancer. (a, a 6) Chronic inflammation and repeated trauma might be associated with immune suppression, which may facilitate viral infection at the buccal mucosa and induce changes in bacterial communities. These effects may be involved in carcinogenesis in the oral mucosa of betel quid chewers. (b, b 1) TGF-β, NF-kB, JNK, and p38MAPK mediated downstream targets acts on fibroblasts and leads to its proliferation, inducing collagen synthesis. (b, b 2) Areca nut chemicals increase collagen synthesis, decrease its breakdown, and activates proliferative cascades in fibroblasts; these factors may be involved in the pathogenesis of OSF. (c, c 1) Areca nut contents act on endothelial cells and inhibit their growth, survival, and proliferation. These effects may induce vascular impairment and lead to hypoxia. Hypoxia may further influence oral carcinogenesis by inducing hypoxia-mediated pathways. ROS, reactive oxygen species; TGF-β, transforming growth factor-beta; NF-kB, nuclear factor kappa B; p38MAPK, p38 mitogen-activated protein kinases; JNK, c-Jun N-terminal kinases; αvβ6, alpha v beta 6; TSNAs, tobacco-specific nitrosamine; OSF, oral submucous fibrosis

of 7-methylguanine, O6-methylguanine (O6-MeG), and O4-methylthymidine in DNA [51, 52]. These effects are induced by DNA hypermethylation followed by the downregulated expression of genes involved in the inhibition of carcinogenesis, thereby leading to genomic instability and invasive carcinogenesis [54]. Accordingly, a cumulative effect of betel quid and tobacco has been observed with a significant increase in the risk of oral cancer when these two factors were combined together [55]. Additionally, oxidation of thiol group of antioxidants such as glutathione-S-transferase, glutathione reductase, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) was reported to be mediated by nitrosamines derived from betel quid and tobacco [2, 56]. These effects have induced oxidative stress and cellular proliferation resulting in a higher frequency of mutations [51–53]. Moreover, betel quid chewing with tobacco has been associated with changes in oral microbial communities and can contribute to oral carcinogenesis [57••, 58•,

59••]. These findings provide evidence and molecular explanation for the synergistic effects of betel quid with tobacco on the development of precancerous oral lesions that may develop into oral cancer.

Betel Quid Chewing and Alcohol Consumption

Alcohol abuse (defined as more than 21 standard drinks in 1 week) is the second largest risk factor for the development of oral cancer [56, 60]. The habit of alcohol consumption increases the risk of oral cancer in habitual chewers [56, 61]. Alcohol may cause damage to epithelial cells and increase the permeability of the epithelial cell membrane, which helps in the penetration of carcinogens into the epithelium [56, 60, 61]. Microtrauma caused by coarse fibers in betel quid could enhance mucosal permeability. Additionally, liver-metabolizing activity enhanced by alcohol might activate carcinogenic substances [60, 61]. These findings indicate that the end product of the metabolism of arecoline and arecoline N-oxide is a human carcinogen [36, 62]. However, further studies are needed to clarify this phenomenon. Meanwhile, recent investigations reported that alcohol with betel quid chewing might alter oral microbial compositions [57••, 59••]. Beta diversity of oral microbiomes was significantly different between the chewers and non-chewers with excessive alcohol consumption [57••, 59••]. Notably, in the presence of ethanol, certain bacterial species are able to produce high levels of acetaldehyde, an oral carcinogen [63]. These findings may support the idea that alcohol consumption increases the risk of oral cancer in habitual betel quid chewers. However, the actual mechanisms responsible for this occurrence and their relationship with the development of cancer remain poorly understood. Further investigations are warranted to clarify these mechanisms.

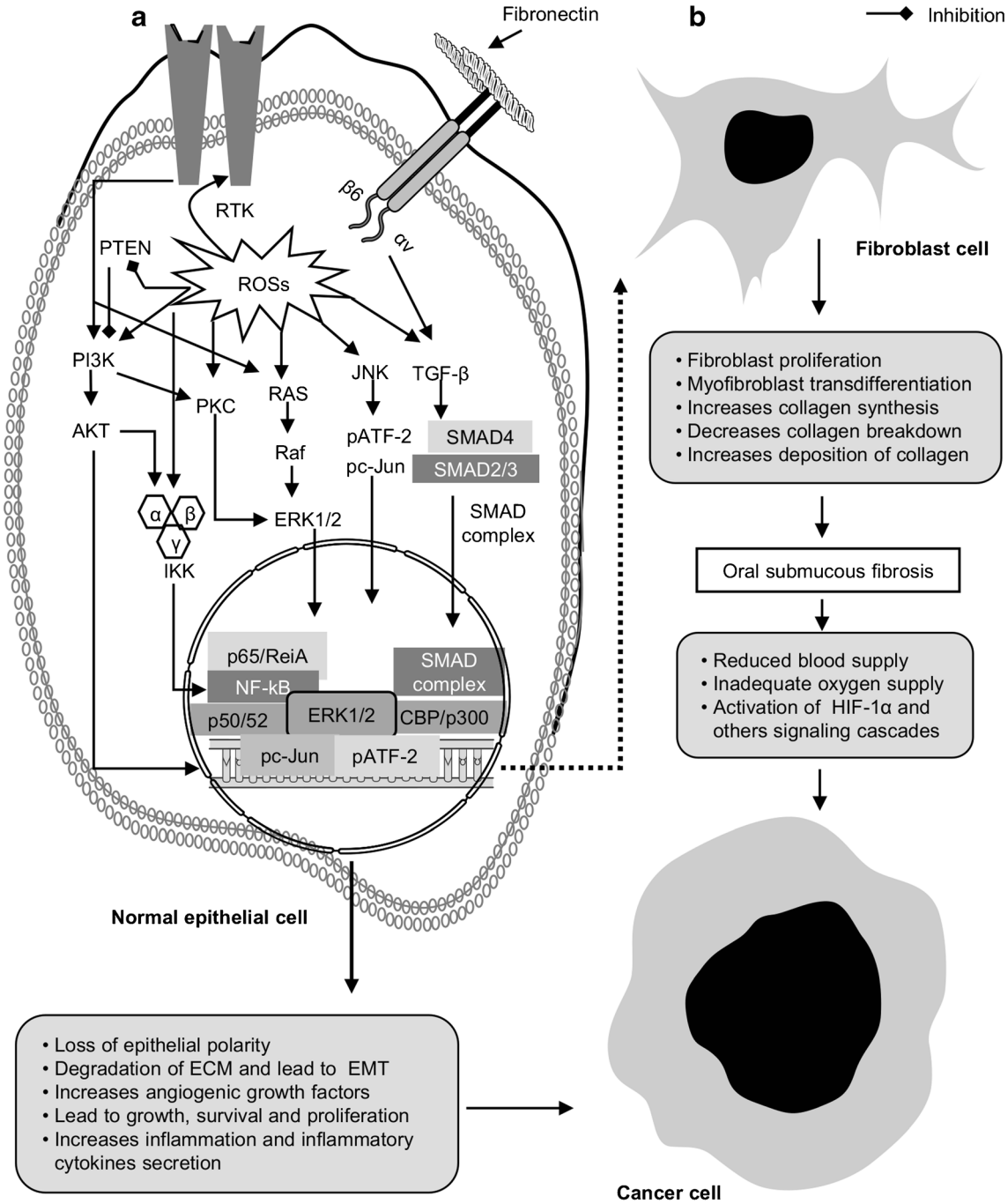
Betel Quid Chewing, Tobacco, and Alcohol Consumption

The incidence of oral cancer was 123-fold higher when these three factors were combined together than in abstainers [64]. The dehydrating effects of alcohol on cell walls may enhance the ability of betel quid and tobacco-derived carcinogens to permeate the oral epithelium [56, 61]. Additionally, nutritional deficiencies associated with heavy drinking can lower the body's natural ability to use antioxidants to prevent the development of cancer [56, 60, 61]. Thus, the oral mucosa may be more vulnerable to carcinogens associated with tobacco, alcohol, and betel quid.

Betel Quid Chewing and Oncogenic Virus

In addition to betel quid chewing and both tobacco as well as alcohol consumption, oncogenic viruses including human papillomavirus (HPV) and Epstein-Bar virus (EBV) might play an important role in oral carcinogenesis [2, 65, 66]. HPV has a well-documented role in the pathogenesis of oral cancer, especially HPV type 16 and 18, which have been shown to be associated with oral cancer in different parts of the world [65]. A high prevalence of HPV has been detected in tissues from oral cancer induced by betel quid chewing [67]. The highest prevalence of HPV-18 was reported in oral leukoplakia induced by betel quid chewing; moreover, HPV infection is thought to increase the risk of betel quid-related oral cancer [68]. A previous study in Sri Lanka has confirmed that the prevalence of oral cancer in habitual betel quid chewers infected with HPV in the buccal mucosa is high [69]. Nonetheless, the relationship between betel quid chewing and the prevalence of HPV is poorly understood. Chronic inflammation with repeated trauma in the oral mucosa of betel quid chewers may expose germinal basal cells to HPV infection [70]. In addition, the suppression of the immune system caused by betel quid components may contribute to HPV infection [71]. Further investigations are required to evaluate the reason for the increase in the frequency of HPV infection in betel quid chewers. The relationship between EBV, an oncogenic double-stranded DNA virus, and oral cancer remains controversial [72]. Betel quid chewing appeared to enhance EBV prevalence in oral cancer, suggesting that EBV may be an important etiological factor for betel quid-

Fig. 3 ROS-mediated oral carcinogenesis induced by betel quid chewing. ▶ (a) Betel quid-induced ROS can phosphorylate RTK and lead to the activation of PI3K/Akt and Ras-Raf-ERK pathways. Activated PI3K/Akt can induce the PKC and NF-kB pathways. PKC can influence the activation of the ERK pathway. ROS also concurrently inactivates PTEN, a negative regulator of the PI3K pathway. ROS can directly activate NF-kB by activating IKKs, which phosphorylate and lead to the degradation of IkB and activation of NF-kB. ROS can also lead to the activation of JNK through phosphorylation of ATF-2 and c-Jun. It can activate TGF-β, via phosphorylation of its ligands, and Smad2/3. Phosphorylation of Smad2/3 leads to the formation of the Smad complex, which translocates to the nucleus and activates its downstream targets. Activated TGF-β can further increase the formation of αvβ6 dimer and mediate keratinocyte migration and invasion. Additionally, a stable dimer of αvβ6 integrin further increases the activity of TGF-β in the oral epithelium. (b) ROS-mediated activation of NF-kB, JNK, Ras-Raf-ERK, and TGF-β in the epithelium can affect the fibroblasts and lead to the pathogenesis of OSF by inducing collagen synthesis and decreasing its breakdown. OSF causes reduction in blood supply and induces tissue hypoxia. These activities result in increased survival signaling within the altered premalignant cells and contribute to oral carcinogenesis. ROS: reactive oxygen species; RTK: receptor tyrosine kinase; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; AKT: protein kinase B; ERK: extracellular signal-regulated kinase; PKC: protein kinase C; NF-kB: nuclear factor kappa B; PTEN: phosphatase and tensin homolog; IKKs: IkB kinase; IkB: inhibitor of kappa B; JNK: c-Jun N-terminal kinases; ATF2: activating transcription factor 2; TGF-β: transforming growth factor-beta; αvβ6 integrin: alpha v beta 6; OSF: oral submucous fibrosis; Smad: mothers against decapentaplegic homolog; CBP/p300: CREB binding protein/E1A-associated protein p300; ECM: extracellular matrix; EMT: epithelial-mesenchymal transition



related oral cancer [66]. The precise molecular mechanisms behind the etiological role of EBV in betel quid-induced oral cancer need to be evaluated in more detail.

Betel Quid Chewing and Oral Microbial Communities

Although bacterial infection is a risk factor for carcinogenesis, [73] the potential role of the oral microbiome in betel quid-induced oral carcinogenesis is relatively unexplored. Betel quid chewers often experience poor oral hygiene and chronic periodontitis, both of which have been linked to changes in oral bacterial compositions, and may increase the risk of oral cancer [74]. Prolonged use of betel quid may disrupt the richness and evenness of the epithelium causing changes in the relative abundance of common bacteria [57••, 58•, 59••]. This disruption in the normal oral microflora may impede its ability to counter betel quid-induced inflammation in the oral mucosa resulting in increased susceptibility to malignant transformation. Increase in the relative abundance of *oribacterium*, *streptococcus infantis*, and *Streptococcus anginosus* has previously

Table 2 Clinical trials using anti-oxidants for treating oral leukoplakia (OLP) [85–88, 89•]

Treatment	Study design	Side effects	Treatment outcomes	Reference
Vitamin A vs placebo	6 months, oral administration, 2000.000 IU/week	Not reported	Complete remission in 57.1% of participants, and total suppression of the development of new leukoplakia	Stich et al. [85]
Group 1: beta-carotene Group 2: beta-carotene and vitamin A vs placebo	6 months, oral administration, beta-carotene—180 mg/week, vitamin A—1000.000 IU/week	Not reported	Decrease micronucleated mucosal cells and inhibit the development of new lesions in the combined group than alone	Stich et al. [86]
Spirulina fusiformis vs placebo	1-year, oral administration, 1 g/day	Headache, muscular pain	Response rates were less than 50%, malignant transformation and recurrence were reported	Mathew et al. [87]
Group 1: Vitamin A vs placebo Group 2: beta-carotene vs placebo	1-year, oral administration, 300.000 IU/week 1-year oral administration, 360 mg/week	No major side effects were observed	The positive clinical response has reported, though, a high rate of relapse has observed	Sankaranarayanan et al. [88]
Curcumin vs placebo	6 months, oral administration, 3.6 g/day	Anemia, skin/subcutaneous tissue disorder, and hypertension	Response rates were more than 50%, though, the malignant transformation was reported	Kuriskose et al. [89•]

been detected in OPMDs in the oral mucosa of betel quid chewers [59••]. Betel quid chewing has been shown to increase the levels of these microbial communities indicating their potential role in the development of oral cancer [59••]. *S.anginosus*, an anaerobic bacteria detected in the tumor tissues, induced the synthesis of inflammatory cytokines and nitric oxide (NO) thereby indicating its potential role in oral carcinogenesis [75]. Cytokine-induced inflammation due to stimulation with bacteria in betel quid chewers may be involved in the development of oral cancer. Furthermore, inflammatory reactions induced by both betel quid and betel quid-related bacteria may participate in betel quid-induced carcinogenesis.

Molecular Pathway Involved in Betel Quid-Induced Oral Carcinogenesis

As stated earlier, ROS may be one of the predominant risk factors for the induction of oral carcinogenesis in betel quid chewers [30]. Other possible molecular mechanisms involved in oral cancer cannot be ruled out; therefore, the ROS-associated signaling pathways in betel quid chewers need to be summarized. ROS are oxygen-free highly reactive molecules and byproducts of aerobic metabolism, and include superoxide anions, hydrogen peroxide, and hydroxyl radicals, all of which have inherent chemical properties that confer to react to different biological targets [76]. ROS are host-defending molecules released by neutrophils for the destruction of foreign pathogens; however, accumulating evidence suggested that ROS play central roles in determining the cell fate by modulating various signaling pathways [76, 77]. The homeostasis of ROS has been maintained by the production of antioxidant enzymes such as SOD, CAT, and GPx [76]. As shown in Fig. 3, betel quid components downregulate these antioxidant proteins and lead to the induction of ROS wherein, ROS activates a wide variety of signaling molecules that can contribute to oral carcinogenesis [38•, 39•, 77–81].

Chemoprevention for Betel Quid-Related Oral Cancer

Oral cancer is well-characterized by the progression from pre-malignant changes to invasive cancer. Thus, chemopreventive interventions should be developed to prevent this malignant transformation. The elimination of ROS may prove most effective because ROS are significant risk factors for the induction of betel quid-mediated oral carcinogenesis [82]. In fact, antioxidants that eliminate oxidative stress induced by ROS are the most widely used chemopreventive agents to treat OPMDs [83]. Antioxidants are a group of chemicals that can deactivate free radicals and prevent their formation [82]. The antioxidant molecules act not only to inhibit the development of cancer cells but also to destroy them through apoptosis, which is caused by the stimulation of cytotoxic cytokines, their action on gene expression, and the prevention of tumor development by affecting the blood supply or by cellular differentiation [82–84]. These apoptotic mechanisms may work as an ideal chemotherapeutic and chemopreventive method

Table 3 Clinical trials using anti-oxidants for treating oral submucous fibrosis (OSF) [90–100]

Treatment	Study design	Side effects	Treatment outcomes	Reference
Group 1: lycopene vs placebo, Group 2: lycopene and biweekly intralesional betamethasone injections vs placebo	2 months, oral administration, lycopene—16 mg/day, betamethasone—1-mL ampules of 4 mg, 2 times/weekly	No major side effects were observed	Mouth opening value were significant increases in the lycopene group	Kumar et al. [90]
Group 1: curcumin capsule Group 2: turmeric oil vs placebo	3 months, oral administration, curcumin capsule—1 g/day, turmeric oil—600 mg/day	Not reported	Mouth opening value has increased Pain and burning sensation has reduced	Deepa et al. [91]
Lycopene tablet contains zinc, selenium and phytonutrients	3 months, oral administration, 2000 μg, 2 times/day	No major side effects were observed	Mouth opening value has significantly increased in the lycopene group	Gowda et al. [92]
Lycopene vs placebo	3 months, oral administration, 8 mg/day	No major side effects were observed	Mouth opening value was significant increases in the lycopene group	Karemore et al. [93]
Group A: lycopene with biweekly intralesional dexamethasone and hyaluronidase Group B: multivitamin capsules with biweekly intralesional dexamethasone and hyaluronidase Group C: biweekly intralesional dexamethasone and hyaluronidase	6 weeks, oral administration, group A: lycopene—16 mg/day, dexamethasone—1.5 ml and hyaluronidase—1500 IU/day group B: multivitamin one capsule, dexamethasone—1.5 ml and hyaluronidase—1500 IU/day group C: dexamethasone—1.5 ml and hyaluronidase—1500 IU/day	No major side effects were observed	Lycopene in combination with intralesional steroids and hyaluronidase is highly efficacious in improving the mouth opening and reducing other symptoms in patients with oral submucous fibrosis	Selvam et al. [94]
Curcumin and piperine tablet	1 month, oral administration curcumin—900 mg/day and piperine—15 mg/day	Not reported	Reduces pain and burning sensation, though no significant in mouth opening	Agarwal et al. [95]
Group 1: intralesional dexamethasone and hyaluronidase injection Group 2: curcumin	3 months, submucosal injection dexamethasone—4 mg/ml/weekly hyaluronidase—1500 IU/weekly oral administration curcumin tablet—600 mg/day	Not reported	Mouth opening value has increased Pain and burning sensation has reduced	Yadav et al. [96]
Curcumin powder and tulsi powder mixed in glycerine	3 months, topical application 4–5 times/day 1 g tulsi and 1 g turmeric mixed in a glycerine base	No effects were observed	Significant improvement was seen in both the burning sensation and mouth opening.	Srivastava et al. [97]
Topical curcumin lozenges vs clobetasol propionate ointment	3 months curcumin—2 g/day clobetasol propionate ointment − 0.05 mg, 3 times/day	No effects were observed	Reduces pain and burning sensation, mouth opening value has increased in the curcumin group	Hazarey et al. [98]
Lycopene tablet vs *Aloe vera* gel	3 months lycopene—8 mg/day aloe vera gel—5 mg, 3 times/day	No effects were observed	Clinical improvements in mouth opening and tongue protrusion was significant	Patil et al. [99]
Curcumin oral paste	3 months, topical administration 4% curcumin oral paste, 3 times/day	No effects were recorded	Reduces pain and burning sensation, mouth opening value has increased compared with baseline data	Devaraju et al. [100]

for cancer treatment without side effects [82–84]. Therefore, antioxidants are most effective for the prevention of betel quid-related oral cancer. The most widely used chemopreventive agents to treat betel quid-induced oral lesions are shown in Tables 2 and 3.

Conclusions

Betel quid chewing habit is considered to be one of the predominant risk factors for the induction of oral cancer in South Asian countries. Each component in betel quid may individually, synergistically, and coordinately participate in carcinogenesis. Besides betel quid, carcinogens derived from tobacco and alcohol may also act synergistically. Additionally, prolonged habitual betel quid chewing may result in chronic inflammation caused by multiple ulcerations and microbiome dysbiosis in the oral cavity. These multiple factors are likely to act synergistically and cause neoplastic transformation in the oral mucosa of betel quid chewers. These observations highlighted in this review will encourage the development of new areas of research, especially with regard to the factors that modify the disease and the therapeutic targets for the treatment of oral cancer.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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