Ocimum sanctum (Tulsi) as a Natural Chemopreventive Agent in Oral Cancer: Pathway Modulation and Clinical Relevance

Ashwini Badhe

Swalife Biotech Ltd North Point House, North Point Business Park, New Mallow Road, Cork (Republic of Ireland)

Corresponding author: drpravinbadhe@swalifebiotech.com
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Abstract

Oral cancer remains a major global health challenge, particularly in developing countries, where high morbidity and mortality rates persist due to late diagnosis and limited therapeutic success. Chemoprevention using natural phytochemicals offers a promising complementary strategy. *Ocimum sanctum* (Tulsi), a revered medicinal herb in Ayurveda, exhibits significant chemopreventive potential attributed to its rich bioactive profile, including eugenol, ursolic acid, and rosmarinic acid. These compounds modulate key molecular pathways involved in carcinogenesis by scavenging reactive oxygen species, suppressing inflammation (via NF-κB and COX-2 inhibition), inducing apoptosis, and regulating tumor suppressor genes such as p53. Experimental evidence from in vitro and in vivo studies supports Tulsi's ability to inhibit oral tumor initiation and progression. Despite encouraging findings, further clinical investigations are essential to validate its efficacy, optimize dosage, and enhance bioavailability. Tulsi represents a promising natural agent for oral cancer prevention through multi-targeted pathway modulation.

1.Introduction

1.1 Epidemiology of Oral Cancer

Oral cancer represents one of the most formidable global health challenges, ranking as the 16th most common cancer worldwide with approximately 389,846 new cases diagnosed annually. The disease demonstrates stark geographical disparities, with South Asian countries bearing a disproportionate burden. Globally, oral cancer accounts for approximately 5% of all malignancies, yet this percentage escalates dramatically in certain regions. In India, oral cancer constitutes approximately 40% of all cancer cases, representing the most prevalent malignancy among men. The age-standardized incidence rate in India reaches 20 per 100,000 population, significantly higher than the global average.[1,2,3]

The epidemiological landscape reveals alarming mortality statistics, with 188,438 deaths attributed to oral cancer worldwide in 2022. India alone accounts for nearly one-third of global oral cancer incidence and mortality, with an estimated 79,979 deaths annually. The disease demonstrates a pronounced male predominance, with age-standardized rates of 14.7 per 100,000 in Indian men compared to 5.0 per 100,000 in women. Particularly concerning is the trend toward younger age of onset in Indian populations, with patients presenting at significantly younger ages compared to Western populations.[1,2,3,4]

Regional variations within India are substantial, with the highest incidence rates observed in the central region, reaching 64.8% in males and 37.2% in females at 70 years of age. The five-year survival rate remains dismally low at approximately 50-60%, with squamous cell carcinoma accounting for 90% of all oral cancers. Recent projections indicate that oral cancer incidence is expected to rise by 65% by 2050, with the most dramatic increases anticipated in low and medium Human Development Index countries.[5,6,7]

The primary risk factors driving this epidemic include tobacco consumption in various forms, alcohol consumption, and human papillomavirus (HPV) infection. A recent analysis revealed that one in three cases of oral cancer globally can be attributed to smokeless tobacco and areca nut use, with over 120,000 cases in 2022 caused by these factors alone. The synergistic effects of tobacco and alcohol create multiplicative risk increases, with current smokers demonstrating an 11.6-fold increased risk and heavy alcohol consumers showing a 2.7-fold elevation in risk. HPV infection, particularly high-risk types, has emerged as an independent risk factor, increasing oral cancer risk by 2.4-fold even in never-smokers and non-drinkers.[8,9,10,11]

1.2 Need for Chemoprevention

The limitations of conventional oral cancer therapies underscore the urgent need for effective chemopreventive strategies. Current treatment modalities—including surgery, radiotherapy, and chemotherapy—are associated with significant morbidity, mortality, and quality of life impairments. Surgery, typically the primary treatment for non-metastatic oral squamous cell carcinoma, often requires extensive resections that result in functional impairment, disfigurement, and compromised quality of life. The complexity of oral anatomy necessitates intricate reconstructive procedures that may not fully restore normal function.[5,12]

Radiotherapy and chemotherapy present their own challenges, including severe acute and chronic toxicities. Oral complications from these treatments include mucositis affecting 59-100% of patients, with severe forms occurring in 23-81% of cases. Additional complications encompass xerostomia, dysgeusia, radiation caries, osteoradionecrosis, and trismus, many of which persist long after treatment completion. These side effects not only compromise quality of life but also contribute to treatment interruptions, dose reductions, and suboptimal therapeutic outcomes.[13,14]

Recurrence remains a persistent challenge in oral cancer management, with local and regional recurrences accounting for up to 90% of treatment failures. Recurrence rates vary from 18-76% in patients who received standard treatment, with median time to recurrence of 7.5 months. The five-year survival rate for recurrent disease is particularly poor, with only 30-45% of patients eligible for salvage surgery achieving favorable outcomes. Patients ineligible for salvage procedures typically receive palliative chemotherapy with limited efficacy and rare potential for cure.[15,16]

The financial burden of oral cancer treatment is substantial, with direct and indirect costs creating significant economic strain on patients, families, and healthcare systems. In India, the total cost of lost productivity due to premature oral cancer mortality was estimated at $5.6 billion in 2022, representing 0.18% of the combined gross domestic product. These economic considerations, combined with the high morbidity and mortality associated with conventional treatments, emphasize the critical need for preventive strategies that can reduce cancer incidence and improve patient outcomes.[4,5,17]

1.3 Natural Products in Cancer Prevention

The limitations of conventional cancer therapies have catalyzed renewed interest in natural products as complementary and alternative approaches to cancer prevention and treatment. Natural compounds have demonstrated significant promise due to their diverse chemical structures, multi-target mechanisms of action, and generally

favorable safety profiles compared to synthetic drugs. Approximately 30 natural compounds are currently under clinical investigation for cancer treatment, reflecting the growing recognition of their therapeutic potential.[18,19]

Herbal medicines offer several distinct advantages in cancer prevention and treatment, including selective toxicity toward cancer cells while sparing normal tissues. This selectivity minimizes the adverse effects associated with conventional chemotherapy, enabling patients to complete treatment regimens more effectively. Natural products also demonstrate synergistic effects when combined with conventional treatments, potentially enhancing therapeutic efficacy while reducing toxicity. Clinical studies have shown that herbal medicines can alleviate chemotherapy-induced side effects, improve treatment tolerance, and maintain quality of life in cancer patients.[18]

The mechanisms of action of natural anticancer compounds are diverse and include apoptosis induction, cell cycle arrest, angiogenesis inhibition, metastasis suppression, and immune system modulation. Bioactive compounds such as flavonoids, alkaloids, terpenoids, and polyphenols exhibit these multifaceted effects through various cellular pathways. For example, curcumin from turmeric demonstrates anticancer properties through NF-κB pathway inhibition, while compounds like paclitaxel from Pacific yew and vincristine from Madagascar periwinkle have become standard components of modern cancer treatment protocols.[18,19]

Historical success stories of natural product-derived drugs provide compelling evidence for their therapeutic potential. Vinca alkaloids (vinblastine and vincristine), introduced in the 1960s, are widely used to treat various cancers including breast cancer, lymphomas, and leukemias. Paclitaxel, derived from Taxus brevifolia, is a cornerstone therapy for breast, ovarian, and lung cancers. These examples demonstrate that natural products can serve as direct therapeutic agents or as templates for synthetic drug development.[18]

The integration of natural products into modern cancer care represents a promising approach to address the limitations of conventional therapies while capitalizing on centuries of traditional medicinal knowledge. This integration requires rigorous scientific validation, standardization of extracts, optimization of delivery systems, and comprehensive safety assessment to ensure therapeutic efficacy and patient safety.[18,20]

1.4 Role of Ocimum sanctum

Ocimum sanctum, commonly known as Tulsi or Holy Basil, occupies a unique and revered position in traditional Indian medicine and culture, making it an ideal candidate for chemopreventive research. This aromatic herb of the Lamiaceae family has been utilized for over 3,000 years in Ayurvedic medicine and holds profound cultural and religious significance in Indian society. The plant is considered sacred in Hinduism and is worshipped as an earthly manifestation of the goddess Lakshmi, symbolizing purity, prosperity, and divine protection.[21,22,23]

The traditional significance of Tulsi extends beyond its medicinal applications to encompass spiritual and cultural dimensions. Ancient Hindu texts, including the Vedas and Puranas, mention Tulsi as a plant of divine origin. The annual ceremony of Tulsi Vivah, celebrating the sacred marriage of Lord Vishnu to Tulsi, demonstrates the deep cultural integration of this plant in Indian society. Tulsi plants are commonly cultivated in Indian households, where they serve as protective and auspicious symbols while providing readily accessible medicinal resources.[21]

The broad pharmacological spectrum of *Ocimum sanctum* has been extensively documented through centuries of traditional use and modern scientific investigation. Ayurvedic texts categorize Tulsi as a Rasayana (rejuvenating herb) and Dashemani Shwasaharni (anti-asthmatic agent), reflecting its adaptogenic and respiratory protective properties. The plant demonstrates remarkable versatility in treating diverse conditions including respiratory disorders, digestive ailments, skin diseases, fever, arthritis, and various infectious conditions.[22,23]

Modern pharmacological research has validated many traditional uses of Tulsi and revealed additional therapeutic properties. Scientific studies have confirmed that Tulsi possesses antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, cardioprotective, hepatoprotective, neuroprotective, anti-diabetic, anti-stress, and notably, anticancer activities. The plant's unique combination of bioactive compounds, including eugenol (the primary active constituent comprising 40-71% of essential oil), flavonoids, phenolic acids, and terpenoids, contributes to its multifaceted therapeutic effects.[22,23,24]

The rationale for exploring Tulsi's chemopreventive potential in oral cancer is multifold. First, its traditional use in treating oral and respiratory conditions provides ethnobotanical evidence for oral cavity-specific effects. Second, the plant's established anti-inflammatory, antimicrobial, and immunomodulatory properties align with key mechanisms involved in cancer prevention. Third, preliminary studies have demonstrated significant anticancer activity against various cancer cell lines, including oral cancer models.[23,24,25,26]

The accessibility and safety profile of Tulsi make it particularly suitable for long-term chemopreventive applications. Unlike many pharmaceutical interventions, Tulsi can be safely consumed as a dietary supplement or herbal preparation with minimal adverse effects. Clinical studies have consistently reported favorable outcomes with no significant adverse events, supporting its potential for widespread preventive use. The plant's cultivation throughout India and increasing global availability ensure practical feasibility for large-scale implementation of chemopreventive strategies.[21,24]

Furthermore, the multi-target nature of Tulsi's bioactive compounds addresses the complex, multistep process of carcinogenesis through simultaneous modulation of multiple pathways involved in cancer initiation, promotion, and progression. This comprehensive approach contrasts with single-target pharmaceutical interventions and may provide superior preventive efficacy against the heterogeneous nature of oral cancer.[18,22]

The convergence of traditional knowledge, cultural significance, extensive pharmacological research, and preliminary anticancer evidence positions *Ocimum sanctum* as a promising candidate for oral cancer chemoprevention, warranting comprehensive investigation of its mechanisms, efficacy, and clinical applications in preventing this devastating disease.

2. Phytochemical Profile of *Ocimum sanctum*

Ocimum sanctum (Tulsi) harbors a diverse array of bioactive phytochemicals that underpin its therapeutic potential.

2.1 Major Bioactive Compounds

The essential oil of Tulsi is dominated by eugenol, constituting 40–71% of its volatile constituents. Ursolic acid, a pentacyclic triterpenoid, ranges from 0.7–3.5% in leaf extracts and contributes to anti-inflammatory and anticancer activities. Rosmarinic acid, a potent phenolic antioxidant, is present at 1.2–2.8% and exerts free-radical scavenging effects. Flavonoids such as apigenin (0.3–0.6%) and linalool (1–5%) impart anti-proliferative and neuroprotective actions. Carvacrol, albeit in lower abundance (0.1–0.4%), enhances antimicrobial efficacy. Additional constituents include ursolic acid derivatives, orientin, vicenin, and methyl eugenol, collectively contributing to a multi-targeted pharmacological profile.[22,23]

2.2 Extraction and Standardization

Tulsi extracts are obtained via various methodologies to maximize yield and reproducibility. Hydrodistillation and steam distillation are standard for essential oil isolation, with yields of 0.5–1.2%

(w/w) and compositional verification by gas chromatography–mass spectrometry (GC-MS). Solvent extraction using ethanol or methanol (70–95%) at room temperature for 24–48 hours yields phenolic-rich extracts; total phenolic content is quantified by spectrophotometry (Folin–Ciocalteu assay) and HPLC. Supercritical CO_2 extraction offers solvent-free recovery of lipophilic triterpenoids like ursolic acid, with parameters optimized at 300 bar and 40 °C to obtain selective fractions. Standardization protocols employ marker-based quantification: eugenol content is standardized to ≥50 mg/g by GC, ursolic acid to ≥5 mg/g by HPLC, and rosmarinic acid to ≥10 mg/g by UV detection.[23,27]

2.3 Pharmacokinetic and Bioavailability Aspects

Key phytochemicals in Tulsi exhibit variable absorption and metabolism. Eugenol displays rapid gastrointestinal absorption with peak plasma concentrations within 30–60 min and systemic bioavailability of ~70% after oral administration; it undergoes extensive phase II metabolism (glucuronidation and sulfation) in the liver, with renal excretion of conjugates. Ursolic acid suffers poor oral bioavailability (<15%) due to low aqueous solubility and extensive first-pass metabolism; lipid-based formulations and nanoparticle carriers have been shown to improve bioavailability by 3–5-fold in preclinical models. Rosmarinic acid attains moderate plasma levels (Cmax ~1.2 μg/mL) within 2 hours; it is metabolized to caffeic acid derivatives and conjugates, displaying a terminal half-life of ~4 hours. Flavonoids such as apigenin exhibit limited absorption (<5%) with enterohepatic recycling prolonging systemic exposure up to 12 hours. Linalool and carvacrol are rapidly absorbed and eliminated, with half-lives of 1–2 hours, indicating the need for frequent dosing or sustained-release formulations to maintain therapeutic levels.[22,23]

Collectively, the phytochemical richness of Tulsi—combined with standardized extraction techniques and ongoing advancements in formulation science—supports its development as a reproducible, multi-targeted chemopreventive agent.

3. Mechanisms of Chemopreventive Action of *Ocimum sanctum*

3.1 Antioxidant and Free Radical Scavenging

Oxidative stress, characterized by excessive reactive oxygen species (ROS), plays a pivotal role in oral carcinogenesis by inducing lipid peroxidation, protein oxidation, and DNA damage. *Ocimum sanctum* exerts potent antioxidant effects primarily through its phenolic constituents, with eugenol serving as the principal free radical scavenger. Eugenol donates hydrogen atoms to neutralize ROS, thereby reducing superoxide anion generation and lipid peroxidation markers such as malondialdehyde (MDA) in oral keratinocytes. Rosmarinic acid complements this activity by chelating transition metals and inhibiting oxidative DNA strand breaks in comet assays, demonstrating a 60% reduction in DNA tail length compared to untreated controls. Flavonoids like apigenin and orientin further bolster endogenous antioxidant defenses by upregulating superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, restoring redox homeostasis in oral cancer cell lines challenged with hydrogen peroxide.[23,25,26,28]

3.2 Anti-inflammatory Activity

Chronic inflammation fosters a microenvironment conducive to tumor initiation and progression. *Ocimum sanctum* mitigates inflammatory signaling through multi-faceted inhibition of key mediators. Eugenol suppresses cyclooxygenase-2 (COX-2) expression by ~70% at 25 μM concentrations in phorbol ester–stimulated epithelial cells, thereby reducing prostaglandin E_2 synthesis and inflammatory edema. Concurrently, Tulsi extracts inhibit nuclear factor-κB (NF-κB) activation by preventing inhibitor κBα (IκBα) phosphorylation and subsequent p65 nuclear

translocation, leading to a 60–80% decrease in TNF-α, IL-6, and IL-1β secretion in macrophage and oral cancer co-culture models. Ursolic acid enhances these effects by downregulating mitogen-activated protein kinase (MAPK) pathways—specifically p38 and JNK phosphorylation—thereby amplifying suppression of pro-inflammatory cytokines and matrix metalloproteinases implicated in tumor-associated inflammation.[26,28,29,30]

3.3 Modulation of Apoptosis and Cell Cycle

Dysregulated apoptosis and unchecked cell cycle progression underpin tumor growth. *Ocimum sanctum* bioactives orchestrate apoptotic and cell cycle arrest pathways to eliminate transformed cells. Eugenol activates the mitochondrial (intrinsic) apoptosis cascade by upregulating tumor suppressor p53 and pro-apoptotic Bax, while downregulating anti-apoptotic Bcl-2, resulting in an increased Bax/Bcl-2 ratio and cytochrome c release. This cascade culminates in caspase-9 and caspase-3 activation, with a 3-fold increase in caspase-3 activity observed in treated oral squamous carcinoma cells. Moreover, rosmarinic acid potentiates extrinsic apoptosis via Fas receptor upregulation, synergistically enhancing cell death. Regarding cell cycle regulation, Tulsi extracts induce G1/S arrest through p21^WAF1/Cip1 upregulation and cyclin D1 downregulation, as well as G2/M arrest by inhibiting cyclin B1 and Cdc2 kinase activity, thereby preventing mitotic entry in transformed cells.[26,31,32]

3.4 Anti-proliferative and Anti-metastatic Effects

Inhibition of tumor cell proliferation and metastasis is critical for chemoprevention. *Ocimum sanctum* bioactives target proliferative signaling and extracellular matrix remodeling. Eugenol and ursolic acid attenuate PI3K/Akt signaling by reducing Akt phosphorylation levels by 50% at 20 μM, leading to decreased mTOR activation and cell proliferation rates. Concurrent suppression of the MAPK/ERK pathway further inhibits proliferation, evidenced by reduced ERK1/2 phosphorylation in oral cancer cell lines treated with Tulsi extracts. Anti-metastatic effects arise from downregulation of matrix metalloproteinases MMP-2 and MMP-9 gene expression by ~60%, impairing extracellular matrix degradation and cancer cell invasion. Additionally, wnt/β-catenin signaling is disrupted via reduced β-catenin nuclear accumulation and TCF/LEF transcriptional activity, curbing migratory and invasive phenotypes. Tulsi's anti-angiogenic potential is manifested through VEGF suppression; eugenol decreases VEGF secretion by 55%, inhibiting microvessel formation in chick embryo chorioallantoic membrane assays.[26,29,30]

3.5 Epigenetic and Genomic Modulation

Emerging evidence highlights Tulsi's role in modulating epigenetic regulators and non-coding RNAs implicated in carcinogenesis. Rosmarinic acid and eugenol inhibit DNA methyltransferase (DNMT) activity by 40–50%, leading to re-expression of tumor suppressor genes silenced by promoter hypermethylation, such as p16^INK4a and RASSF1A, in preclinical oral cancer models. Histone acetylation is enhanced via inhibition of histone deacetylases (HDACs), increasing acetylation marks on H3 and H4 tails and promoting open chromatin conformation conducive to gene transcription. Concurrently, Tulsi phytochemicals modulate microRNA (miRNA) expression: upregulation of tumor-suppressive miR-34a and miR-200c and downregulation of oncogenic miR-21 and miR-155 have been documented, contributing to apoptosis induction, reduced epithelial-mesenchymal transition, and suppression of cancer stem cell–like properties.[31]

Collectively, the multifaceted mechanisms of *Ocimum sanctum*—spanning antioxidant, anti-inflammatory, pro-apoptotic, anti-proliferative, anti-metastatic, and epigenetic modulation—underscore its promise as a natural chemopreventive agent in oral cancer. Continued elucidation of these pathways and translation into clinical studies will be essential for validating Tulsi's preventive efficacy.

4. Experimental Evidence

4.1 In Vitro Studies

Multiple studies have evaluated *Ocimum sanctum* extracts and isolated phytochemicals for cytotoxicity and mechanistic effects in oral cancer cell lines. SCC-9 and KB cells are among the most frequently used models.

In SCC-9 cells, ethanolic extracts of Tulsi leaves exhibited dose-dependent cytotoxicity, with IC_{50} values ranging from 15 to 35 µg/mL after 24 hours of treatment. This cytotoxicity was significantly higher than that observed in normal oral keratinocytes, indicating selective tumor cell targeting. Similar results were obtained in KB cells, where essential oil–rich fractions yielded IC_{50} values of 10–25 µg/mL, attributable primarily to eugenol and ursolic acid constituents.[25,26,32]

Apoptotic induction was confirmed through annexin V/propidium iodide staining and flow cytometric analysis. SCC-9 cells treated with 25 µg/mL ethanolic extract displayed a 4.5-fold increase in early apoptotic events compared to controls, accompanied by a 3.2-fold rise in late apoptotic/necrotic populations. Western blot analysis revealed upregulation of p53 and Bax proteins concurrent with downregulation of Bcl-2, resulting in an increased Bax/Bcl-2 ratio. Caspase assays demonstrated a 2.8-fold elevation in caspase-3 activity and a 2.5-fold increase in caspase-9 activity in treated SCC-9 cells, confirming engagement of the intrinsic apoptotic pathway.[26,32]

Oxidative stress parameters further elucidated Tulsi's antioxidant effects. ROS levels, measured by dichlorofluorescein diacetate fluorescence, were reduced by 55% in KB cells treated with 20 µg/mL of rosmarinic acid–enriched extracts, compared to vehicle-treated cells. Lipid peroxidation, quantified by malondialdehyde (MDA) assays, showed a 48% decrease following treatment with 30 µg/mL eugenol. Concurrently, activities of antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—increased by 1.7-, 1.9-, and 2.1-fold respectively, restoring redox balance in cancer cells.[25,26,28]

Cell cycle analyses using propidium iodide staining indicated Tulsi extracts induce G1/S and G2/M arrest. SCC-9 cells exposed to 20 µg/mL of ursolic acid–rich fractions exhibited an accumulation of 65% of cells in G1 phase versus 48% in controls, with concomitant downregulation of cyclin D1 and CDK4 proteins. At higher doses (30 µg/mL), G2/M arrest was observed in 42% of cells, associated with reduced cyclin B1 and Cdc2 expression.[26,31]

Migration and invasion assays (wound healing and transwell) demonstrated that Tulsi bioactives inhibit metastatic phenotypes. KB cells treated with 15 µg/mL eugenol exhibited a 62% reduction in wound closure after 24 hours compared to controls, and Matrigel invasion assays revealed a 58% decrease in invasive cell counts. Gelatin zymography confirmed MMP-2 and MMP-9 activities were suppressed by 65% and 70% respectively at 25 µg/mL of ethanolic extract.[30]

4.2 In Vivo Studies

The chemopreventive efficacy of *Ocimum sanctum* has been validated in DMBA-induced oral carcinogenesis models, predominantly in the hamster buccal pouch system.

In Syrian golden hamsters, topical application of 0.5% DMBA thrice weekly induced papillomas and squamous cell carcinomas within 10–12 weeks. Co-treatment with 100 mg/kg body weight of Tulsi ethanolic leaf extract reduced tumor incidence from 100% in DMBA-only controls to 40% in treated animals. Tumor multiplicity decreased from an average of 4.5 tumors per animal in controls to 1.8 tumors per animal in the Tulsi group, representing a 60% reduction in tumor burden.

Histopathological examination of buccal mucosa revealed that DMBA-only hamsters exhibited moderate to severe dysplasia, carcinoma in situ, and invasive carcinoma in 85% of sections. In contrast, Tulsi-treated animals demonstrated only mild dysplasia in 25% of sections, with no evidence of carcinoma in situ or invasion. Immunohistochemical analysis showed a significant decrease in Ki-67 proliferation index (from 45% in controls to 15% in Tulsi group) and reduced VEGF expression by 50%, indicating impaired angiogenesis.[33]

Biochemical assessments revealed a 2.5-fold increase in hepatic and buccal mucosal SOD and CAT activities in Tulsi-treated hamsters compared to DMBA-only animals, corroborating in vitro antioxidant findings. Pro-inflammatory cytokines TNF-α and IL-6, quantified by ELISA in serum samples, were reduced by 68% and 55% respectively following 12 weeks of Tulsi administration, reflecting systemic anti-inflammatory effects.[33]

Dose–response studies indicated that 50 mg/kg Tulsi extract conferred 45% tumor inhibition, whereas 200 mg/kg achieved 75% tumor inhibition, with no observable hepatotoxicity or alterations in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. These findings support a favorable safety profile and dose-dependent chemopreventive efficacy.

4.3 Clinical Studies

Human clinical evidence for Tulsi's chemopreventive role in oral cancer remains limited, but safety and tolerability data support translational potential.

A randomized, double-blind, placebo-controlled trial evaluated the safety of standardized Tulsi leaf extract (500 mg twice daily) in 60 healthy volunteers over 12 weeks. No serious adverse events were reported, and only mild gastrointestinal symptoms occurred in 8% of participants, resolving without intervention. Pharmacokinetic profiling in this cohort showed peak plasma eugenol concentrations of 4.3 μg/mL at 1 hour post-dose, with a half-life of 2.1 hours and complete elimination by 24 hours.[24]

A pilot study in 30 tobacco chewers with oral leukoplakia administered 1 g of Tulsi powder orally twice daily for 6 months. Lesion size regressed by ≥30% in 40% of subjects, and complete resolution occurred in 10%. Biopsy specimens post-intervention demonstrated decreased dysplasia grades in 50% of participants and reduced Ki-67 expression by 35% compared to baseline. No hepatic or renal toxicity was observed, and patient-reported outcomes indicated improved oral comfort and reduced lesion-associated pain.[34]

Case reports describe adjunctive use of Tulsi mouthwash (0.2% essential oil solution, thrice daily) in 10 patients undergoing radiotherapy for oral carcinoma. Patients reported reduced mucositis severity, with a mean mucositis grade reduction from 3 to 1 on the WHO scale, and decreased analgesic requirements. These anecdotal findings warrant controlled trials to evaluate efficacy in preventing radiation-induced oral complications.[34]

Overall, clinical data underscore Tulsi's favorable safety profile and preliminary efficacy signals in oral premalignant conditions. However, well-powered randomized controlled trials focusing on high-risk populations (e.g., tobacco users, patients with leukoplakia) are essential to establish definitive chemopreventive benefits and optimal dosing regimens.

5. Comparative Efficacy of *Ocimum sanctum* and Other Herbal Chemopreventives

Among prominent herbal chemopreventives, *Ocimum sanctum* (Tulsi), curcumin, green tea polyphenols (GTPs), and resveratrol exhibit overlapping yet distinct efficacy profiles. Tulsi's eugenol-rich extracts produce IC_{50} values of 10–35 μg/mL in oral cancer cell lines, comparable to curcumin's 5–20 μM range (≈1.8–7.2 μg/mL) and GTP-EGCG's

15–50 μM (≈6.8–22.7 μg/mL) in similar models. Resveratrol demonstrates IC_{50} of 20–60 μM (≈4.6–13.8 μg/mL) in head and neck squamous carcinoma cells.[18,26]

Mechanistically, all four agents inhibit NF-κB and COX-2 pathways; Tulsi and curcumin both reduce TNF-α and IL-6 by 60–80%, while EGCG and resveratrol achieve 50–70% suppression of these cytokines in oral keratinocyte models. Apoptosis induction via p53 upregulation and caspase-3 activation is observed across the board, with Tulsi and curcumin eliciting 2.5–3-fold increases, EGCG 1.8–2.2-fold, and resveratrol 2–2.5-fold. Anti-metastatic effects—MMP-2 and MMP-9 downregulation by 60–70%—are comparable for Tulsi and resveratrol, whereas EGCG exhibits 50% reduction and curcumin 55%.[18,26,29,30]

Synergistic combinations hold promise: Tulsi + curcumin co-treatment yields enhanced ROS reduction (up to 80% vs. 55% individually) and apoptosis (4-fold vs. 2.8-fold) in SCC-9 cells, attributed to complementary antioxidant and pro-apoptotic spectra. Tulsi + EGCG demonstrates additive inhibition of PI3K/Akt signaling, reducing Akt phosphorylation by 75% vs. 50% alone. Resveratrol + Tulsi combination augments miR-34a upregulation by 2-fold compared to monotherapies, potentiating epigenetic reprogramming toward apoptosis.[30,31]

Overall, *O. sanctum* exhibits efficacy on par with established phytochemicals, with synergistic potential when combined, supporting multi-agent strategies for oral cancer chemoprevention.

6. Pathway Modulation Summary

Table 1. Summary of Signaling Pathways Modulated by *Ocimum sanctum*

Pathway	Modulation by Tulsi	Key Bioactives	Downstream Effects
NF-κB	Inhibition	Eugenol, ursolic acid	↓ IκBα phosphorylation, ↓ TNF-α, IL-6, IL-1β
PI3K/Akt	Inhibition	Eugenol, ursolic acid	↓ Akt phosphorylation, ↓ mTOR activation, ↓ proliferation
Nrf2	Activation	Rosmarinic acid, apigenin	↑ Nrf2 nuclear translocation, ↑ HO-1, NQO1, antioxidant enzymes
p53	Activation	Eugenol, rosmarinic acid	↑ p53 expression, ↑ Bax/Bcl-2 ratio, ↑ caspase-3
MMP-2/MMP-9	Suppression	Ursolic acid, eugenol	↓ ECM degradation, ↓ invasion and metastasis

Pathway	Modulation by Tulsi	Key Bioactives	Downstream Effects
COX-2	Inhibition	Eugenol, rosmarinic acid	↓ PGE_2 synthesis, ↓ inflammatory edema
Wnt/β-catenin	Inhibition	Apigenin, carvacrol	↓ β-catenin nuclear translocation, ↓ EMT

7. Clinical Relevance and Future Perspectives

7.1 Translational Potential: From Bench to Bedside

The robust preclinical evidence for *Ocimum sanctum* underscores its promise for clinical application in oral cancer chemoprevention. Standardized Tulsi extracts or isolated bioactives such as eugenol and ursolic acid could be developed into adjunctive oral formulations (capsules, mouthwashes) aimed at high-risk populations, including tobacco users and individuals with premalignant lesions. The demonstrated safety profile in healthy volunteers and preliminary efficacy in leukoplakia patients support the feasibility of early-phase clinical trials.

7.2 Challenges in Dosage Standardization

Variability in phytochemical content across cultivars and extraction methods poses a significant barrier to reproducible dosing. Establishing pharmacopeial standards—such as defining minimum eugenol (≥50 mg/g), ursolic acid (≥5 mg/g), and rosmarinic acid (≥10 mg/g) levels—is imperative. Batch-to-batch consistency must be verified through validated analytical techniques (GC-MS, HPLC).

7.3 Bioavailability and Formulation Improvements

Key phytochemicals like ursolic acid exhibit poor aqueous solubility and limited oral bioavailability (<15%), necessitating advanced delivery systems. Nanoformulations—lipid nanoparticles, polymeric micelles, and solid lipid carriers—have shown 3–5-fold bioavailability enhancements in preclinical models. Co-crystal and phytosome technologies may further improve absorption of hydrophilic phenolics such as rosmarinic acid and apigenin.

7.4 Regulatory Approval Pathways

As a botanical drug, Tulsi-based products must navigate stringent regulatory frameworks. In the United States, the FDA's Botanical Drug Development Guidance requires demonstration of consistent extract composition, nonclinical toxicology, and well-controlled clinical trials. Similar guidelines exist in EMA and CDSCO regulations, emphasizing Good Manufacturing Practice (GMP) compliance and robust quality control.

7.5 Need for Controlled Clinical Trials

Definitive evidence of chemopreventive efficacy demands randomized, placebo-controlled Phase II trials with endpoints including lesion regression, biomarker modulation (e.g., Ki-67, oxidative stress markers), and safety. Stratification by risk factors (tobacco use, HPV status) will clarify targeted benefits. Long-term follow-up for recurrence and survival outcomes is essential.

7.6 Ethical and Safety Considerations

While Tulsi exhibits minimal toxicity, comprehensive assessment of herb–drug interactions, particularly with conventional chemotherapy agents, is critical. Ethical trial design must ensure informed consent, especially in vulnerable populations, and incorporate rigorous adverse event monitoring. Environmental sustainability of large-scale Tulsi cultivation should also be considered to prevent overharvesting and preserve biodiversity.

Conclusion

Ocimum sanctum (Tulsi) embodies a promising natural chemopreventive agent against oral cancer, owing to its multi-targeted mechanism—including antioxidant, anti-inflammatory, pro-apoptotic, anti-proliferative, anti-metastatic, and epigenetic modulatory actions. Key bioactives such as eugenol, ursolic acid, and rosmarinic acid synergistically inhibit NF-κB and PI3K/Akt signaling, activate p53 and Nrf2 pathways, and suppress MMP-mediated invasion, all while exhibiting a favorable safety profile in preclinical and preliminary clinical studies. To realize Tulsi's translational potential, integrative research must prioritize standardized extract formulations, enhanced bioavailability via advanced delivery systems, and well-designed, controlled clinical trials in high-risk populations. By bridging rigorous laboratory investigations with clinical implementation, Tulsi can be positioned as an evidence-based component of comprehensive oral cancer prevention strategies.

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