# A COMPREHENSIVE ASSESSMENT OF MUCOSITIS DUE TO RADIATION FOR HEAD AND NECK CANCER: PATHOPHYSIOLOGY, CLINICAL AND PATIENT-REPORTED OUTCOMES, AND A PROPOSAL FOR A NOVEL TREATMENT BASED ON AYURVEDIC MEDICINE

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by

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ABSTRACT

Introduction: Head and neck cancers require aggressive multi-modal treatment, including a course of radiation therapy. Mucositis is a common side effect of radiation therapy with short- and long-term symptoms and disabilities. The objectives of this body of work were to characterize the myriad of clinical manifestations of mucositis and to describe patient-reported scales currently available to measure patients' symptoms (Manuscript #1) and to describe the pathological mechanisms that result in these clinical signs and symptoms and propose a new treatment based on Ayurvedic medical principles and formulations (Manuscript #2).

Methods: In-depth reviews of articles published in PUBMED were done; articles were identified using the following search terms: radiation therapy; head and neck cancer; and patient symptoms/reported problems/subjective feelings/complications/side-effects. Review of traditional Ayurvedic texts was conducted to identify plant compounds effective for general stomatitis and, using a bio-prospecting methodology, recent literature was reviewed to ascertain mechanisms of action of these compounds.

Results: Multiple clinician-rated scales use inconsistent terminology and scoring rubrics to rate mucositis and often do not correlate with patient-reported symptoms. Only several patient-reported scales currently exist and they measure diverse and limited symptoms. However, multiple studies indirectly describe a plethora of acute and chronic symptoms resulting in marked disability and suffering. There are multiple metabolic pathways that drive the phases of radiation-induced mucositis and current medications are ineffective in controlling symptoms and progression. This results in patient suffering and cancer therapy

protocol interruption. Using bio-prospecting, seven compounds met the following criteria for a novel treatment based on Ayurvedic medicine: can be used internally, daily, and as a mouthwash; has anti-bacterial and anti-fungal effects; and enhances saliva production, pH balance, and wound healing.

Discussion: Radiation-induced mucositis is characterized by complex pathophysiology and clinical manifestations. There is a great need to develop uniform methods to describe symptoms, which would, in turn, enhance the assessment of new interventions to treat mucositis as well as enhance adherence to aggressive therapeutic protocols. Patient-reported scales should be developed and should capture the diverse experience of mucositis using patients' language (see poem below). In addition, clinicians' measurements should be standardized in terminology and content. A new mouthwash that is based on traditional Ayurvedic knowledge, supported by current scientific data, and targets multiple pathways, offers a new possibility to treat radiation-induced mucositis. This proposal is a paradigm shift in the drug discovery mechanism in that drug development need not always be confined to new molecular entities. Instead, bio-prospecting of plants utilized by ancient knowledge and gained from indigenous medicines may provide a new strategy for drug development.

**Putting the Patients' Experience in Their Own Words**: ***Stomatitis***
Anita Hart Balter
N Engl J Med 1990;322:704

"A fish hook lodges in my throat.
Spittle, kindergarten paste, thickens everything - even vision.
Mouth pocked with sores and blisters, swollen ulcerated tongue.
Topside sandpapered with number 7 coarsest grade.
Taste buds, saliva glands, seared.
Cool water, corrosive acid now.
The tongue rests; teeth become enemies,
Coiled steel razored wire atop dentate prison walls.
Only moans escape my lips. I cannot eat or speak.
Inside, a howl festers.
Pain lengthens time.

## BIOGRAPHICAL SKETCH

Rajesh Ramnath earned his Bachelor of Ayurvedic Medicine and Surgery degree from University of Calicut, Kerala, India and Masters Degree from University of Liverpool. He is currently working in herbal drug development and at P S Mission Hospital. He is interested in demystifying the age of Ayurvedic medicine and in drug development based on traditional Ayurvedic Knowledge supported by robust scientific data.

Dedicated to my dad,
for his motivation and encouragement

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**Manuscript #1**

# Measuring Effects of Radiation-Induced Mucositis in Head and Neck Cancers: A Review of Clinical Manifestations and Patient-Reported Scales

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CONFLICT OF INTEREST NOTIFICATION PAGE

NONE TO DECLARE

**ABSTRACT**

Introduction: Radiation-induced mucositis is a common and serious complication of therapy for head and neck cancer. There are multiple clinician-rated staging systems to describe mucositis but few patient-reported scales to describe the impact of mucositis on function and quality of life. The objectives of this literature review were to compile patient-reported symptoms of mucositis in order to propose what symptoms and side-effects should be included in future patient-reported scales.

Methods: A PUBMED search was conducted to identify patient-reported scales that address radiation-induced mucositis using the following search terms: radiation therapy; head and neck cancer; and patient symptoms/reported problems/subjective feelings/complications/side-effects.

Results: Multiple clinician-rated scales use inconsistent terminology and scoring rubrics to rate mucositis and often do not correlate with patient-reported symptoms. Only several patient-reported scales currently exist and they measure diverse and limited symptoms. However, multiple studies indirectly describe a plethora of symptoms including pain, xerostomia, dysphagia/odynophagia, altered saliva viscosity, gagging/regurgitation, aspiration, lost or altered taste, loss of appetite, fatigue, poor nutrition, weight loss, prolonged time to eat, hoarse voice, trouble being understood, limited food choices, over sensitivity to hot, cold, spicy, sweet and acidic foods, sensation that teeth are loose and cracking/chipping, trouble with dentures, over sensitivity when brushing teeth, limitations in jaw and shoulder/neck movement, and long-term osteoradionecrosis.

Discussion: Both patient-reported scales and clinician-measured features should be combined to provide a comprehensive assessment of radiation-induced

mucositis for head and neck cancer. Uniformity would enhance the establishment of new interventions to treatment the disability and suffering associated with mucositis as well as enhance adherence to aggressive therapeutic protocols. Patient-reported scales should be developed and rigorously tested for validity, reliability and responsiveness and should capture the diverse experience of mucositis using patients' language. In addition, clinicians' measurements should be standardized in terminology and content. When paired, such measures will capture radiation side effects fully throughout the course of treatment, from acute to long-term chronic effects.

## INTRODUCTION

### ***Background***

Despite advances in early detection and effective treatment, cancer remains one of the most feared diseases due to its association not only with death but also with diminished quality of life. Although research is producing new insights into the causes and cures of cancer, efforts to manage the symptoms of the disease and its treatments have not kept pace.[1]

Head and neck cancer is the fifth most common neoplasm with at least 500,000 new cases reported every year.[2] Of these, 40% occur in the oral cavity, 25% in the larynx, 15% in the pharynx, and 7% in the salivary glands.[3] The treatment protocol involves simultaneous or sequential use of surgery, radiation and chemotherapy. Concurrent chemotherapy and radiation have become the current standards of care and are associated with considerable toxicity, particularly xerostomia and mucositis.[4,5] These side effects not only significantly impair quality of life but also result in severe long-term oral disorders among patients who have remission from their cancers.[4]

Oral mucositis commonly occurs during head and neck cancer treatment and is expected to increase in prevalence to 100% as radiation dose intensification

becomes part of routine care.[6,7,8] Mucositis caused by radiation differs from that due to chemotherapy in that radiation damage often is permanent.[9] Currently, two thirds of patients treated for cancers of the larynx and hypopharynx suffer from mucositis.[10] The severity of oral mucositis varies from erythema and burning mucosal discomfort, to large areas of deep coalescing ulcers that require high doses of opioids to manage pain. [11,12] The etiology of mucositis is the unavoidable toxicity to normal cells throughout the gastrointestinal track that have a rapid rate of turnover, such as the oral mucosa. [13]

Apart from the pain associated with mucositis, additional serious morbidity include difficulties in swallowing, chewing, and speaking. This is particularly prevalent when the pharyngeal mucosa is involved. It is estimated that 93% of patients have disturbances in eating. Chronic pain is also prevalent with an estimated 79% of patients having sleep disturbances[14,15].

In summary, oral mucositis is associated with a range of acute and chronic symptoms that exert a substantial negative impact on quality of life. The pain due to mucositis can cause functional difficulties in eating, drinking, swallowing, speaking, and even sleeping.[16,17,18] These effects, in turn, can lead to weight loss, anorexia, cachexia, dehydration, and the need for parenteral feeding[19,20].
The control of cancer-related symptoms is a vital aspect of medical care and an important clinical goal for patients and health care professionals.[21] However, measurement of mucositis is not a routine part of medical care. For example, a survey of 46 transplant centers in 16 European countries conducted by the research subcommittee of the European Blood and Marrow Transplant Nurses Group found that only 59% of centers used standardized assessments for mucositis. In addition, there are no standardized scales or protocols for assessment, and thus no methods to gauge success of management. The first step to address this gap is to understand what scales currently exist to measure mucositis and whether they adequately address patients' reports of symptoms and suffering.[22]

***Issues Related to Measurement***

Mucositis is a complicated disorder to classify as it involves a complex and overlapping cascade of compounds from multiple pathways of injury. In addition to complex interactions in the mechanism of injury there also are feedback loops that create new vulnerabilities. Often there also are areas of mucositis at varying stages, making the process of measurement more difficult. Developing a model of mucositis is important because it at least gives a framework to measure both symptoms and effects of new anti-mucositis agents. While biological measurement is important, it is critical also to keep the patients' point of view and symptoms in focus. Thus a robust assessment tool needs to address both biological and patient-reported perspectives, and ideally would be useful clinically and for research. Such a measurement tool could provide much needed insight into the effectiveness of anti-mucositis agents and predict the clinical course and prognosis of mucositis.

In addition to identifying the necessary content of a measurement scale, another major hurdle is the lack of a globally accepted validated scoring system. The widely used World Health Organization (WHO) and National Cancer Institute (NCI) systems were developed to describe toxicities associated with a particular chemotherapeutic agent or regimen. These scales combine objective signs of mucositis (erythema and ulcer formation) with subjective and functional outcomes (pain and inability to eat). Although the developers of these scales intended the scorer to consider lesions painful if analgesia masked the pain, in practice many scorers ignore this differentiation, most likely resulting in underreporting and underscoring of mucositis. At the other end of the spectrum, there is a scale developed by oncology nurses which has a more holistic approach and integrates elements such as evaluation of the integrity of the oral mucosa and functional and subjective outcomes such as speech quality, avoidance of spicy foods, swallowing, lip and mucosal dryness, infection, bleeding, and cleanliness. Finally, some

researchers attempted either to eliminate subjective findings completely or to evaluate them independent of objective findings and then integrate them into a single comprehensive score.

***Objective Scales and Mixed Scales***

There are multiple clinical tools that consider signs, symptoms and functional disturbances secondary to mucositis toxicity. They can be broadly divided into scales that focus on patient-reported items (considered 'subjective'), scales that assess observable physician-rated features (considered 'objective'), and mixed scales that have elements of both. Some scales focus only on one feature, such as pain, dysphasia, burning sensation and discomfort. A comprehensive summary of existing physician-rated scales and their scoring rubrics are listed below.

- World Health Organization (WHO) Scale In 1979 the WHO proposed a classification of mucositis according to severity based on 0-4 grades. Apart from objective signs of erythema and ulceration, the subjective symptom of ability to eat is also rated. This is currently the most widely used scale and it addresses three components of mucositis: objective signs (such as ulceration), subjective symptoms (such as soreness), and functional disturbances (such as inability to eat). The WHO scale is widely regarded as the gold standard despite the fact that it has not been extensively tested. The scale is divided into the follow grades: grade 0 - absence of mucositis; grade 1 - presence of a painless ulcer, erythema or mild sensitivity; grade 2 - presence of painful erythema or ulcers that do not interfere with the patient's ability to take food; grade 3 - confluent ulcerations that interfere with the patient's ability to take solid food; and grade 4 – severe symptoms requiring enteral or parenteral support.[23]

- Radiation Therapy Oncology Group/European Organization Research and Treatment of Cancer (RTOG/EORTC) Scale The RTOG/EORTC Scale is a simple scale that considers mucositis based on its absence to the appearance of ulceration and/or necrosis. The RTOG/EORTC is scored similar to the WHO scale in that it ranges from a grade of 0 to 4 as follows: grade 0 – no change over baseline; grade 1 - mild pain but does not require analgesics; grade 2 - patchy lesions that may have serosanguinous discharge, pain requiring analgesics, patches less than 1.5 cm and non-contiguous; grade 3 - confluent fibrinous patches, may include severe pain requiring narcotics, will be greater than 1.5 cm in size; grade 4 - necrosis or deep ulceration with or without bleeding.[24,25]

- National Cancer Institute/Common Toxicity Criteria (NCI/CTC) Scale The NCI/CTC scale was updated in 1998 and classifies mucositis according to the zone of appearance. It similarly is organized into the following grades: grade 0 - no pain, ulcers, erythema or soreness; grade 1 - painless ulcers, erythema or mild soreness; grade 2 - painful erythema, edema, ulcers, but can eat; grade 3 - painful erythema, edema, ulcer, cannot eat; grade 4 - patient requires parenteral or enteral support. The NCI-CTC is used commonly to evaluated mucositis; however, this scale is characterized by wide inter-rater variability.[26]

- Scale of the Western Consortium Cancer Nursing Research (WCCNR) The Western Consortium Cancer Nursing Research developed a system to evaluate mucositis induced by chemotherapy. This is a 4-point scale with detailed descriptions of each stage of mucositis. This scale is administered by a nurse. The authors consider advantages of this system to be its low complexity (compared with scales of multiple variables) and the fact that the description for each stage has been selected to describe technical and very intuitive general progression of mucositis.[27]

- Oral Mucositis Scale Rating (ORMS) The ORMS was designed as an instrument to quantify mucosal changes associated with bone marrow transplant. The scale delineates specific degrees of mucosal tissue injury and considers the visual measurement of erythema and ulceration.[28]

- MacDibbs Questionnaire This is a 15-item questionnaire composed of 9 subjective questions and 6 objective measurements. The 9 subjective questions are scored on a 5-point rating scale from 0 to 4. Pain is rated twice - with and without swallowing. The objective items consider number of ulcers in the oral cavity, the size of the largest ulcer in millimetres, and the presence of vesicles, reddened areas, and white patches.[29]

- Vander Schueren Scale This scale addresses only objective items and is divided into the following 5 grades: grade 0 - no erythema; grade 1 - slight erythema; grade 2 - pronounced erythema; grade 3 - spotted mucositis; and grade 4 - confluent mucositis patches which are greater than 0.5 cm in size.[30]

- Byfield Scale This scale considers both patient-reported and clinical features according to the following grades: grade 1 - mucositis with minimal dysphasia, thining but no overt break in mucosal integrity; grade 2 - mucositis has significant dysphasia, focal mucosal vesicles or denuded patches, symptoms with ingestion of only semisolid foods; grade 3 - only fluids are tolerated and there are obvious large confluent patches of mucosal denudation; and grade 4 - only parenteral fluids are tolerated and there is severe confluent mucosal denudation with bleeding.[31]

- Seto Scale This scale considers both patient-reported and clinical features according to the following grades: grade 1 - localized erythema with no pain; grade 2 - generalized erythema without pain or localised erythema or ulcers with

mild pain; grade 3 - multiple ulcers or generalized erythema with moderate pain; and grade 4 - generalized erythema or ulcers but with moderate to severe pain.[32]

- Eilers Scale This scale has 8 objective components in which voice, swallowing, lips, capacity to speak, saliva, mucous membranes, gingiva and dentition are assessed with a score of 1 (normal) to 3 (definitively compromised). Mucositis is then divided into three grades: grade 1 - pink and moist mucositis; grade 2 – presence of reddened areas or white film without ulcerations; and grade 3 - ulceration with or without bleeding.[33]

- Beck Scale This scale assesses several objective items, specifically lips, tongue, mucosa, gingiva, saliva, dentition, and the capacity to speak, and one subjective item, the capacity to swallow.[34]

- Spikjervet Scale This scale was developed for bone marrow transplant patients and uses quantitative determinants for some variables and qualitative determinants for others variables; these are then summed for a global score. Features considered are atrophy, erythema, pseudomembranous lesions, ulceration, hyperkeratosis, and oedema, which are evaluated on a 0-3 scale (0 - normal/no change; 1 - slight; 2 - moderate; 3 - severe). Mucositis is then graded by an observer as follows: grade 0 - no discoloration or erythema; grade 1 - white discoloration; grade 2 - erythema; grade 3 - pseudomembranes; and grade 4 - ulceration.[35]

- Maceijewski Scale This scale considers percent of area involved with the following grades: grade 0 - normal; grade 1 - mild erythematous area less that 25; grade 2 - severe erythematous area 25-50%; grade 3 - area involved greater than 50%; and grade 4 - confluent mucositis.[36]

- Lindquist/Hickey Scale This scale includes clinical features and ability to eat/drink according to the following grades: grade 0 - normal; grade 1 - presence of whitish gingiva with slight burning sensation or discomfort; grade 2 - presence of moderate erythema and ulceration or white patches, with pain but patient able to eat, drink and swallow; and grade 3 - presence of severe erythema with ulceration or white patches, with severe pain and inability to eat, drink or swallow.[37]

- Fred Hutchinson Cancer Research Center (FHCRC) Scale This scale rates mucositis in a Likert format: none, mild, moderate, severe, and life threatening.[38]

- Oral Mucositis Index (OMI) This scale was developed in 1992 by dental professionals to assess mucositis associated with bone marrow transplantation. The OMI is composed of 34 items, including erythema, ulceration, atrophy, and oedema and is scored during a dental examination. A shorter 20-item version of the OMI also is available.[39]

- Walsh Scale This scale includes more variables for both subjective and functional evaluation, such as mucosal integrity, saliva changes, saliva flow, oral hygiene, swallowing difficulty, drugs administered, sensation of oral dryness, and severity of pain[40]

- Acute Radiation-Induced Salivary Gland Morbidity Score (ARISGM) Patients receiving cancer therapy often develop transient or permanent xerostomia (subjective symptom of dryness) and hyposalivation (objective reduction in salivary flow). Hyposalivation can further aggravate inflamed tissues, increase risk for local infection and make mastication difficult. Many patients complain of thickened secretions due a decrease in the serous component of saliva. The ARISGM Score considers side effects related to salivary gland morbidity and

xerostomia according to the following grades: grade 1 - mild dryness, slightly thickened saliva, and slightly altered or metallic taste; grade 2 - moderate to complete dryness, thick, sticky saliva, and markedly altered taste; grade 3 - not defined for acute xerostomia; and grade 4 - acute salivary-gland necrosis.[25]

- Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 Criteria for Xerostomia This is a grading system for more severe symptoms that includes the requirement for total parenteral nutrition (TPN): grade 1 - dry or thick saliva without significant dietary alterations, and unstimulated saliva flow rate >0.2 mL/min; grade 2 - dry or thick saliva with significant oral intake alterations including copious water intake, use of other lubricants, diet limited to purees and/or soft moist foods, and unstimulated saliva flow rate 0.1–0.2 mL/min; and grade 3 – severe symptoms leading to inability to adequately aliment orally requiring intravenous fluids, tube feedings, or TPN, and unstimulated saliva flow rate <0.1 mL/min.[41]

Of all the above scales, the WHO and the NCI scales are most often used because they require little time to complete; however, they do require clinical experience and expertise in assessing the oral cavity in order to have inter-rater reliability. This is particularly true in cases where the mouth is too painful to allow complete evaluation and when mucositis extends from one area to another, or when there are multiple areas of mucositis.

The above scales heavily weigh physicians' perspectives and include only some aspects of patient-reported symptoms. Radiation therapy has both short term acute side effects and long-term side effects. However, patients often are not aware of the long-term effects and thus do not attribute these symptoms to radiation and do not report them. As such, physicians may not assess patients for

mucositis routinely and instead focus their attention on monitoring for persistent or recurrence cancer. Thus mucositis is often underreported or not reported.

***Patient-Reported Scales: the Gap in the Assessment of Mucositis***

A recent cohort study of head and neck cancer patients assessed both clinician measurement of oral and pharyngeal mucositis using the National Cancer Institute (NCI) and the Oral Mucositis Assessment Scale (OMAS), and then compared the findings with patient- reported experiences of oral mucositis as measured by the Patient Reported Oral Mucositis Symptom (PROMS) scale. There were marked differences in clinician and patient assessments for 2 groups of patients: 1) those with high manifestations and minor complaints, called "stoical sufferers", and 2) those with minor manifestation and high complaints called "complaining sufferers". The authors concluded that clinical observations can differ substantially from individual patient's experiences of mucositis. [42]

There are multiple possible reasons for these discrepancies. For example, variables that are hypothesized to increase mucositis include older age, poor oral hygiene, lifestyle, co-morbidities, and smoking history. It has also been suggested that some individuals with genotype variations are susceptible to mucositis. Human papilloma virus also can be partially responsible for amplified mucositis and associated symptoms.[42]

Patient reported outcomes and experiences can augment clinical data and may help in assessing the effectiveness of interventions in cancer care. In clinical cancer research, the use of patient reported outcomes has been recommended for patients with prostate, ovarian, gynaecologic, oesophageal, and head and neck cancer, among other types. Patients' reports can be used to monitor symptoms such as oral pain, skin changes, dental health, dry mouth, taste, saliva quality and quantity, difficulties with swallowing and mouth opening, shoulder disability or immobility, vocal problems (including hoarseness), and various social and

functional domains of well-being. In addition to delineating the side effects of therapy, patient-reported scales might also be useful to help predict the development of mucositis and identify those patients most at risk. Thus it is of paramount importance that patient-reported outcomes be included in interventional studies of mucositis, and that they be used to document within-patient progression as well as between-patient differences.

***What Is Known about Patients' Symptoms: Objectives of this Review***

Patients with significant mucositis present with a variety of symptoms, including pain, dysphagia, odynophagia, excess oropharyngeal mucus, gagging and regurgitation, aspiration, difficulties in eating/swallowing, weight loss, and respiratory symptoms (a comprehensive summary is provided in Appendix 1).

To truly understand the symptom burden, scales addressing function and quality of life are needed. This is necessary in the light of studies which have demonstrated that patient- reported symptoms tend to be more diverse and severe than physician-reported symptoms.[43-45] For example, in a recent study to closely monitoring the objective and subjective development of mucositis, investigators characterized patients' experiences with terms such as anxiety, distress, pain, exhaustion, fatigue and nausea. They also concluded that there were further functional issues related to coping styles, level of distress, personality indices, comorbidity, and health related quality of life inventories. In another study, investigators found that 78% of patients with taste disturbances had a positive culture for Candida spp. This was 2.58 times more frequent as compared to patients without taste disturbances. In addition, patients with proven Candida spp. had a dry mouth in 88% of cases, i.e. 3.11 times more often than patients without Candida spp. Plaques were the most prevalent clinical finding, irrespective of other reported symptoms or microbiological results. These authors concluded that

correlating symptoms and findings appears to be helpful as certain symptoms are associated with a specifically treatable finding. [46]

Thus incorporating patients' perspectives is a critical component in the comprehensive evaluation of patients undergoing treatment for cancer that results in oral mucositis. The objective of this review, therefore was 1) to assess side effects of radiation therapy, particularly with respect to mucositis and its associated symptoms and pathways; 2) to review different symptoms reported by patients during different stages of radiation therapy; and 3) to review existing patient-reported scales and consider what symptoms may be missing from these scales.

## METHODS

To determine the spectrum of *patient-reported symptoms*, a systematic literature search was undertaken in PUBMED to find patient-reported scales that address radiation-induced mucositis using the following search terms: radiation therapy; head and neck cancer; and patient problems/symptoms/reported problems/subjective feelings/complications/side-effects. Specifically the search used the following terms and options:
("radiation"[MeSH Terms] OR "radiation"[All Fields] OR "electromagnetic radiation"[MeSH Terms] OR ("electromagnetic"[All Fields] AND "radiation"[All Fields]) OR "electromagnetic radiation"[All Fields]) AND ("Head Neck"[Journal] OR ("head"[All Fields] AND "and"[All Fields] AND "neck"[All Fields]) OR "head and neck"[All Fields]) AND ("patients"[MeSH Terms] OR "patients"[All Fields] OR "patient"[All Fields]) AND ("diagnosis"[Subheading] OR "diagnosis"[All Fields] OR "symptoms"[All Fields] OR "diagnosis"[MeSH Terms] OR "symptoms"[All Fields]).

Only scales that were exclusively patient-reported were included. Few studies assessed patient-reported symptoms and none considered long-term symptoms. The Boolean search yielded 6213 studies.

## RESULTS

### ***Etiology of Mucositis and Associated Symptoms***

Radiotherapy is performed in an ionizing fashion - ionic medium is ionized making it electrically unstable. This affects nuclear DNA resulting in loss of reproduction and in cell death. Radiation affects cells in mitotic division and also adjacent cells. Tolerance of the adjacent cells is a major dose limiting factor. Most patients are treated with 50-70 Gy of radiation. This is fractionated by giving 2 Gy per day 5 days a week over 5 to 7 weeks. The radiation is fractionated so as to reduce early side effects due to damage to tissues with rapid turnover and to reduce late side effect due to damage to tissues with late turnover. [3]

It was traditionally held that mucositis was due to the direct effect of chemotherapy or radiation on muscosal tissues. More recently the following 5-stage rubric was proposed. [47-54]

1) *Initiation of tissue injury* due to the direct effect of radiation on the basal epithelium begins within hours of therapy. As a result of radiation, highly reactive oxygen species or free radicals are generated as a by-product of oxygen metabolism and cause direct cellular damage. These reactive oxygen species also initiate a cascade of injurious molecular events extending to replicating cells and causing damage to DNA. 2) *Up-regulation of inflammation* occurs via generation of messenger signals due to free-radical activation of messengers that transmit signals from receptors on the cellular surface to the inside of cell. These in turn up-regulate pro-inflammatory cytokines leading to tissue injury and cell death, as well as to damage to epithelium and connective tissues. These events are associated with symptomatic sensations of burning and pain and the start of erythema. 3) *Signalling and amplification* from up-regulation of pro-inflammatory cytokines, such as TNF-a and interleukin 6 produced mainly by macrophages, occur and cause injury to mucosal cells. Different feedback loops are generated in this phase with the same cytokines that target tissues for direct damage also stimulate genes

that are responsible for cytokine production. These feedback loops sustain and escalate the severity of mucosal injury even after cytotoxic cancer therapy has been discontinued. 4) *Ulceration and inflammation* occur based in part on metabolic by-products of newly colonizing oral microflora. These organisms replace normal colonized micro-organisms which usually help establish and maintain a homeostatic environment (i.e. "colonization resistance") and now are eradicated due to radiation. Production of pro-inflammatory cytokines also is further up-regulated as a result of this secondary infection. This is the stage in which there is classic expression of mucositis. Since there is often concurrent neutropenia and compromise of oral flora, bacteria colonize and further stimulate macrophage-directed inflammation. 5) *Healing* is characterized by epithelial proliferation, as well as cellular and tissue differentiation and restoration of the integrity of the epithelium. This phase typically starts 2 to 4 weeks after discontinuation of cancer treatment. Healing is governed primarily by regulatory proteins expressed by the extracellular matrix.

Multiple proteins and extra-cellular species are actively involved throughout this process, such as factor Kappa B (stage 1), sphingomyelinases and NF-kB transcription factor (stage 2), COX-2 cyclooxygenase, TNF-alpha activated NF-kB and C-JUN (stage 3),
keratinocyte growth factor, matrix metalloproteinases (stage 4), and pro-angiogenic and epithelial growth factors that may be distinct from normal tissue (stage 5).[47-53,11]

Although this classification rubric is highly descriptive, it does not completely capture the complex mechanism of mucositis which is also characterized by overlap and integration of injury. In particular, the cascade also involves multiple pathways, which in turn present great challenges to therapeutic management. Signs and symptoms of mucositis in different phases can be seen in the following Figure 1.1.

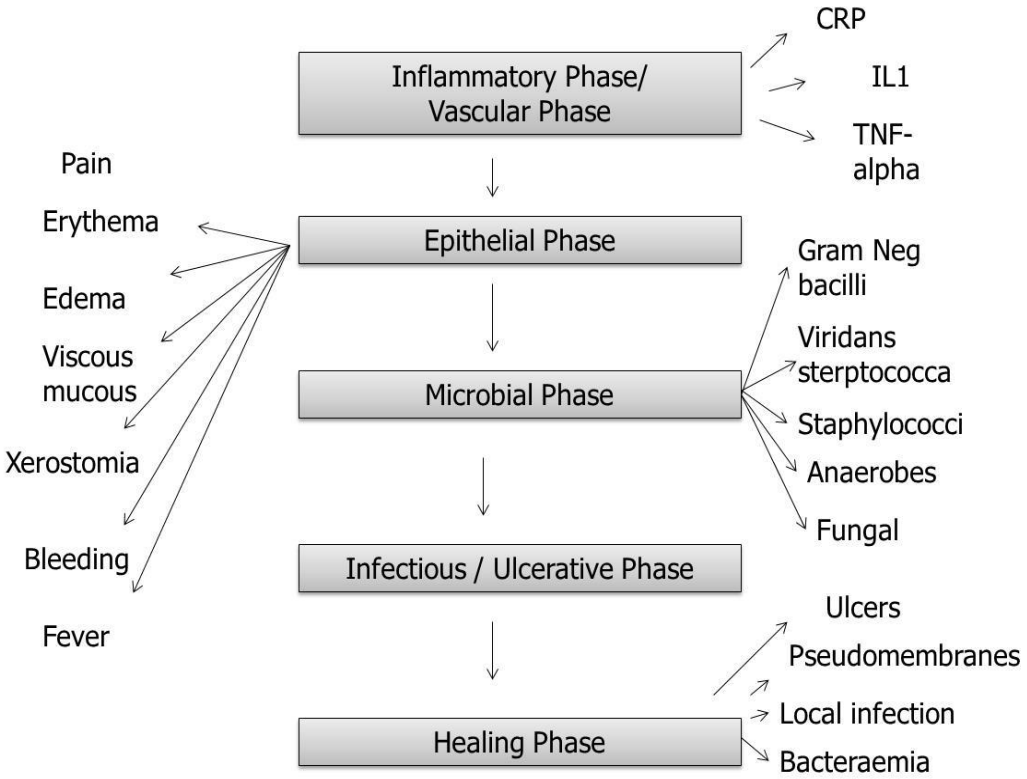


**Figure 1.1 Stages of muscositis, associated symptoms and clinical features** [54]

***Other Symptoms Associated with Radiation Therapy Determined from Reported Studies***

Currently it is relatively common to administer radiation in an outpatient setting. Unfortunately this leaves the responsibility on the patient to detect, report, and manage oral side effects. However, patients may not attribute all side effects to radiation treatment, as was found in one study in which mouth pain and dryness were underreported by patients.[55] Another study also demonstrated that occurrence of mucositis was under documented in medical records compared to interview. [56] Other symptoms also are seldom reported by patients. For example, excessive, viscous mucus in the mouth and throat is seldom reported but has been shown to be one of the most burdensome symptoms[57]. Paradoxically, it is not uncommon for patients to complain of both dry mouth and excessive throat mucus.[58,57] In another series of early studies from 1986 and 1989, multiple symptoms were

associated with radiation, including mucositis, accumulation of mucus, candidiasis, dysphagia, radiation-induced caries, periodontal deterioration, xerostomia, reduced maxillomandibular opening, decrease in resiliency of perioral tissues, and intrinsic bone changes. [59,60]

The Care Study Group Systematic Reviews, MASCC/ISOO has documented some additional complications due to radiation, including biphosphonate osteonecrosis, dysgeusia, oral fungal infection, oral viral infection, dental disease, osteoradionecrosis of the mandible and maxilla, and trismus. In addition to xerostomia, salivary gland hypofunction also is associated with lip dryness and crusting, fissures of lip commissures, atrophy of dorsal tongue surface, atrophic and fragile oral mucosa, difficulties in speaking, chewing and swallowing, oral burning sensation, taste disturbances, increased thirst, and sensitivity and pain in response to spicy foods and strong flavorings. Increased salivary viscosity, on the other hand, is associated with decreases in flushing and clearance of acid production after sugar exposure resulting in demineralization of teeth and subsequent dental decay. Compromise of salivary pH leads to increased pathogenicity of oral flora and results in dental caries and erosion.[61,62]

A review of recent literature shows a preponderance of symptoms such as: pain, dysphagia/odynophagia, weight loss, oropharyngeal mucus associated gagging/regurgitation, and aspiration[11]; mouth and throat sores, difficulty swallowing, pain, lost or altered taste (ageusia, dysgeusia), excessive secretions leading to gagging, nausea, and vomiting; loss of appetite, fatigue, weight loss, and aspiration[63]; xerostomia, ageusia or dysgeusia[64]; xerostomia and excessive throat mucus[65]; long-term osteoradionecrosis, [66] xerostomia, dysgeusia, dysphagia, halitosis, and pain[67] ; xerostomia[68]; increased salivary viscosity, taste changes, dysphagia, pain, sore throat, osteoradionecrosis and trismus [60,44]; and xerostomia, fibrosis, trismus, dermatitis, photosensitivity, radiation caries, soft tissue necrosis, and osteoradionecrosis. [69]

Other studies assessed physiological parameters and found a decrease in pH during irradiation, with the lowest value being reached 3 months after the beginning of radiation therapy and increasing gradually during subsequent months[70]. However, salivary pH continued to be slightly acid (pH = 6.87) 12 months after treatment, and stimulated salivary flow was reduced by 93% compared to the beginning of treatment.

Concurrent with mouth dryness others have demonstrated decreased resting and stimulated salivary secretion rates [71,72]and increased salivary viscosity due to radiation.[73] In addition, the NCI has divided complications of head and neck cancer into 2 groups based on occurrence – acute (enumerated above) and chronic, which includes mucosal fibrosis and atrophy, decreased saliva secretion and xerostomia, accelerated dental caries related to compromised saliva secretion, infections and soft tissue necrosis and osteonecrosis, taste dysfunction (dysgeusia/ageusia), muscular and cutaneous fibrosis, and dysphagia.

Overall, the most common symptoms reported using our search terms with the corresponding number of citations were: mucositis (831); mouth dryness (770); xerostomia (762); hyposalivation (774); pain (410); dysphagia (385); weight loss (339); caries (193); aspiration (183); taste disturbance (163); trismus (89); fatigue (57); sore throat (25); odynophagia (10); sleep disturbance (18); regurgitation (4); gagging (2); and perioral tissue changes, halitosis, and perioral tissue changes (1). Thus, this search yielded terms which are generally not considered for mucositis such as hyposalivation, caries, trismus, fatigue, regurgitation, gagging, halitosis, viscid mucus and perioral tissue changes.

***Patient-Reported Scales***

Because of the diverse array of manifestations characterizing mucostis, establishing a comprehensive patient-reported scale to measure symptoms has been challenging. This is compounded by the fact that patient- and clinician-reported

measurements may not agree. Currently the 5 following scales measure only patient-reported symptoms.

- M.D. Anderson Symptom Inventory - Head and Neck (MDASI-HN) module
This 28-item module consists of 3 subscales: 1) 13 core MDASI questions that rate the severity of general symptoms associated with cancer; 2) 6 questions assessing how severely symptoms interfere with activities of daily life; and 3) 9 questions specific to head and neck cancer that address region-specific symptoms, specifically mucus in mouth and throat, difficulty swallowing/ chewing, choking/coughing, difficulty in speech/voice, skin pain/burning/rash, constipation, problems with tasting food, mouth/throat sores, and problems with teeth and gums.[57]

- Functional Assessment of Cancer Therapy-Head and Neck Version 4 (FACT-HN module) This 39-item module consists of 2 subscales: 1) 27 questions assess general quality of life issues related to physical, social/family, emotional, and functional domains; and 2) 12 items assess head and neck cancer-specific quality of life issues.[74]

- Oral Mucositis Daily Questionnaire (OMDQ) This is 10-item questionnaire addresses general health (1 question) and the remaining 9 questions focus on head and neck cancer. [75]

- Oral Mucositis Weekly Questionnaire for Head and Neck Cancer (OMWQ-HN)
This 12-item questionnaire addresses general health (2 questions) and the remaining 10 questions focus on head and neck cancer.[76]

- Patient-Reported Oral Mucositis Symptom (PROMS) Scale In this 10-item scale all questions pertain to head and neck cancer with a visual analogue response format.[77]

The symptoms addressed in each scale and their overlap are summarized in Figure 1.2.

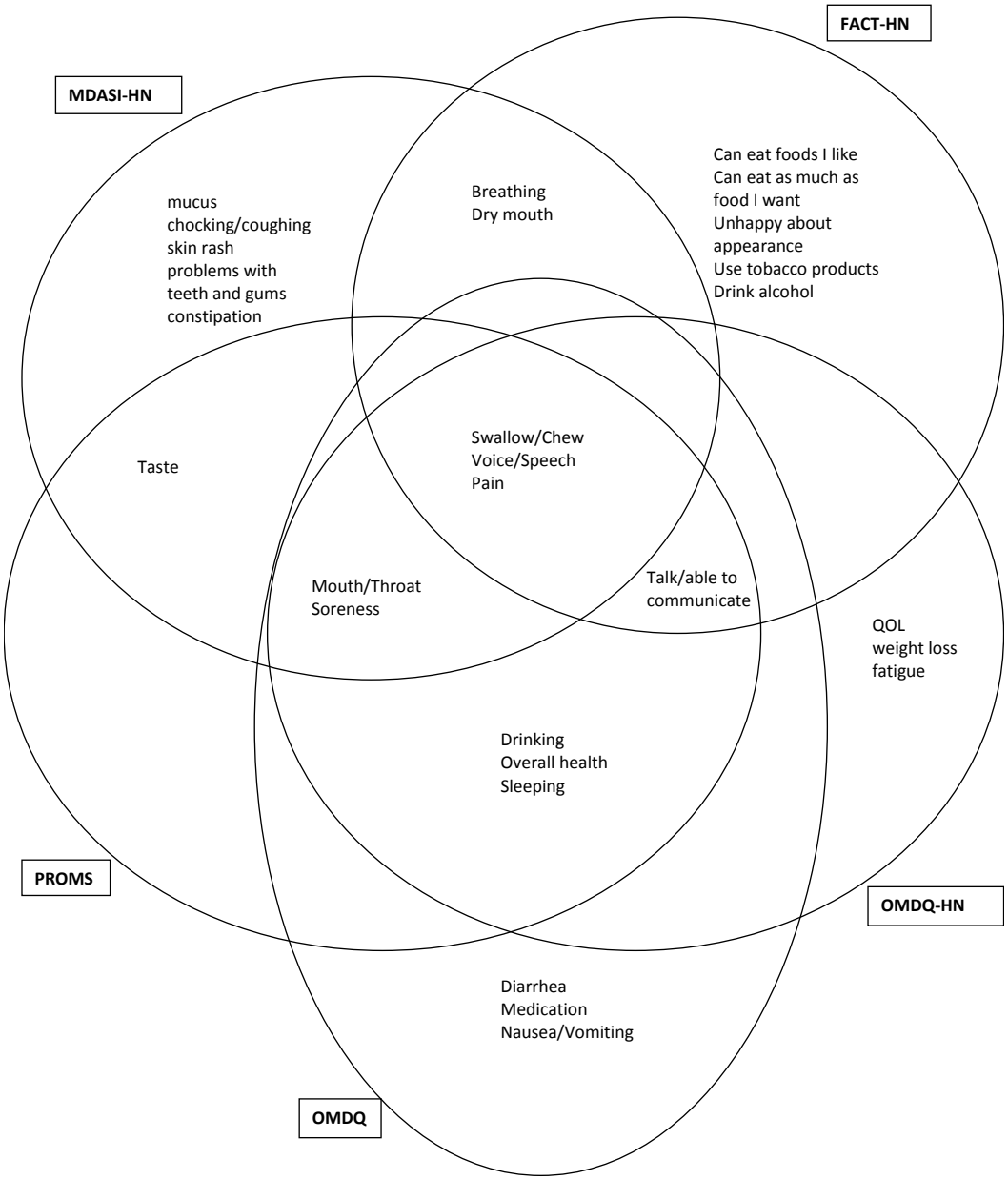


**Figure 1.2 Summary of items addressed in patient-reported scales**

All 5 scales address issues related to radiation side effects to varying degrees. The MDASI-HN includes the most symptoms, while the other scales address different quality of life, social and functional issues.

Given the broad array of potential symptoms, investigators in one study queried patients about the most troublesome and debilitating side effects they experienced from radiation therapy. The most frequently reported side effects were lethargy, weakness, dry mouth, mouth sores, pain, and taste changes. When patients were asked to identify the one most debilitating side effect, 20% patients stated it was painful sore throat, 18% stated mouth sores and pain, and 14% stated dry mouth. Patients reported these symptoms were accompanied by burning causing significant discomfort and inability to eat, drink or swallow.[17] In this study 90% of patients also reported taste alteration, including complete loss of taste (54%), distorted taste (33%), and reduced taste (13%). 75% reported changes in mouth conditions, with most reporting mouth sores, loss of saliva, dry mouth, pain, irritation, and sores and blisters on the tongue; 88% also reported changes in the throat or oesophagus.

The overall effect of oropharyngeal mucositis was devastating. 88% of patients reported they could not eat or drink due to mucositis or did so with extreme difficulty. 83% experienced weight loss from 12 to 79 pounds leading to gastric tube placement in 29%. 29% reported speaking difficulties, 38% reported depression, 25% reported sleep disturbances, and 13% required hospitalization due to mucositis.

Other investigators reported additional symptoms, some of which are seemingly remote from mucositis, such as fatigue, feeling drowsy, feeling distressed, lack of appetite, numbness or tingling, shortness of breath, difficulty remembering, nausea, vomiting, choking or coughing, constipation, hair loss, skin pain, burning, rash, diarrhea, trismus, hearing loss, distorted smell, and limited range of motion.[57] Other reports cite the following additional patient-reported

symptoms: poor nutrition, weight loss, difficulty regulating weight, requiring liquid supplements, difficulty drinking thin liquids, food getting stuck in the mouth and throat, coughing after swallowing, prolonged time to eat, hoarse voice, trouble being understood, limited food choices, over-sensitivity to hot, cold, spicy, sweet and acidic foods, sensation that teeth are loose and cracking/chipping, trouble with dentures, over-sensitivity when brushing teeth, limitations in jaw and shoulder/neck movement, and mood changes including anxiety and depression.

To better understand and predict mucositis it is important to asses and grade mucositis with a comprehensive scoring system that considers all patient-related factors. Additional factors that should be included are detailed appraisals of pain, such as its location, duration, and pattern (i.e. continuous or intermittent). Quality of pain also should be recorded according to patients' qualitative experiences and should be recorded with a) descriptors preferably associated with nociceptive pain (e.g., sore, dull, hurting, tender, throbbing) and b) descriptors preferably associated with neuropathic pain (e.g., burning, stabbing, tingling, shooting, radiating). Variations in pain, (e.g., due to swallowing, chewing, talking, changing body position, and associated with time of day), disturbances in daily function due to pain, effects of analgesics, and alternative methods to relieve pain (e.g., warmth, cold) also should be included. Finally, current smoking, abuse of alcohol and illegal drugs, and comorbidity and medication use should be included. Figure 1.3 incorporates these factors and depicts a plan for comprehensive evaluation of patients with mucositis.

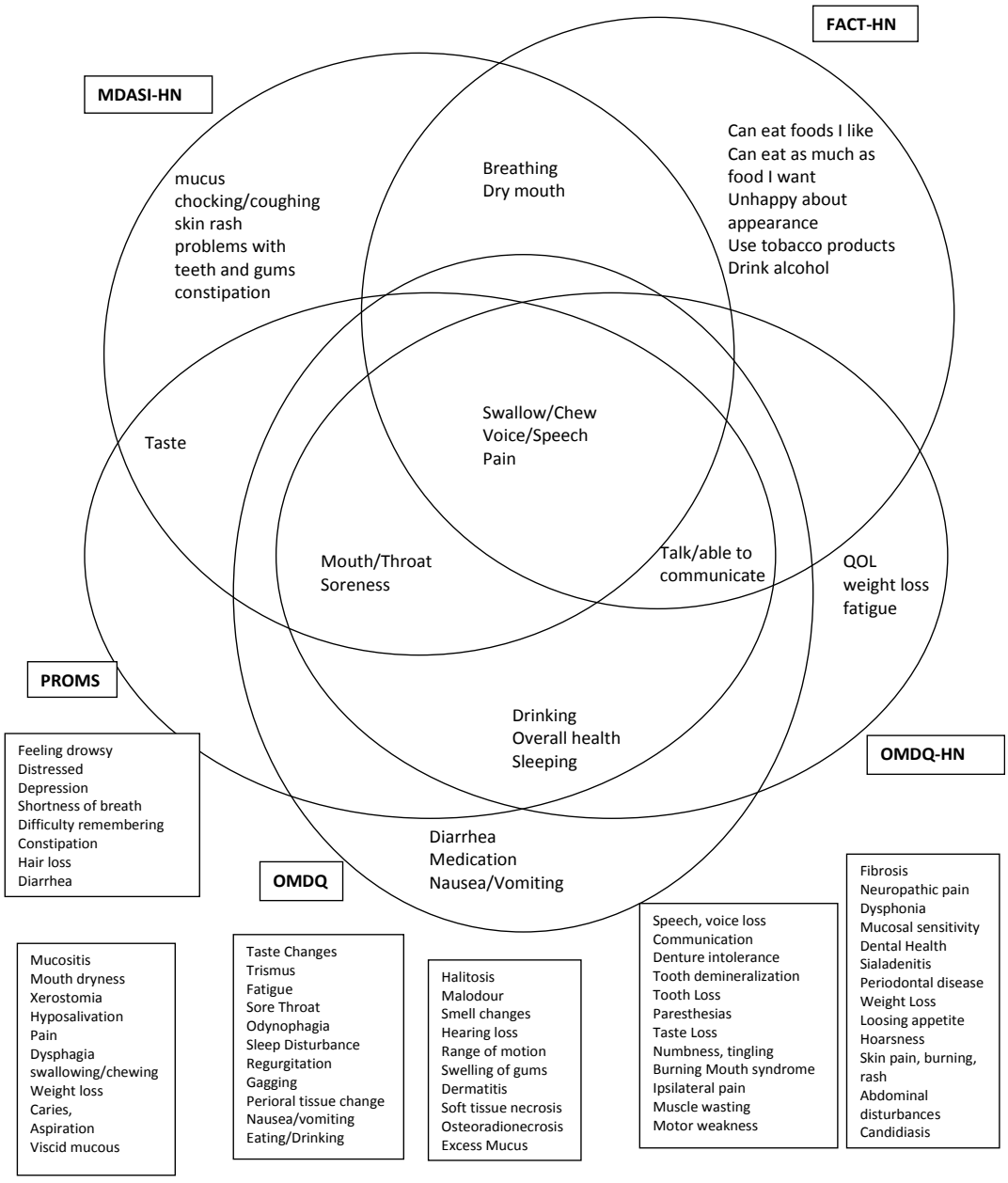


**Figure 1.3 A potential rubric for the assessment of symptoms of mucositis that could be part of a comprehensive patient-reported scale and a new scoring system**

In order to advance clinical care and research in this area, more specific patient reported quality of life measurements are needed to assess longitudinal functional status. Scales currently used to assess functional status are generic and measure global health. This is particularly important because radiation-induced

side effects are both acute and long-term. Longitudinal functional status measures would be better suited to capture chronic side effects of radiation. Longitudinal measures also are useful to cancer researchers and physicians who should incorporate baseline and subsequent frequency, severity and distress caused by side effects into overall assessments in order to develop effective interventions to ameliorate all symptoms.

Another critical component to consider is the high level of mental distress and psychiatric morbidity during treatment and how these impact the interpretation of symptoms. Various mood disturbances undoubtedly contribute to patients' coping strategies and these should be assessed along with other measurements of mucositis. When considered longitudinally, individual change in these assessments can help clinicians tailor mental health interventions to each patient's specific needs.

Mucositis dramatically impacts patients and if not addressed aggressively could result in delaying therapy while waiting for symptoms to subside. This can result in compromising the delivery of curative therapy which, in turn, may result in significant mortality and morbidity. Because examining the oral cavity for mucositis is complicated and it may be impractical to routinely visualize all potentially involved mucosa, the use of patient-reported scales becomes a vital component in the decision to proceed with a therapeutic protocol.

## DISCUSSION

### *Long-Term Use of Scales and How They Should Guide Treatment*

Given the numerous symptoms associated with mucositis it is important to assess each symptom to better understand the differential impact of various therapies. There is also a need to develop scales that will incorporate uniform terminology to better describe outcomes.

Despite these needs, mucositis researchers have not reached consensus regarding an easy to administer, accurate, and reproducible scoring system designed specifically for investigative applications.

In addition to measuring acute effects, an ideal scale also would address long-term side effects of radiation. Such a scale would provide not only an assessment of symptoms, but would also educate patients regarding what to monitor for as a radiation side effect.

Most of the oral symptoms associated with radiation-induced mucositis are associated with development and progression of mucositis. In particular, symptoms like hyposalivation, sore mouth and throat, dry mouth, and taste change are associated with progression of mucositis. Some symptoms like having viscid mucus, taste changes and hyposalivation can be present throughout the development of mucositis and could persist up to one year after radiation. Therefore it is important to assess the plethora of symptoms associated with mucositis and to adopt therapeutic approaches that prioritize the patients' symptoms over diagnostic findings.

A good scoring system would be one which will consider all patient-related factors. In particular, measurement of symptoms must take into account the subjective nature of symptoms and the nuances of language and expressions of distress. Measurement should not be limited to severity or frequency of symptoms but also should be linked to functional consequences. This is supported by the well-established clinical observation that although some symptoms may be infrequent and appear to be mild in severity, they still can be quite bothersome and debilitating to patients.

A good scoring system also would take into account late side effects. These side effects are less often documented for various reasons. For example, the patient does not recognize or attribute these late complications to radiation therapy, there are no patient- reported scales to systematically record symptoms, and

follow-up visits focus on residual cancer or recurrent disease, thus giving patients less time to voice their concerns related to quality of life. In addition, patients can become adjusted to their new situation and learn to cope with many side effects of treatment. This propagates a vicious cycle where lack of pertinent information results in lack of awareness on the part of clinicians, which then leads to failure to diagnose and manage these side effects. Patients then do not attribute these symptoms to their treatment and thus continue to fail to report these symptoms, forming the last part of the vicious loop.

From the point of view of cancer researchers, inconsistent use of scales and inconsistent terminology have hindered progress in understanding mucositis and have prevented comparisons across studies or combining data for meta-analysis. The true estimate of the impact of mucositis or the assessment of treatment effectiveness can only be ascertained if patients' experiences are included in the overall assessment based on validated and established scales. In addition, rigorously obtained symptoms can impact clinical care. For example, one study found that control of pain due to mucositis could reduce weight loss; this study provides a good example of the potential link between an objective outcome and a symptom-control intervention.[78]

In summary, both patient-reported scales and clinician-measured features should be combined to provide a comprehensive assessment of radiation-induced mucositis for head and neck cancer patients. Patient-reported scales should be developed and rigorously tested for validity, reliability and responsiveness and should capture the diverse experience of mucositis using patients' language. In addition, clinician measurements should be standardized in terminology and content. When paired, such measures will capture radiation side effects fully throughout the course of treatment, from acute to long-term chronic effects.

APPENDIX 1

**Description and Frequency of Symptoms Due to Side Effects of Radiation Therapy**

***Saliva*** Saliva is one of the most versatile fluids of the human body meeting a broad spectrum of needs, such as maintaining pH, protecting the oral cavity, helping with digestion, and protecting the gastrointestinal epithelium. The total volume of saliva produced varies from 0.5 to 1.5 litre per day and the pH oscillates between 6.5 to 7.4. [3]

Additional functions of saliva are cleaning the oral cavity, clearing food scraps and bacteria, helping in digestion, aiding the sense of taste, lubricating the oral cavity, and promoting chewing, swallowing and speaking. Salvia also helps to protect teeth, aid in the formation of enamel, neutralize acids, act as a first line of defence against microorganisms, and provide buffering of gastric reflux. [79,3]

Saliva undergoes qualitative and quantitative changes during radiation treatment resulting in reduction of amylase activity, change in pH and buffering capacity, and alteration in electrolytes such as calcium, potassium, sodium, and phosphate.

***Appetite and weight loss*** Approximately 38% of patients report weight loss and more patients report moderate to severe weight loss in the early recovery stage.[57] In addition, 68% of patients report loss of appetite. This decreases to 30% by 6 months post treatment. Loss of appetite also has been shown to lead to physical and psychological symptoms such as anxiety and depression. [80]

***Swallowing and eating*** Approximately 78% of patients report taking longer time to eat, with moderate to severe difficulty for 68% of patients. Effortful swallowing was reported in 82% of patients in the early phases of treatment and in 70% of patients in later phases. [57]

***Xerostomia*** Close to 90% of patients report some degree of xerostomia and 66% report moderate to severe symptoms. Xerostomia related functional deficits are reported by many patients, such as chewing/swallowing difficulty (85%) with 59% reporting moderate to severe difficulty, and moderate to severe and talking difficulty 36%.

Xerostomia is the most common complication of radiation to the head and neck. The intensity varies but the onset is very pronounced and rapid. Up to 50% of the salivary flow is lost during the first week of radiation and there can be up to 95% loss of salivary flow during the course of the radiation.

Xerostomia is both and acute and late side effect of radiation, and is often permanent affecting taste, chewing, swallowing, and speech and causing secondary infections, change in pH, dental caries, and osteoradionecrosis. Unfortunately these changes can lead to nutritional deficiencies which are difficult to reverse. Xerosomia also can be severe enough that it affects sleep[58]; approximately 28% of patients report awaking from sleep because of dry mouth. Currently-used observer-based measurements tend to underestimate the severity of xerostomia and under-report xerostomia.

***Mucositis*** A retrospective study of head and neck cancer reported oral mucositis to occur in 83% of patients [82] whereas a prospective study reported mucositis in 99% for patients with oral cavity, oropharyngeal, laryngeal and hypopharyngeal tumours.[66]

Mucositis typically occurs within the first hour of radiation and progressively worsens during the course of treatment due to the inflammatory cascade. Moderate to severe pain from mouth sores has been reported in 38% and moderate to severe dysphagia has been reported in 52% of patients. Mucositis will occur in 100% of patients with increases in radiation dose. Damage with mucosal erythema progresses to pseudomembranous degeneration, frank ulceration,

haemorrhage, and secondary infections.[57] In one study 100% of patients developed some pain during the second week of therapy and severe pain occurred in the fifth week of therapy.[78] In a retrospective study pain persisted in 52% of patients and was reported as severe.[82] Although there can be healing, the regenerated mucosa does not have the same integrity of normal mucosa.

***Excess mucus*** According to various studies, thick mucus is reported by 82% of patients and results in choking or gagging in 58% of patients, swallowing difficulty in 61%, and difficulty with sleep in 49% of patients. Patients also report soiling of clothes and odour.[57] Halitosis was also reported by 33% of patients in one study. [83]

***Speech and communication*** Difficulty speaking, difficulty being understood, and hoarseness have been reported in 61%, 59%, and 64% of patients, respectively. [57]

***Taste alterations*** Approximately 83% patients report taste alterations, 52% report decreased desire to eat, 65% report alterations in food choices, and 54% note decreased amount of food taken. Patients also experience persistent taste phantoms, such as metallic better, sweet, sour and salty taste. [57]

***Dental health issues*** Approximately 41% of patients report difficulty chewing attributed to their teeth or dentures. 50% report their teeth are particularly sensitive to hot, cold or sweet foods, 37% report the sensation of loose teeth, 36% report cracking or chipping, and 42% report difficulty with dentures. [57]

***Mucosal sensitivity*** Approximately 46% of patients report a burning pain in the lining of their throat or mouth and 81% report sensitivity to spicy, hot or acidic food. In 61% of patients mucosal sensitivity affected the types of food they ate.

The proposed mechanism is peripheral neuropathy secondary to sensitization of nerve endings by inflammation. [57]

***Range of movement*** Limitations in jaw and shoulder/neck movement have been reported in 54% of patients. Trismus (i.e. the uncontrolled inability to open the mouth or jaw) and associated fibrosis and dermatitis are late side effect of radiation and can be so severe that they impede eating. Scars of muscle bundles and subcutaneous tissues also can result in permanent deformity.

***Radiation caries*** Radiation caries typically are seen only after one year of radiation therapy. Although they are relatively painless, they are aggressive and rapidly compromise teeth.

***Soft tissue necrosis*** Diminished blood supply from scarring and thickening of arteriole walls results in fibrosis and subsequent soft tissue necrosis. The risk of necrosis increases as fibrosis and xerostomia progress. Sharp tooth brushes and abrasive brushing techniques can trigger necrosis. In addition, trauma of any kind, including a sunburn to the radiated area, can result in necrosis.

***Osteoradionecrosis*** Fibrosis affecting arterioles and bone marrow space can compromise the production of osteoblasts and osteoclasts, and results in osteonecrosis (occurring in 4% to 20% of patients). The mandible is most prone to osteoradionecrosis.

***Dysphonia*** Approximately 91% of patients report difficulty speaking[83]. Radiation-induced dysphonia is always seen during the treatment of laryngeal cancer. Among non-laryngeal cancer, the prevalence of dysphonia is dose dependent and seen when radiation is more than 50 Gy. Patients can present with

dysphonia early and this typically worsens within 5 to 15 days. The onset is sudden and can persist for years. Dysphonia is thought to be due to oxidative injury, mucosal oedema, necrosis, epithelial sloughing, and fibrosis. Objective measurements such as grade, roughness, breathiness, asthenia and strain are measured. In addition, patients can have decreased volume, pitch range and maximum phonation time associated with increased shimmer, jitter, and subglottic air pressure.[84]

***Neuropathic pain and chronic drainage*** Neuropathic pain and chronic drainage are late adverse effects of radiation[85]. In a retrospective study in which the median time since radiation was 56 months, investigators found that 12% of patient reported symptoms of brachial plexus neuropathy[86]. The most common symptoms were ipsilateral pain (50%), numbness and tingling (40%), and motor weakness with or without muscle wasting (25%). These investigators also found that brachial plexus related neuropathy was under-reported in head and neck cancer patients.

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**Manuscript #2**

# Pathophysiology, Treatment Options, and a Proposal for a Novel Ayurvedic Treatment for Radiation-Induced Mucositis in Head and Neck Cancer

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CONFLICT OF INTEREST NOTIFICATION PAGE

NONE TO DECLARE

**ABSTRACT**

Introduction: Radiation-induced muscositis is a common and debilitating side effect of treatment for head and neck cancer that often interrupts treatment protocols. There currently are no effective medications to ameliorate the multiple manifestations of radiation-induced mucositis. Ayurvedic medicine has traditional formulations (mostly mouthwashes) that are effective to varying degrees in treating general stomatitis. The objectives of this review were: to describe the clinical phases, metabolic pathways, and current treatments for mucostis; to conduct detailed bio-prospecting from treatments of general stomatitis from Ayurvedic traditions based on formulation and bio-activity; and to identify potential plants that might be useful in new medications to treat mucositis and to describe their traditional uses and contemporarily-ascertained biologic activity.

Methods: Articles that address current allopathic treatments for mucositis and mechanisms of mucosal damage were identified from PUBMED searches. Eight different Ayurvedic texts were consulted to compile information about bio-prospecting of plants that might be useful to treat radiation-induced mucositis. A rubric of eight key properties potentially critical for the management of mucositis was define and seven plants were identified and described regarding their effectiveness and known mechanism of action.

Results: Multiple inflammatory pathways are involved in radiation-induced mucositis and contribute to a plethora of symptoms associated with different stages of damage and healing. There currently are no effective medications to arrest mucositis. Novel potential compounds that might have efficacy for radiation-induced mucusitis were identified by using a bio-prospective method to assess mouthwashes used for general stomatitis in Ayurvedic medicine. First, eight key criteria were stipulated: can be used internally, daily, and as a mouthwash; has

anti-bacterial and anti-fungal effects; and enhances saliva production, pH balance, and wound healing. Second, compounds had to have strong evidence for at least four of these eight properties, some evidence for other properties, be cited in Ayurvedic texts, and be described in a mouthwash formulation. Seven plants met all these criteria: *Acacia catechu, Azadiracta indica, Glycyhhhiza glabra, Centalla asiatica, Emblica officinalis, Terminalica belerica,* and *Terminalia chebula.*

Discussion: Despite its frequency, symptomatic impact, and health and economic costs, there are no effective interventions for radiation-induced mucositis. In this review we propose a mouthwash that is based on traditional Ayurvedic knowledge and supported by scientific data that has the potential to ameliorate this adverse effect of cancer treatment. Given there are multiple pathways associated with mucositis, therapies that target a single pathway most likely will continue to be ineffective. Our multi-targeted approach offers a plausible alternative. This study supports a paradigm shift in the drug discovery mechanism in that drug development need not always be confined to new molecular entities. Instead, bio-prospecting of plants utilized by ancient knowledge and gained from indigenous medicines may provide a new strategy for drug development.

## INTRODUCTION

### ***Background and Significance***

The incidence of head and neck cancer in India is 30/100,000 in males and 10/100,000 in females[1]. This accounts for 23% of all cancers in males and 6% in females in India, while it accounts for only 3% of all tumours in developed countries.[2] Only the Indian state of Kerala has banned smoking since 1999; however, this has resulted in an increase in oral tobacco use.[3,4]

Radiation therapy is the mainstay of treatment for head and neck cancers in India.[5] The most common side effect of radiation therapy is oral mucositis; two-thirds of patients starting treatment for larynx and hypopharygeal cancer suffer from mucositis shortly after starting therapy.[6,7] Symptoms typically begin 1 to 2 weeks after the initiation of radiation therapy and are characterized to varying degrees by erythema, burning mucosal discomfort, and large areas of deep coalescing ulcers. Patients often require high doses of opioids to control pain, especially as symptoms worsen with dose escalation.[8,9]

Virtually every patient undergoing oral cancer therapy with radiation will develop some form of mucositis[10]; grade 3 mucositis (range 0-4) occurs in about 60% patients. Among these, 11% require treatment interruption, which has a detrimental effect on cancer control and potential cure. Mucositis, in turn, often triggers a chain of events characterized by difficulty swallowing and chewing, poor nutritional and fluid intake, weight loss, sleep disturbances, and psychological distress. In addition, damaged mucosal tissues in the setting of inhibited local host responses easily develop lesions caused by microorganisms, including fungi, herpes viruses, and a wide variety of bacteria. This leads to secondary infections which further complicate clinical management.[11-13] Additional adverse and costly outcomes in severe cases include placement of parenteral feeding tubes, more emergency room visits, more unplanned office visits, and longer hospital stays.[14]

Maximizing the therapeutic effectiveness of radiation therapy often involves increasing total radiation dose and daily fractions and utilizing concomitant chemotherapy. However, chemotherapy also causes erosion of mucosal epithelium and thus exacerbates radiation-induced mucositis. Thus mucositis not only is associated with pain and suffering, but also results in reliance on parenteral nutrition, administration of narcotics, hospitalization, and sub-optimal cancer treatment due to interruption in treatment protocols.[15,16]

The objectives of this review were: to describe the clinical phases, metabolic pathways, and current treatments of radiation-induced mucositis; to conduct detailed bio-prospecting from treatments of general stomatitis from Ayurvedic traditions based formulation and bio-activity; and to identify potential plants that might be useful in new medications to treat mucositis and to describe their traditional uses and contemporarily-ascertained biologic activity.

## METHODS

Articles that addressed current allopathic treatments for mucositis and mechanisms of mucosal damage were identified from PUBMED searches. Eight different Ayurvedic texts were consulted to compile information about bio-prospecting of plants that might be useful to treat radiation-induced mucositis. A rubric of eight key properties potentially critical for the management of mucositis was define and seven plants were identified and described regarding their effectiveness and known mechanism of action.

## RESULTS

### ***Mechanism of Mucositis***

A healthy oral mucosa not only serves as a physical barrier to microorganisms but also acts as a chemical barrier limiting absorption of many compounds into the epithelium.[17,18] Oropharyngeal cells undergo rapid renewal over a 7-14 day cycle; radiation therapy interferes with mitosis resulting in a reduced ability of oral mucosa cells to regenerate. Although cells that survive have an increased turnover rate, they cannot keep pace with cell death and thus patchy then confluent denuded areas emerge. This gradually leads to pseudomembranes and ulceration. Since this process decreases oral intake, poor nutritional status further interferes with mucosal regeneration. The severity of the mucositis

depends on the type of ionizing radiation, the volume of irradiated tissue, the daily dose, and the cumulative dose.[19-25]

Until recently radiation mucositis was thought to result as the direct effect of radiation on epithelial tissue. Only lately clinical studies have revealed that the mechanism is more complex and involve trans-tissue toxicity with the submucosa playing an important role. In addition, other co-factors such as oral hygiene, genetic predisposition, and an inflammatory cascade can influence this process.[26-28]

Five sequential stages in the development of mucositis have been proposed - initiation, up-regulation and message generation, amplification and signalling, ulceration, and healing (Figure 1).[29-30]

- During ***initiation***, radiation causes direct injury to basal epithelial cells resulting in damage and death of underlying tissues. This process begins within hours of radiation exposure. Indirect cell damage also occurs which is initiated through a non-DNA mechanism mediated by the generation of reactive oxygen species. Reactive oxygen species initiate a cascade of several injury-producing pathways affecting epithelial cells and fibrobasts.

- In addition to causing direct cell death, free radicals activate messengers that transmit signals from receptors on the cellular surface to the inside of cell. These in turn up-regulate pro-inflammatory cytokines and transcription factors such as nuclear factor-κB (NF-κB) and NRF-2, which then leads to ***up-regulation*** of genes that modulate the damage response. This in turn stimulates macrophages to produce pro-inflammatory cytokines such as tumor-necrosis factor-α (TNF-α) and interleukin-6 which then lead to further tissue damage. At this point, the oral mucosa becomes symptomatic with sensations of burning and pain, and erythema is also seen.

- Up-regulation of pro-inflammatory cytokines causes injury to mucosal cells and also activates molecular pathways that ***amplify*** mucosal injury. Different feedback loops are generated in this phase, the same cytokine that targeted tissues for direct damage will also further stimulate genes that are responsible for cytokine production. For example, cytokines such as TNF-alpha can up-regulate and amplify the transcription factor NF-kappa B or activate enzymes responsible for activating the ceramide pathway that leads to apoptosis. These feedback loops sustain and escalate the severity of mucosal injury even after cytotoxic cancer therapy has been discontinued.

- The significant inflammatory infiltrate coupled with colonizing oral microflora then leads to ***ulceration***. Secondary infection in the setting of immunocompromise and neutropenia, in turn, up-regulates further inflammation. Offending organisms often are gram positive bacteria, viruses such as herpes simplex, and fungal species such as candida. In addition, the oral mucosa is a habitat for several colonized micro-organisms that help to establish and maintain a homeostatic environment and prevent colonization of exogenous pathogens. This potent defence mechanism called "colonization resistance" is practically destroyed by radiation and thus secondary infection is facilitated.

- Epithelial proliferation and cellular and tissue differentiation ultimately occur restoring the integrity of the epithelium and thus initiate the process of ***healing***. This phase typically starts 2 to 4 weeks after discontinuation of cancer treatment. This healing is governed actively by regulatory proteins expressed by the extracellular matrix. [26-34]

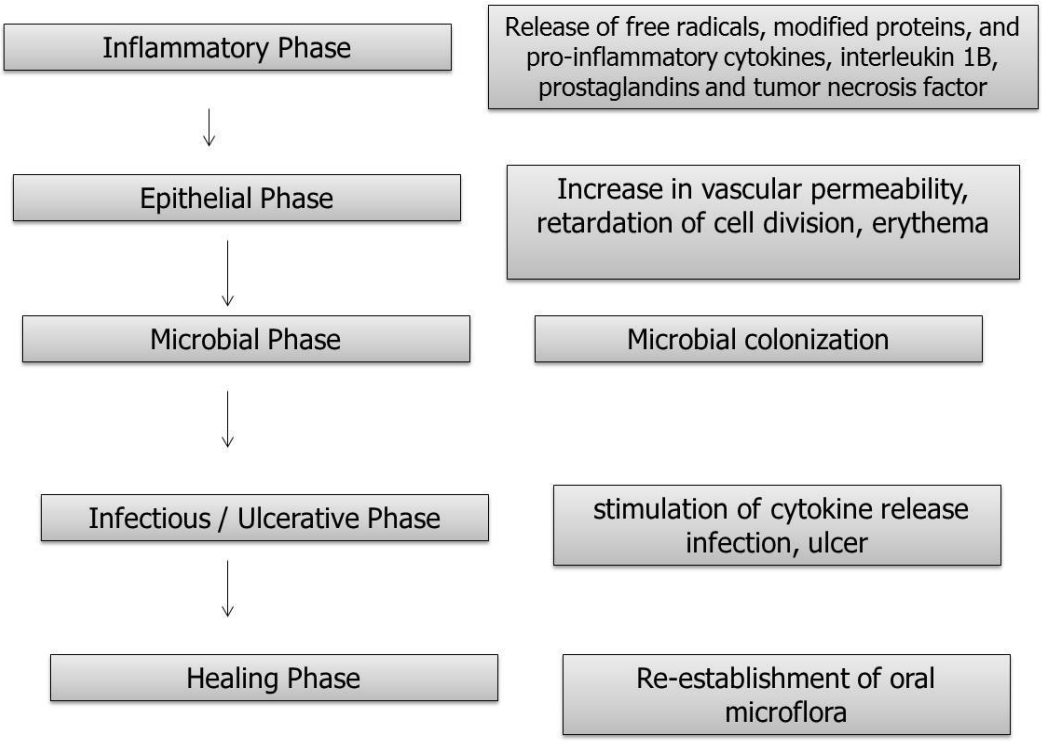



**Figure 2.1 Proposed phases in the development of mucositis**

In addition to these physiological phases, oedema and alterations in vasculature occur. In particular, thickening of the tunica intima and destruction of the elastic and muscle fibers of blood vessels result in reduction in lumen size and blood flow. If there is concurrent thrombocytopenia, then oral bleeding can occur complicating the clinical scenario.[35]

Although this schema is highly regarded, the actual process is more complex and multifaceted with considerable overlap and integration of mechanism of injury that are currently not known. Because many pathways are involved, it is unlikely that a single drug intervention could fully mitigate the expression of clinically significant mucositis. However, this conceptual model provides a preliminary road map for the development of anti-mucositis agents. This schema also has led to closer observation and monitoring of mucositis and to improvements in objective and subjective functional assessment tools.

Multiple symptoms emerge during the different phases of mucositis. In particular, radiation induces transient or permanent xerostomia and hyposalivation,

which in turn aggravate inflammation and increase the risk of local infection. Patients also report difficulty chewing and thickening of salivary secretions due to a decrease in the serous component of saliva. Different inflammatory markers also are associated different phases. For example, release of CRP, IL-2 and TNF alpha occur during the inflammatory phase and secondary infection from bacteria, viruses, and fungi occur in the microbial phase. Figure 2.2 shows various symptoms, signs, and pathological features for each phase.

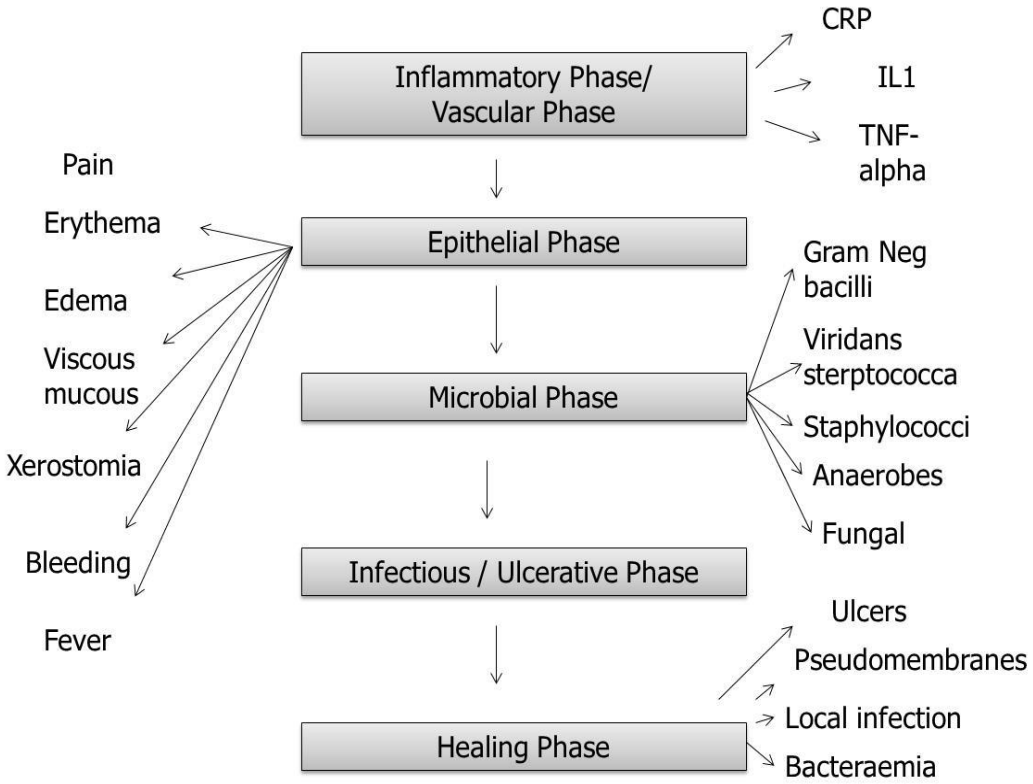



**Figure 2.2 Various symptoms, signs, and pathological features for each phase**

There are several patient-related risk factors for mucositis. These include older and younger age, body mass index greater than 25, female gender, and African race. Trauma from ill-fitting dental devices, damaged teeth, poor dental hygiene, and oral retainers can increase the risk of mucositis. Genetic polymorphisms also may be associated, such as TT polymorphism in the MTHFR gene which metabolizes methotrexate, as well as certain comorbidity, such as

Addison's disease are associated with increased risk. Interestingly, patients with other comorbidity, such as psoriasis, may have a lower risk.[36-39]

***Role of Saliva***

We produce about 600-1000 ml of saliva per day.[41] Saliva contains several electrolytes (Na+, K+, Cl-, HCO3-, Ca2+, Mg2+, HPO42-), and over 2000 different proteins and peptides, including amylase, proline-rich proteins, mucins, albumin, lipase, alpha-amylase, histatin, cystatin, peroxidase, lysosome, lactoferrin, defensins, cathelicidin-LL37, and immunoglobulins (e.g. secretory IgA, IgG, IgM). There are several other small organics such as glucose, urea, uric acid and lipids, as well as epidermal growth factor, insulin, cyclic adenosine monophosphate-binding proteins and serum albumin.[42,43]

Cleaved salivary proteins have multiple different functions which are complex and synergistic. Saliva is secreted by major and minor salivary glands and content and volume vary for each gland. In addition, content varies according to circadian rhythms, with total protein peaking at the end of afternoon and sodium and chloride peaking at the beginning of the morning. There are also seasonal variations with lowest volumes in summer and highest volumes in winter.[43-46]

Saliva protein content also varies according to sympathetic/parasympathetic stimulation, with sympathetic tone producing low volume high protein content and parasympathetic tone producing high volume low protein content.[47] Several physiological and pathological conditions can modify saliva production quantitatively and qualitatively, e.g., smell and taste stimulation, chewing, psychological and hormonal status, drugs, age, hereditary factors, oral hygiene and physical exercise.[48-50] In one study salivary protein concentration, flow rate, buffering capacity, and pH were estimated in normal subjects and those with gingivitis and periodontitis. The investigators found a significant rise in total protein and albumin concentration in gingivitis and periodontitis subjects. An

overall decrease in salivary flow rate also was observed among the elderly and among women.[51]

Saliva is the first barrier to infection and the first point where digestion takes place.[17,18] During radiation there are changes in salivary flow and salivary pH.[46,47] In one study of children with actue leukemia undergoing bone marrow transplant, patients with mucositis had significantly lower levels of salivary myeloperoxidase, peroxidase, and immunoglobulin A, and almost double the amount of total protein in saliva. The investigators postulated that although ulcerative lesions favour granulocytic infiltration, especially with bacterial infection, the lack of granulocytes in peripheral circulation causes a low concentration of neutrophils in saliva and subsequently low myeloperoxidase levels.[52]

Other researchers also noted a decrease of secretory immunoglobulin A that persisted up to 5 years after chemotherapy. In one study, participants who died during the study had lower concentrations of serum immunoglobulin compared to survivors.[53] Albumin abnormalities were found in another study in which higher concentrations of albumins in whole saliva were found compared to parotid saliva prior to the occurrence of mucositis.[54]

***Pathways of Mucositis***

Radiation damage depends on radiation type, dosage, dose rate, and region exposed. Radiation causes several modes of cell death - necrosis, apoptosis, and autophagy, in addition to accelerated normal senescence (during which a cell is viable but has altered functions and no longer is capable of proliferation). Apoptosis can be divided broadly into intrinsic and extrinsic pathways (the extrinsic is triggered by extracellular signals transduced by extracellular death receptors and the intrinsic pathway is initiated inside the cell and affects

mitochondrial integrity). Both pathways converge ultimately at initiation of protein lysis and DNA fragmentation. [55]

The pathophysiology of mucositis is complex and involves at least the following 14 metabolic pathways: nitrogen metabolism, toll-like receptor signalling, NF-kB signalling, B-cell receptor signalling, P13K/AKT signalling, G2/M DNA damage checkpoint receptor, P38 map signalling, Wnt/B catenin signalling, glutamate receptor signalling, integrin signalling, VEGF signalling, IL-6 signalling, death receptor signalling, and SAPK/JNK signalling. These pathways overlap, cross over and form feedback loops and thus foster multiple changes in epithelia cells, macrophages, cell membranes, connective tissues, mitochondria, the nucleus and genes.[14,56-60]

***Treatment of Mucositis***

Different strategies have been attempted to prevent and heal radiation induced mucositis. In a study to identify national treatment practices for chemotherapy- and radiotherapy-induced oral mucositis, researchers mailed a survey to clinical pharmacists in 200 hospitals throughout the United States. 31% were completed and returned from 42 states with most of the respondents from university-based medical centers and 45% of hospitals ranged in size from 500-750 beds. There was a wide range of agents used for both prophylaxis and treatment; 69% of respondents reported they had no standard protocols for mucositis and, among those that did, 82% of protocols included a single agent or combination of ingredients that lack proven efficacy, such as mouthwash mixtures with hydrogen peroxide, saline, water, salt, soda, and nystatin. [61]

As part of this review, a literature search was done to characterize current treatments for mucositis. More than 50 randomized trials aimed at prevention, palliation, or reduction of radiation-induced mucositis in patients with head and neck cancer were identified. The treatment modalities reported can be broadly

divided into locally applied non-pharmacological methods, locally applied pharmacotherapies, and systemically applied pharmacotherapies. The modalities used and results from the trials are listed in Table 2.1 and their hypothesized locations of action are shown in Figure 2.3.

**Table 2.1 Treatment modalities for mucositis reported in randomized trials**

**Table 2.1**

| | Author | Year | Treatment | Number of patients | Result |
|---|---|---|---|---|---|
| 1 | Maiya[62] | 2006 | He-Ne laser therapy | 50 | Helped in healing of muositis |
| 2 | Bensadoun[63] | 1999 | He-Ne laser therapy | 30 | Reduced severity |
| 3 | Oguchi[64] | 1998 | AD film | 52 | No benefit |
| 4 | Stokman[65] | 2003 | Selective elimination of oral flora | 64 | No effect |
| 5 | Carvalho[66] | 2011 | Low level laser therapy | 70 | Reduced severity |
| 6 | Etiz[67] | 2000 | Sucralfate | 44 | Recommended |
| 7 | Carter[68] | 1999 | Sucralfate | 102 | No difference |
| 8 | Epstein[69] | 1994 | Sucralfate | 27 | No significant difference |
| 9 | Makkonen[70] | 1994 | Sucralfate | 40 | No significant difference |
| 10 | Franzen[71] | 1995 | Sucralfate | 48 | Different but not statistically |
| 11 | Meredith[72] | 1997 | Sucralfate | 111 | No effect |
| 12 | Lievens[73] | 1998 | Sucralfate | 83 | No benefit |
| 13 | Cengiz[74] | 1999 | Sucralfate | 28 | No benefit |
| 14 | Dodd[75] | 2003 | Sucralfate | 30 | Not effective |
| 15 | Okuno[76] | 1997 | Antibiotic lozenges | 54 | No compelling evidence |
| 16 | Spikjervet[77] | 1991 | Antibiotic lozenges | 30 | Decreased severity |
| 17 | El-Sayed[78] | 2002 | Antimicrobial lozenges | 137 | No impact |
| 18 | Ferretti[79] | 1990 | Chlorhexidine | 70 | Not effective |
| 19 | Foote[80] | 1994 | Chlorhexidine | 52 | Detrimental |
| 20 | Madan[81] | 2008 | Chlorhexidine vs PI vs Soda | 80 | PI better |
| 21 | Satheesh Kumar[82] | 2010 | Triclosan | 24 | Help delay |
| 22 | Rahn[83] | 1997 | Providone-iodine | 40 | Reduced incidence and severity |
| 23 | Adamietz[84] | 1998 | PVP-iodine solution | 40 | Reduced incidence and severity |
| 24 | Barber[85] | 2007 | Gelclair | 20 | Not effective |
| 25 | Kazemian[86] | 2009 | Benzydamine | 100 | Effective |
| 26 | Epstein[87] | 2011 | Benzyamine HCL | 69 | No effect |
| 27 | Leborgne[88] | 1998 | Corticosteroids | 66 | No significant difference |
| 28 | Veness[89] | 2006 | Topical misoprostol | 83 | Unable to find any benefit |
| 29 | Naidu[90] | 2005 | MF 5232 (Mucotrol) | 30 | Positive evidence |
| 30 | Hanson[91] | 1995 | Prostaglandin E1 analog, Misoprostol | 69 | Promising |
| 31 | Hanson[92] | 1997 | Prostaglandin E1 analog, Misoprostol | | Controversial |
| 32 | Ferreira[93] | 2004 | Alpha-tocopherol (VitE) | 54 | Not effective |

**Table 2.1** Continued

| | Author | Year | Treatment | Number of patients | Result |
|---|---|---|---|---|---|
| 33 | Schneider[94] | 1999 | Filgrastim (r-metHuG CSF) | 14 | May decrease severity |
| 34 | Makkonen[95] | 2000 | Granulocyte macrophage colony stimulating factor (GM-CSF) and sucralfate | 40 | No evidence |
| 35 | Wu[96] | 2009 | Human epidermal growth factor (RhEGF) | 113 | May have effect |
| 36 | Ryu[97] | 2007 | GM-CSF | 130 | No effect |
| 37 | Kannan[98] | 1997 | GM-CSF | 10 | Side effects |
| 38 | Rosso[99] | 1997 | GM-CSF | 29 | Worse |
| 39 | Nicolatou[100] | 1998 | GM-CSF | 17 | No effect |
| 40 | Rovirosa[101] | 1998 | GM-CSF | 12 | Less severe mucositis |
| 41 | Saarilahti[102] | 2002 | GM-CSF sucralfate vs sucralafate | 40 | GM-CSF sucralfate Slightly better |
| 42 | Schneider[103] | 1999 | Filgrastim (r-metHuG-CSF) | 14 | May decrease severity |
| 43 | Makkonen[104] | 2000 | GM-CSF and Sucralfate | 40 | No evidence |
| 44 | Trotti[105] | 2004 | Iseganan HCL oral solution | 545 | No effect |
| 45 | Antonadou[106] | 2002 | Amifostine | 50 | Slightly reduced mucositis and dysphagia |
| 46 | Bourhis[107] | 2000 | Amifostine | 24 | Reduction |
| 47 | Buntzel[108] | 1998 | Amifostine | 39 | Significant Reduction |
| 48 | Koukourakis[109] | 2000 | Amifostine | 39 | Significant Reduction |
| 49 | Huang[110] | 2000 | Glutamin | 17 | Reduction |
| 50 | Kaushal[111] | 2001 | Placentrex | 120 | Appears to control subjective symptoms |
| 51 | Mose[112] | 1997 | Immunoglobin injection IM | 20 | More severed |
| 52 | Mehta[113] | 2004 | Aloe vera | 54 | No effect |
| 53 | Amruthesh[114] | 2010 | Tinospora Cordifolia | 14 | Significant Reduction |
| 54 | Das et al[115] | 2011 | Yashti madhu (Glycyrrhiza glabra) | 75 | Food intake not affected, no treatment interruption |
| 55 | Maddocks-Jennings[116] | 2009 | Manuka and Kanuka | 19 | Helps, needs more studies |
| 56 | Carl[117] | 1991 | Kamilosn liquidium | 20 | Worse |
| 57 | Putwatana[118] | 2009 | Payayor | 60 | Prolonged time of onset |
| 58 | Rothwell[119] | 1990 | Hydrocortisone, nystatin, tetracycline, dipheyhydrarmine to a cherry syrup containing sorbitol, magnesia, and alumina suspensions and vitamins | 12 | Effective |
| 59 | Gujral[120] | 2001 | Hydrolytic enzymes | 100 | Useful |

**Table 2.1** Continued

| | Author | Year | Treatment | Number of patients | Result |
|---|---|---|---|---|---|
| 60 | Symonds[121] | 1996 | PTA (polymyxin E, tobramycine, amphotericin B) | | No difference |
| 61 | Wijers[122] | 2001 | PTA | 77 | No difference |
| 62 | Biswal[123] | 2003 | Honey | 50 | Reduced severity |
| 63 | Khanal[124] | 2010 | Honey | 40 | Useful |
| 64 | Rashad[125] | 2009 | Honey | 40 | Supports prophylactic use |
| 65 | Danilenko[126] | 1999 | Keratinocyte growth factor | Preclinical | Promising |
| 66 | Dorr[127] | 2007 | Wobe-Mugos E (enzyme) | 69 | No benefit |
| 67 | Ertekin[128] | 2004 | Zinc sulphate | 30 | Beneficial further studies required |
| 68 | Lin[129] | 2010 | Zinc supplements | 40 | Delayed mucositis |
| 69 | Abdulrhman[130] | | Honey, beeswax, olive oil, propolis extract | 90 | No benefit |

As can be seen from the table, most of these modalities were not effective. Thus despite the frequency, symptomatic impact, and health and economic costs, there are no known effective interventions for radiation-induced mucositis.

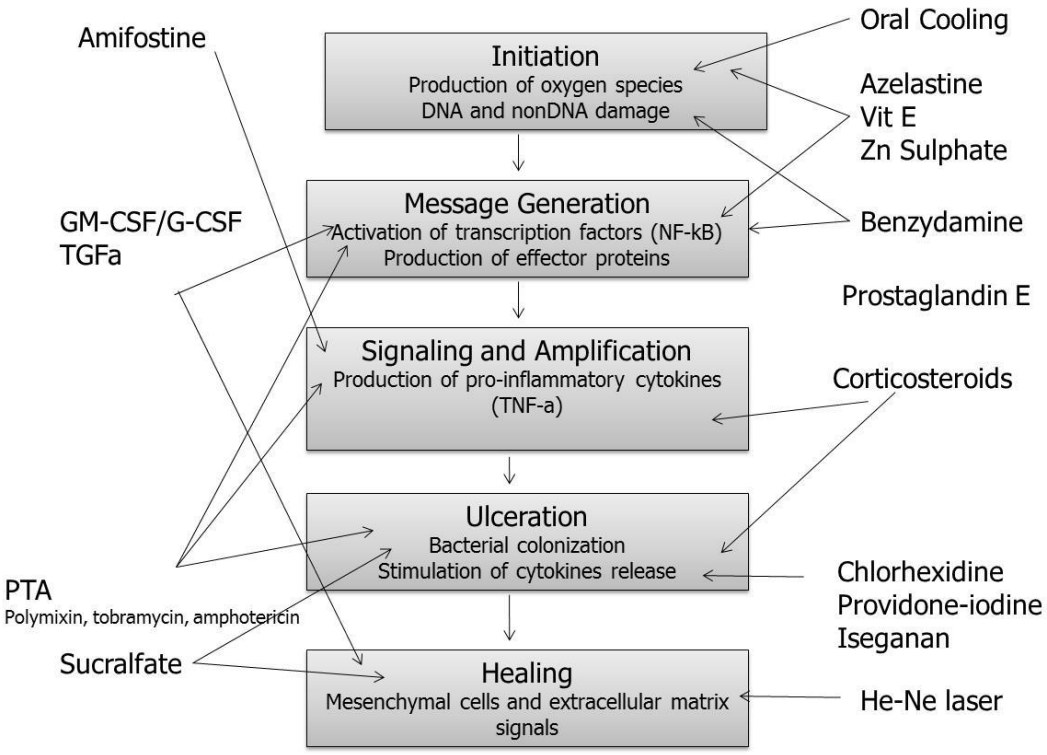



**Figure 2.3 Proposed location of action of various treatment modalities**

As seen in Figure 2.3, several agents are hypothesized to act during more than one stage of mucositis. This is relevant because different areas of the mouth will have mucositis in different stages; thus therapies should have a broad spectrum of action and ideally treatment existing mucositis and also prevent emergence of mucositis in new locations. For example, an effective modality would be one that would inhibit inflammatory pathways and inhibit feedback loops that prevent healing.

***Long-Term Sequelae of Radiation Mucositis***

Chemotherapy-induced and radiation-induced mucositis differ in that mucositis induced by radiation often has permanent features.[131, 132] For example, healed mucosa after radiation may appear pale and be atrophic. The degree of abnormality depends on the extent of injury, extent of mesenchymal cell depletion, healing time and recovery rate. Some ulcers also take a non-healing route and progress to soft tissue or bony necrosis.[133, 134]

Multiple adverse outcomes result if secondary infection occurs with bacteria, viruses or fungi, such as pain hindering adequate nutrition, interruption of cancer therapy, decreased quality of life, and systemic infection (Figure 4). Potentially fatal bacteraemia in the myelosuppressed state is a possibility in severe cases. The challenge of timely differentiating infected and non-infected mucosisits further complicates effective management.[135]

There are short- and long-term complications of radiation-induced mucositis. The short term complications start from erythema and can lead to long term complications such as malnutrition, fatigue, taste changes, dental caries and abdominal disturbances.

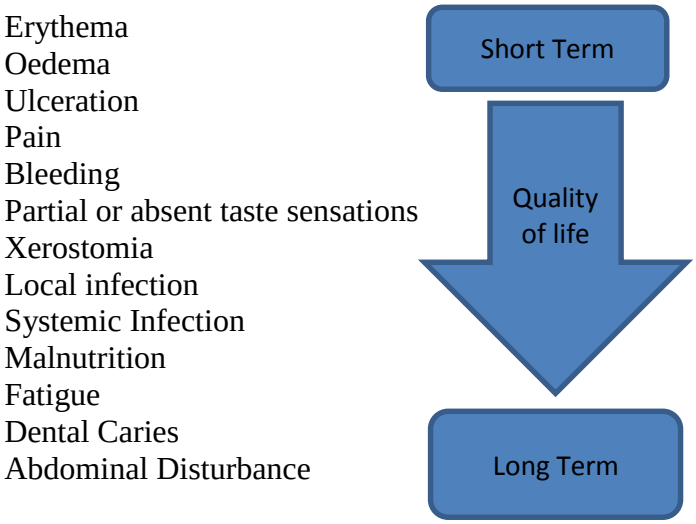



**Figure 2.4 Short- and long-term adverse sequelae of radiation-induced mucositis**

***Moving Forward: A New Paradigm to Treat the Multiple Short- and Long-Term Phases of Mucositis Based on Ayurvedic Principles***

It seems unlikely that there will be a single therapeutic agent that will be effective in all phases of mucositis, thus a sequenced-therapy approach might be more effective. Such an approach would block or partially block multiple pathways at the same time.

We propose that drug discovery and drug development need not always be confined to new molecular entities. A paradigm shift is proposed that focuses on revisiting Ayurvedic medical practices.

Ayurveda is a legitimate medical system in India for which practitioners receive state-licensed, institutionalized medical training. It utilizes a pharmacopeia that has thousands of herbal combinations; often 100 – 200 herbs are combined in a single formulation. The methodology of formulation and preparation is geared to enhance bioavailability and synergism. Currently Ayurveda is used routinely by approximately two-thirds of India's rural population and also is used to some

extent in urban populations. Currently Ayurveda is used in 2458 country hospitals with 44,820 beds, in 15,353 dispensaries, and in 495 teaching colleges in India. In addition, there are 7494 drug manufacturing units affiliated with the AYUSH, a department of alternative medicine in India with an annual financial growth of 26.8%.[136]

Ayurveda is an ancient tradition based on a holistic approach. The goals are treatment effectiveness through synergetic poly-herbal formulations that are safe and devoid of genotoxicity and mutagenic activity. The emphasis on multiple herbal combinations that improve bioavailability offers numerous advantages in drug development including reduction of dose and treatment cost. A reverse pharmacology approach inspired by traditional medicine and Ayurveda can offer a smart strategy for the identification of new drugs that have multi-site activity (Figure 2.5). [137]

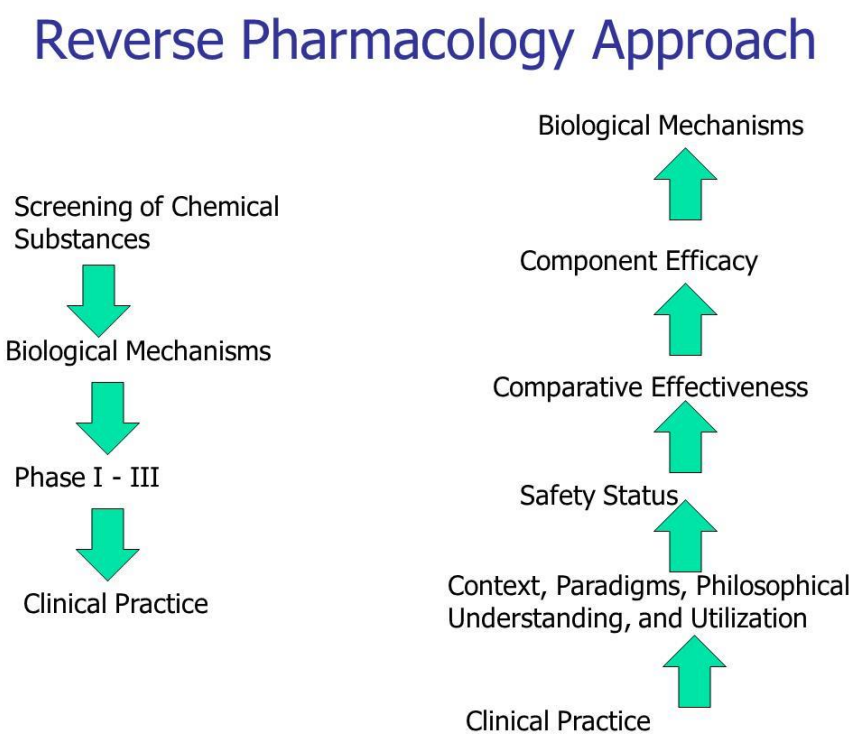



**Figure 2.5 A comparison of allopathic and Ayurvedic approaches to pharmacology**

Allopathic medicine (left side, Figure 5) uses a phase-wise system of drug development characterized by first proposing a molecular substance and then testing the substance in a sequence of clinical experiments. Ayurveda (right side, Figure 5) starts with substances known to be clinically effective in some way, and then seeks to identify what elements and in what ways these various components are effective; the ultimate goal is then to refine the effective elements. This latter process, termed ***bio-prospecting***, is a cost effective method to identify active components as opposed to random screening methods. However, most importantly, this method incorporates the knowledge that isolated compounds from plants do not necessarily always exhibit the same properties as the whole plant, as the other compounds in the plant may facilitate the absorption, assimilation and utilization of the 'active' compound. In addition, when multiple plants are used in the same preparation, various compounds in one plant may provide this enabling role for the 'active' ingredient in another plant. Furthermore, this process may also play a role in the safety of the preparation, such as moderating side effects.

***Potential Management of Mucositis with Ayurvedic Medicine***

The Ayurvedic pharmacopeia has mouthwashes derived from herbal preparations that are used for oral hygiene and for general stomatitis and ulcerations. Knowledge of these herbal preparations could be utilized in bio-prospecting of multi-herbal combinations for radiation-induced mucositis. We consulted the following texts for various herbal preparations (details of various texts are included in Appendix 2): Charak Samhitha, Sushruta Samhita, Ashtanga Hridaya, Sharangdhara Samhita, Chakradattam, Sahasra Yogum, and Yogamrutham.

Mucositis or stomatitis has been studied as a disease in traditional Ayurveda. However, there are no contemporary discussions of radiation-mucositis and possible preparations or herbs that might be effective. Thus, we conducted a

comprehensive review of the above Ayurvedic text for herbs that have been traditionally used (from time immemorial) for mucositis in general. A total of 56 herbs were identified, 47 are listed in Table 2.2.

**Table 2.2 Herbs identified from Aruyvedic texts traditionally used for general mucositis**

| | HERB NAME | Used internally | Used Daily | Used as mouthwash | Anti bacterial | Anti fungal | Saliva Stimulant | pH balance | Wound healing |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Cypreus rotundus | | | | | | | | |
| 2 | Terminalia arjuna | | | | | | | | |
| 3 | Mesua Ferrea | | | | | | | | |
| 4 | Zingiber offcinale | | | | | | | | |
| 5 | Ficus glomerata | | | | | | | | |
| 6 | Ficus microcarpa | | | | | | | | |
| 7 | Ficus Bengalinosis | | | | | | | | |
| 8 | Ficus Religiosa | | | | | | | | |
| 9 | Symplocos racemosa | | | | | | | | |
| 10 | Anethum graveoleus | | | | | | | | |
| 11 | Cacsalpia sapan | | | | | | | | |
| 12 | Erythrina Vasigeate | | | | | | | | |
| 13 | Acorus Calamus | | | | | | | | |
| 14 | Celastrus Panaculatus | | | | | | | | |
| 15 | Cyclia pelteta | | | | | | | | |
| 16 | Picorisa curora | | | | | | | | |
| 17 | Alpinia galanga | | | | | | | | |
| 18 | Jasminum gradiflorum | | | | | | | | |
| 19 | Cucuma longa | | | | | | | | |
| 20 | Coscinium fenestratum | | | | | | | | |
| 21 | Aconitum hetrophyllum | | | | | | | | |
| 22 | Adathoda vasica | | | | | | | | |
| 23 | Aegle marmelos | | | | | | | | |
| 24 | Piper nigrum | | | | | | | | |
| 25 | Piper longum | | | | | | | | |
| 26 | Brassica juncea | | | | | | | | |
| 27 | Benincasa hispida | | | | | | | | |
| 28 | Solanum xanthocarpum | | | | | | | | |
| 29 | Cinnmamon tamalum | | | | | | | | |
| 30 | Cedrus deodara | | | | | | | | |
| 31 | Vitus vinifera | | | | | | | | |
| 32 | Butea frondosa | | | | | | | | |
| 33 | Syzygium cumini | | | | | | | | |
| 34 | Tephrosa purpura | | | | | | | | |
| 35 | Nardostadys jatamansi | | | | | | | | |
| 36 | Aidei racemosa | | | | | | | | |
| 37 | Cinnamamom camphora | | | | | | | | |
| 38 | Aquilaria agalloca | | | | | | | | |
| 39 | Woodfordia fruticosa | | | | | | | | |
| 40 | Santalum album | | | | | | | | |
| 41 | Rubia cordifolia | | | | | | | | |
| 42 | Sesamum indicum | | | | | | | | |
| 43 | Tinospora cordifolia | | | | | | | | |
| 44 | Cinnamamom zylanicum | | | | | | | | |
| 45 | Eletoria cardamom | | | | | | | | |
| 46 | Santalum indicum | | | | | | | | |
| 47 | Myristica fragrance | | | | | | | | |
| 48 | illicum verum | | | | | | | | |
| 49 | Laccisum lacca | | | | | | | | |

(Green represents often, blue moderate, red little, and black none.) Ref [138-269]

A literature search was then conducted to document how these herbs have been used. Several major properties were hypothesized to be important for radiation-induced mucosits: used internally in different formulations; used on a daily basis; used as a mouthwash; has potential anti-bacterial and anti-fungal effects; stimulates saliva; affects salivary pH; and promotes wound healing.

Although all 56 herbs were used in internal formulations, 4 were used to a lesser extent, 5 herbs had little usage on a daily basis, and 7 herbs had moderate usage. All 56 herbs were used as a mouthwash and only 6 were used moderately.

Regarding anti-bacterial effects, 33 herbs had evidence for strong anti-bacterial properties, 19 had moderate anti-bacterial effects, 2 had weak anti-bacterial properties, and 3 did not have published evidence for anti-bacterial properties. Regarding anti-fungal effects,
29 herbs had strong anti-fungal properties, 25 had moderate effects, 1 herb did not show strong effects, and 1 herb was not assessed for anti-fungal activity.

Regarding saliva production, only 5 herbs had strong evidence for enhancement of saliva production, 24 herbs did not have strong evidence, 12 herbs had moderate evidence, and 15 herbs had no evidence of saliva stimulation.

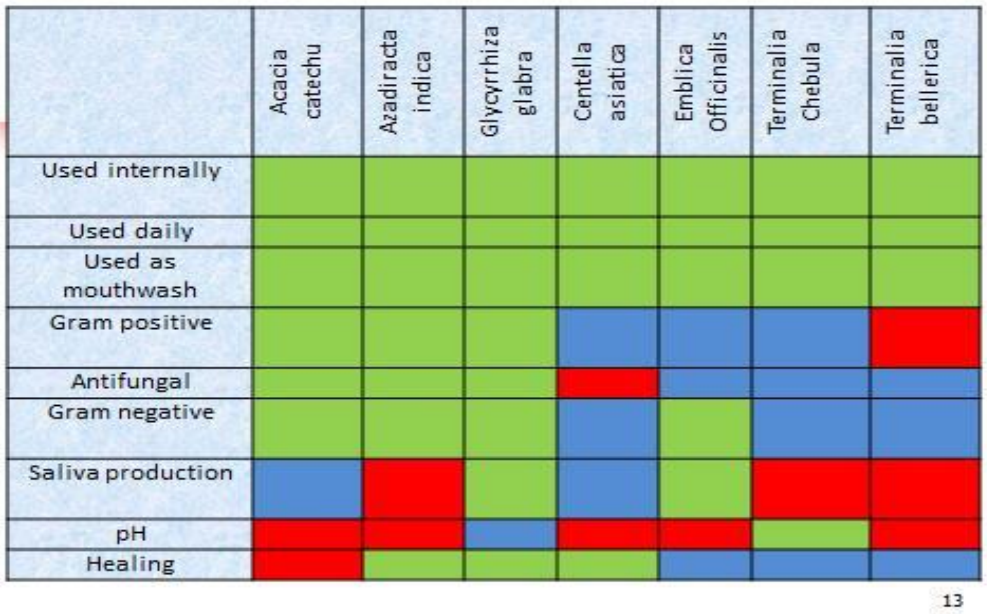
Regarding pH balancing properties, 33 herbs were found to have only weak evidence of pH balance, 18 herbs did not have studies done on pH stimulation, 4 herbs were found to have moderate evidence, and 1 herb was found to have strong evidence of pH balancing property.

Regarding wound healing, 20 herbs had strong wound healing properties, 13 herbs had no evidence to support wound healing, 20 herbs had only moderate evidence for wound healing, and 3 herbs had no evidence of wound healing.

In order to hone in on the most promising compounds for future investigation, we applied several threshold criteria. First, we required that there should be evidence of both in vitro and in vivo action against mucosits and we stipulated that the herb should have strong evidence for at least 4 (50%) of the 8

properties; 40 herbs met this criterion. We then included only those herbs that also had any effectiveness for any of the other properties; 19 herbs met this criterion. We then considered which of these 19 herbs were discussed in all 7 Ayurvedic texts; 13 met this criterion. Because we propose that a mouthwash formulation would be best suited to the management of radiation-induced mucositis, we further stipulated the herb had to be described in all texts in 2 or more mouthwash formulations; 8 herbs met this criterion. We eliminated one herb (curcuma longa or curcumin) because it has already was tested for mucositis and was not effective. Thus, the remaining 7 herbs in combination would address all 8 key properties (Table 2.3). Specifically, the formation could be used internally on a daily basis as a mouthwash, and could provide antibacterial and antifungal activity, prevent xerostomia, enhance saliva production, promote pH balance, and promote healing.

**Table 2.3 Severn herbs meeting all criteria for possible use against radiation-induced mucositis**




Acacia catechu, Azadiracta indica, and Glycyrrhiza glabra showed excellent activity against gram positive and gram negative bacteria and excellent antifungal activity. While Centella asiatica, Emblica offinalis, and Terminallia

chebula exhibited moderate activity against gram positive bacteria, Terminallia bellerica showed very weak activity against gram positive bacteria, and all but Centella asiatica showed moderate antifungal activity.

Glycyrrhiza glabra and Embilica officinalis were found to be excellent saliva stimulators. Terminalia chebula showed excellent pH balancing properties and the same to a moderate extent was exhibited by Glycyrrhyiza glabra. Excellent wound healing activity was found for Azadiracta indica, Glycyrrhiza glabra, and Centella asiatica with moderate activity exhibited *by Emblica officinalis, Terminalia bellerica, and Terminalia chebula* .

Detailed descriptions of these 7 herbs - Acacia catechu, Azadiracta indica, Glycyrrhiza glabra, Centella asiatica, Emblica officinalis, Terminalia chebula, and Terminalia bellerica, are summarized below.

***Acacia catechu*** This compound has significant antipyretic, antidiarrheal, hypoglycaemic and hepatoprotective effects that were demonstrated in a recent study in rats published in 2006.[270] *Acacia* has also shown considerable anti-microbial activity. For example, in an in-vitro study, a 50% *Acacia catechu* solution was found to have significant inhibitory activity against gram-positive cocci and gram-negative bacilli. The strains assessed were Straphylococcus aureus (112 strains), Staphylococcus epidermidis (112 strains), Enterobacter aerogenes (28 strains), Klebsiella pneumoniase (28 strains) and Escherichia coli (28 strains).[271, 272] Other researchers demonstrated that *Acacia catechu* has activity against bacterial (Escherichia coli, Straphylococcus aureus and Salmonella typhi) and fungal strains (Candida albicans and Aspergillus niger). [271, 272 - 274]

*Acacia catechu* also has anti-inflammatory properties in joints, being a dual inhibition of cyclooxygenase (COX I and II) and 5-lipoxygenase (5-LOX)[275] and is a free radical scavenger that might be protective in patients undergoing cancer treatment.[276]

***Azadirachta indica*** This compound has therapeutic potential for controlling gastric hypersecretion and gastroesophageal and gastroduodenal ulcers. [277] It was also found to have anti-ulcer and ulcer healing properties, including in experimental diabetic rats.[278, 279] *Azadirachta indica* has anti-bacterial activity against 21 strains of food borne pathogens[280, 281] and anti-filarial activity [282] as well as anti-viral activity against group B coxsackie viruses.[283]

*Azadirachta indica* has been shown to have anti-proliferative activity against several cancer cell lines[284] such as prostate cancer cells [285] and decreasing CEA levels. [286] *Azadirachta indica* also attenuated alkylation induced carcinogenesis [287] and has chemoprotective effects in murine carcinogenesis model systems and in rat mammary and liver carcinogenesis.[288] Effects have been shown for B 16 melanoma and Ehrlicha carcinoma.[289] An *Azadirachta indica* leave preparation was also found to prevents leukocyte apoptosis mediated by cisplatin plus 5-fluorouracil treatment in Swiss mice [290] and to restore impaired chemotactic activity of peripheral blood mononuclear cells from head and neck squamous cell carcinoma [291]. *Azadirachta indica* also was found to be useful in paracetamol-induced liver damage. [292]

Regarding anti-microbial effects, *Azadirachta indica* mouthwash was found to inhibit Streptococcus mutans and lactobacilli growth,[293] and to have fungicidal properties by impeding both fungal growth and aflatoxin production of Aspergillus parasiticus. [294]

***Centella asciatica*** This compound has been shown to help cartilage formation, collagen synthesis, connective tissue formation, and wound healing. [295, 296, 297] In rat studies, *Centella asiatica* induced proliferation of granulation tissue and increased tensile strength in wounds when locally applied and also decreased skin necrosis caused by burns. [298] Other studies showed reduced scarring and

stimulated skin growth by fostering production of collagen fibers which in turn resulted in decreased inflammation and myofibroblast production. [299]

Another study also showed *Asiaticoside*, a major constituent of the herb, promoted wound-healing by reducing lipid peroxide levels in wounds while increasing enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (vitamin E and ascorbic acid) antioxidant levels.[300] An animal study found that topical application of *Asiaticoside* used in a 0.2% solution improved healing in non-ulcer skin wounds. [301] An overview of three small human clinical trials suggests that topical use of an ointment or powder containing an extract of *Centella asciatica* with active triterpene compounds may speed wound healing in people with slow-healing skin ulcers. [302] These studies used either a topical ointment with a 1% extract concentration or a powder with a 2% extract concentration.

Regarding anti-microbial properties, *Centella asciatica* has been shown to have a broad spectrum of anti-bacterial activities against gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Shigella sonnei*) organisms. Activity against gram-positive bacteria is greater than against gram-negatives. Germacrene compounds in the essential oil of *Centella asciatica* are known to be strong anti-microbial and anti-tumour agents .[295]

*Centella asciatica* has been used to treat leprosy, wounds, fever, syphilis, acne, allergies, and as a psycho-physical regenerator. [295, 303] Traditionally *Centella asciatica* also has been used as a constituent of a brain tonic for the mentally challenged and as an anti-convulsant. [304]

*Centella asciatica* also has considerable anti-tumour properties and showed 100% cytotoxicity to two tumour cell lines (Dalton's ascites tumour cells and Ehrlich ascites tumour cells) after a 3 hour incubation at 37ºC. [305] The acetone fraction of *Centella asciatica* extract, a partially purified fraction (3.5 and 8

μg/mL), inhibited the proliferation of mouse lung fibroblast cells after exposure for 6-7 days at 37°C. The authors suggested it also stimulates the immune system and may involve inhibition of DNA synthesis .[305]

Regarding the mucosal lining, oral administration of *Centella asciatica* water extract and asiaticoside reduced the size of acetic acid-induced gastric ulcers in rats at 3 and 7 days in a dose-dependent manner with concomitant attenuation of myeloperoxidase activity in the ulcer tissues. [306] Cell proliferation and angiogenesis were promoted in this study. *Centella asciatica* also protected the gastric mucosa by improving the integrity of the mucosal lining. This may be due to a reduction of myeloperoxidase, a decrease in the recruitment of neutrophils, or to its free radical scavenging activity. [307]

***Glycyrrhiza glabra*** This compound is probably the most outstanding herbal remedy for mouth sores. *Glycyrrhiza glabra* is licorice root and it is a potent anti-inflammatory agent and tissue healer. In one pharmacological study, the anti-inflammatory effects of *Glycyrrhiza glabra* exceeded hydrocortisone and amidopyrine.

Regarding the mucosa, a biochemical and histochemical study done on rats with *Glycyrrhiza glabra* and *Terminalia chebula* showed improved secretory effects on Brunner's gland and subsequent excellent protection against duodenal ulcer. [308] Flavanoids of *Glycyrrhiza glabra* showed anti-H Pylori effects and may be useful as a chemo-protective agent for peptic ulcer and gastric ulcer. [309]

*Glycyrrhiza glabra* also has a wide range of anti-viral effects with in-vitro studies showing anti-viral activity against HIV-1, SARS related coronavirus, respiratory syncytial virus, arboviruses, vaccinia virus, and vesicular stomatitis virus. *Glycyrrhiza glabra* also reduced hepatocellular damage in chronic Hepatitis B and C and reduced the risk of hepatocellular carcinoma in hepatitis C induced cirrhosis. Reduction in mortality from herpes simplex virus encephalitis and

influenza A virus pneumonia [310] and potent anti-viral activity against Japanese encephalitis virus [311] also have been shown.

*Glycyrrhiza glabra* has known activity against both gram-positive and gram-negative bacteria. [312] Glabridin, an active constituent of *Glycyrrhiza glabra* root, was found to be active against both yeast and filamentous fungi. [313, 314, 315] It may also have effective anti-microbial activity against MRSA. [316]

In-vitro studies also showed *Glycyrrhiza glabra* to be protective against gamma radiation [317] and to protect mitochondrial function against oxidative stresses. [318] It also had anti-oxidant activity with free radicals scavenging effects in another study showing higher anti-oxidant activity compared to commercial anti-oxidants. [319, 320] *Glycyrrhiza glabra* also exhibited free radical scavenging activity, metal chelation and antioxidant power in another study.[321] *Glycyrrhiza glabra* also potentiated anti-tumour and anti-metastatic effects of cisplatin. [306]

***Emblica officinalis, Terminalia bellerica, and Terminalia chebula*** These three berries have been used in *Ayurveda* as an anti-oxidant trio for many diseases.

***Emblica officinalis*** has been shown to have a major role in mucin protection and regeneration through its effects in healing stomach ulcers due to nonsteroidal anti-inflammatory drugs.[322] In a series of studies, acute gastric ulcer models induced in rats by aspirin, ethanol, cold restraint stress, pyloric ligation, and acetic acid in rats, showed that *Emblica officinalis* had significant ulcer protective and healing effects. Effectiveness was attributed to enhancing both offensive and defensive mucosal factors.[323]

In another study, oral administration of *Emblica officinalis* at doses 250 mg/kg and 500 mg/kg significantly inhibited the development of gastric lesions, decreased pyloric-ligation induced basal gastric secretion, and titratable acidity and gastric mucosal injury. It also was protective against ethanol-induced depletion of

stomach wall mucus and reduction in non-protein sulfhydryl concentration. Histopathological analyses have concurred with pharmacological and biochemical findings. [324]

Two independent studies showed *Emblica officinalis* also exhibited anti-oxidant hepatoprotective properties and also offered protection against chemical carcinogenesis. [325,326] In addition, *Emblica officinalis* also showed considerable anti-proliferative activities for in-vitro tumour cell line growth and protection against cytotoxic effects of arsenic.[327, 328] It also demonstrated free-radical scavenging activity. [329,330] *Emblica officinalis* also has some reported antimicrobial activity.

***Terminalia chebula*** This compound has multiple effects. In the GI tract this compound has been shown to improve gastrointestinal motility [331], to have short- and long-term anti-diabetic activity in rats, [332] and to have activity against helicobacter pylori. [333]

*Terminalia belerica* also has anti-cholinergic effects which supports its use in hyperactive gastrointestinal disorders in folklore medicine.[334]

*Terminalia chebula* has been shown to be effective against cancer cells [335] and to be protection against Fe-NTA-induced renal carcinogenesis and oxidative damage. [336] *Terminalia belerica* was also found to have anti-oxidant properties[337]. One recent study provided preliminary data that *Terminalia belerica* may be synergistic with doxoruicine and cisplantin as a growth inhibitory agent. [338] There may also be a role for *Terminalia belerica* in dermal wound healing. [339] In high concentrations *Terminalia belerica* inhibited salmonella activity. [340]

***Triphala*** Is an Ayurvedic formulation composed of *Terminalia chebula, Terminalia belerica and Emblica officinalis*. Its enteroprotective effects in equal formulation (1:1:1) and unequal formulation (1:2:4) were studied and the results

suggested that the unequal formulation provided significantly more protection than the equal formulation against methotrexate-induced damage in rat intestine. [341] As a group, all three herbs have been reported to have antioxidant and free radical scavenging activities [342,343,344] and to have considerable protective effectives against indomethacin-induced stomach ulceration when compared with misoprostol.

Other formulations with multiple compounds also have demonstrated effectiveness. For example, *Acacia catechu, Glycyrrhiza glabra, and Terminalia Chebula* showed high anti-oxidant activity in one study [345] and A*zadirachta indica, Terminalia chebula*, and *Terminalia bellerica* showed some cancer curative properties.[346] Major effects for each compound are summarized in Table 2.4.

**Table 2.4 Summary of active effects of seven identified compounds**

| | |
|---|---|
| ***Acacia catechu*** | Gram positive cocci, gram negative bacilli; 112 strains staphylococcus aureus, 112 strains Staphylococcus epidermidis, 28 strains Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli, Salmonella, Candidata, Aspergillus niger, antifungal, dual inhibition COX 1 and COX 2 |
| ***Azadirachta indica*** | Anti-ulcer, ulcer healing, anti-bacterial, 21 strains food-borne pathogens, fungicidal, chemoprotective effects, prevents leukocyte apoptosis, streptococcus mutans |
| ***Centella asciatica*** | Proliferation of granulation tissue, non-ulcer skin wounds, necrosis, collagen formation, leprosy, anti-tumour, acetic-acid induced gastric lesion |
| ***Glycyrrhiza glabra*** | Chemoprotective agent for peptic and gastric ulcer, anti-viral vesicular stomatitis virus, yeast, filamentous fungi, gram positive and negative in vitro protection of gamma radiation, mitochondrial protection against gastric lesions |
| ***Emblica officinalis*** | Ulcer protection and healing, defensive mucosal factor, mucin protection, healing of NSAID stomach ulcers, radical scavenging activity |
| ***Terminalia bellerica*** | Anti-salmonella, hyperactive gastrointestinal disorders, enteroprotective |
| ***Terminalia chebula*** | Dermal wound healing, improved gastrointestinal motility, anti-caries, increase pH and buffering |

The potential therapeutic role for mucositis for each of these herbs was ascertained from the literature and the possible location of action(s) along the complex biological cascade of mucositis was considered. The herbs were found to act on multiple targets and have multiple anti-inflammatory properties; such as inhibiting pro-inflammatory cytokines such as interleukin 2, COX 1 and 2, interleukin-1 beta, interleukin 6, tumor necrosis factor, interleukin 1, and nuclear factor kappa-B. In addition, some herbs also potentiated keratinocyte growth factor and were excellent anti-oxidants. The Ayurvedic knowledge and literature review suggest that these herbs would have action against radiation-induced mucositis at multiple targets and would be capable of inhibiting multiple pathways. These are summarized in Table 2.5.

**Table 2.5 Array of anti-inflammatory effects of the seven identified compounds**

| | Acacia Catechu | Azadiracta Indica | Glycyrrhiza glabra | Centella asiatica | Embilica officinalis | Terminalia Chebula | Terminalia bellarica |
|---|---|---|---|---|---|---|---|
| Anti-inflammatory | √ | √ | √ | √ | √ | √ | √ |
| IL2/6/10 inhibition | √ | √ | √ | √ | √ | √ | √ |
| COX 1 and2 Inhibition | √ | √ | √ | √ | √ | √ | √ |
| DNA Protection | √ | √ | √ | √ | √ | √ | √ |
| Free Radical Scavenging | √ | √ | √ | √ | √ | √ | √ |
| TNF alpha Inhibition | √ | √ | √ | √ | √ | √ | √ |
| Anti-Oxidant | √ | √ | √ | √ | √ | √ | √ |
| NF-kB Inhibition | √ | √ | √ | √ | √ | √ | √ |
| Cyclooxygenase Inhibition | √ | √ | √ | √ | √ | √ | √ |
| Interferon gamma inhibition | √ | √ | √ | √ | √ | √ | √ |
| ROS neutralization | √ | √ | √ | √ | √ | √ | √ |
| Immuno-Modulator | √ | √ | √ | √ | √ | √ | √ |

***Location of Action of the Seven Identified Compounds***

These seven compounds potentially have multiple sites of action along the inflammatory pathways associated with mucositis.

***Acacia catechu*** This compound was found to have dual inhibitory activity of COX-2 and 5-LOX (5-lipoxygenase). [347] A study that assessed flavacoxid from *Acacia catechu* found that this substance affected COX-2, 5-LOX, tumour necrosis

factor-α (TNF-α), interleukin (IL)-6, IL-10, extracellular-regulated-kinase 1/2 (ERK), JunN-terminal kinase (JNK), NF-κB, and β-arrestin 2 protein expression in mice in a positive way. Flavacoxid also inhibited the mitogen-activated protein kinases (MAPKs) pathway, preserved β-arrestin 2 expression, reduced blood $LTB_4$, $PGE_2$, TNF-α and IL-6, and increased IL-10 and lipoxin $A_4$ serum levels. Treatment with flavocoxid also was found to be protective against the histologic damage induced by CLP, and to reduce the myeloperoxidase (MPO) activity in the lung and liver. [348]

***Azadiracta indica*** This compound was found to have effects on IL-12 production by mediating activation of the P38/MAPK pathway and the ERK1/2 signalling pathway which influences interferon gamma and tumour necrosis factor alpha. In addition this compound has effects on natural killer cell-mediated cytotoxicity of tumour cells associated with CD40–CD40L-mediated endogenous production of interleukin-12.[349] *Azadiracta indica* also down regulates interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α). Nimbolide present in *Azadiracta indica* down regulates IGF-1 (insulin like growth factor 1), proliferating cell nuclear antigen, phosphoinositide 3-kinase, nuclear factor kappa B, extracellular-signal-regulated kinase, Ras, Raf-1, IkB kinase, IKK epidermal growth factor receptor, and matrix metalloproteinases.[350-352]

***Glyccyrrhiza glabra*** This compound inhibits TNF-alpha activated JNK/c-Jun and IκB/NF-κB signaling pathways without affecting extracellular signal-regulated kinase 1/2 and p38. Glyccyrrhiza glabra blocks ERK1/2 activation and inhibits expression and activation of matrix metalloproteinases and the phosphorylation of ERK1/2 and JNK1/2. These inhibitory effects are associated with an up regulation of tissue inhibitor of metalloproteinase-1 and a down regulation of the transcription factors NF-κB and activator protein 1 signalling

pathways. *Glyccyrrhiza glabra* also inhibit migration and invasion of cancer cells by reducing the expression of the P13K/AKT signalling pathway.[353-355]

***Centella asiatica*** Substances in this compound, such as madecassoside attenuate phosphorylation and inhibit p38 MAPK and phosphatidylinositol-3-kinase (PI3K)/AKT signalling. Centella asiatica prevents lipopolysaccharide induced nuclear factor-kappa B (NF-kappaB) translocation from the cytoplasm into the nucleus, and also inhibits phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38. Other *Centella asiatica* substances, such as asiatic acid and asiaticocide, also inhibit lipopolysaccharide induced nitric oxide and prostaglandin E2, as well as inhibit inducible nitric oxide synthase and cyclooxygenase-2. There is a dose-dependent response for asiatic acid and reduction of interleukin-6, IL-1β, interleukin-1β, and TNF-α. [356-357]

***Emblica officinalis*** This compound effectively inhibits UVB-induced photo-aging in human skin fibroblast via its strong ROS scavenging ability.[358] *Emblica officinalis* extracts or purified components also inhibit NF-kappa B. One substance, Quercetin, has been found to prevent epidermal growth factor receptor-induced EMT via the EGFR/PI3k/Akt/ERK1/2 pathway and by suppressing transcriptional repressors.[359-361] Smad 3 inhibition also is exhibited. [362]

***Terminalia bellerica*** This compound shows VGEF reduction which helps in tumour growth inhibition. *T bellerica* was found to down regulate the p13/Akt signalling pathway. Terminalia bellerica effectively inhibits NF-kB/DNA interactions and reduces TNF-alpha and pro-inflammatory IL-8 expression.[363,364]

***Terminalia chebula*** This compound suppresses migration, proliferation and inflammatory mediator production in macrophages. It also reduces nitric oxide

production, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) expression. *Terminalia chebula* has inhibitory effects on metalloproteinase-9 (MMP-9) expression and down regulation of mitogen activated protein kinases (MAPKs) signalling molecules. Smad 3 inhibition also is exhibited.[365] *Terminalia chebula* has also been shown to inhibit NFkB, COX-1, COX-2, 5-LOX, and TNF-alpha. Leutolin found in chebula inhibits Iκ-B-α degradation and subsequently inhibits nuclear factor-κB p65 translocation to the nucleus. In addition, luteolin blocks the phosphorylation of ERK1/2, c-Jun N-terminal kinases, and p38 mitogen-activated protein kinases (MAPKs).[366-368]

***Emblica officinalis, Terminalia chebula,*** and ***Terminalia bellerica*** in combination prevent production of free radicles and decrease lipid peroxidation. These three berries in combination also inhibit pro-inflammatory markers IL-1b, IL-6, TNF-a, VGEF, prostaglandin E2, and COX-2 by blocking the NF-kB mediated inflammatory pathway. [365]

**Toxicity**

With respect to toxicity, none of these 7 compounds has reported toxicity. With respect to adverse effects, only 3 studies have been reported so far: *Centella asiatica* – no side effects [369] and sedation with chronic administration at high doses [370]; and *Azadiracta indica* - infertility at high doses [371]

**DISCUSSION**

Mucositis occurs as a result of a complex cascade of biological events with multiple signalling pathways involving multiple molecules and cytokines. The understanding of these mechanisms is in the early phases; however, research to date has already elucidated complex biological pathways and identified numerous potential therapeutic targets. Overall, it is now known that mucositis develops as

a consequence of related and interacting biologic events, culminating in injury and apoptosis of basal epithelial cells and resulting in the loss of epithelial renewal and in the subsequent development of ulcerations and atrophy. It is also known that patient factors, such as age, nutritional status, and genetic predisposition play important roles in the development of mucositis.

It also seems apparent that while it is promising that multiple pathways and potential therapeutic targets have been identified, blocking all these pathways might be required to inhibit mucositis. In particular, therapeutic agents should have the following properties: have direct and indirect cytoprotective activity; have anti-bacterial and anti-fungal activity; prevent xerostomia; and foster saliva production and pH balance.

In this review bio-prospecting was done using traditional Ayurvedic knowledge and practices as the discovery engine because this is cost effective and potentially more effective compared to random screening methods. Seven potentially useful plants were identified with scientific evidence collated from the literature and supported by Ayurvedic texts that cite their use in mouthwashes. Our review showed a combination of *Acacia catechu, Azadiracta indica, Glycyrrhiza glabra, Centella asiatica, Emblica officinalis, Terminalia bellerica,* and *Terminalia chebula* might be useful to inhibit and moderate radiation-induced mucositis. The Ayurvedic pharmacopeia also is a resource for formulation and preparation of mouthwashes. In the traditional method, herbs undergo decoction, a method of mashing then boiling to retrieve dissolved chemicals from herbal or plant material. The products are then standardised by weight and volume and added to oil and ground herbal paste to formulate a mouthwash. The method permits extraction of water soluble and fat soluble particles and combining them in the same preparation.

A particular advantage of this method is that multiple compounds are included in a formulation. Isolated compounds from plants do not necessarily

exhibit the same properties or activity as unrefined extracts at comparable concentrations of the active component. In addition, other compounds in plants play an important role in facilitation, absorption, assimilation and utilization of the active ingredient. Other compounds also play a major role in providing synergetic action, especially when multiple plants are used in the same preparation. Additional benefits include potential inhibition of multi-drug resistance and decreased cost, as isolates are often expensive and unavailable in bulk quantities compared to whole plant extracts which can be produced locally.

Ayurvedic preparations also are not typically associated with side effects, as they have been used widely and daily for centuries. For example, traditional people from Kerala, a southern state of India, drink water boiled with *Acacia catechu* daily; *Azadiracta indica* is used internally and externally as an antibiotic and antifungal agent; *Glycyrrhiza glabra* is chewed as an adjuvant therapy in diabetes and is given to infants as a fungicide; *Centalla asiatica* is used as a nerve tonic in children and the elderly; *Emblica officinalis* is used in cooking and as a berry; and *Terminalica belerica* and *Terminalia chebula* are used daily to aid digestion. All seven herbs are also used as adjuvants to synthetic ingredients in different mouthwashes currently commercially available on the market. Specifically, the following parts of the plant are used: the dry stems of *Acacia catechu* and *Azadiracta indica*; the dry root of *Glycyrrhiza glabra*; the wet whole plant of *Centella asiatica*; and the dry fruit without the seed of *Emblica officinalis, Terminalia belerica,* and *Terminalia chebula*.

In summary, the consequences of mucositis in the treatment for head and neck cancers are broad and include radiation dose reduction, temporal interruption in radiation protocols, reliance in parenteral nutrition, administration of narcotics, hospitalization and morbidity. Despite its frequency, symptomatic impact, and health and economic costs, there are no effective interventions for radiation-induced mucositis. In this review we propose a mouthwash that is based on

traditional Ayurvedic knowledge and supported by scientific data that has the potential to ameliorate this adverse effect of cancer treatment. Given that the pathobiology of mucositis is not completely understood and that therapies targeted at a single site most likely will continue to be ineffective, our multi-targeted approach offers a plausible alternative. The study also supports a paradigm shift in the drug discovery mechanism in that drug development need not always be confined to new molecular entities. Instead, bio-prospecting of plants utilized by ancient knowledge and gained from indigenous medicines may provide a new strategy for drug development.

APPENDIX 2

**Text of Ayurvedic Medicine**
**Charak Samhitha**
This is one of the oldest classic text of Ayurveda. The compendium by Charak is believed to have been written around 400-600 BC; there are some claims it dates to much earlier (5000 years old). It has 120 chapters with 8 sections:
*Sūtra* (general principles) - 30 chapters deal with healthy living, collections of drugs and their uses, remedies, diet and duties of a physician.
*Nidāna* (pathology) - 8 chapters discuss the pathology of eight chief diseases.
*Vimāna* (specific determination) - 8 chapters address pathology, various diagnostic tools, medical studies and conduct.
*Śārīra* (anatomy) - 8 chapters describe embryology and anatomy of the human body.
*Indriya* (sensorial prognosis) - 12 chapters elaborate on diagnosis and prognosis of diseases of the basic senses.
*Cikitsā* (therapeutics) - 30 chapters deal with special therapy.
*Kalpa* (pharmaceutics and toxicology) - 12 chapters describe usage and preparation of medicine.
*Siddhi* (success in treatment) - 12 chapters describe general principles of 'Panchkarma' (detoxification).
A rational approach to causation and cure of disease and introduction to objective methods of clinical examination are unique scientific contributions made by the author.

**Sushruta Samhita**
This text was written during the 3rd or 4th century BC. It is composed of 184 chapters describing 1,120 illnesses, 700 medicinal plants, and 64 preparations based on mineral sources and 57 preparations based on animal sources. The text also discusses in detail surgical techniques including incision, probing, extraction of foreign bodies, hernia repair, alkali and thermal cautery, tooth extraction, caesarean section, haemorrhoid and fistula management, laparotomy, management of perforated intestine, fracture types and management, traction, manipulation, apposition, stabilization and fitting of prosthesis, and cataract surgery.

**Ashtanga Samgraha**
This text is an encyclopedic compendium of all the eight specialized branches of Ayurveda. Vegbhata, who flourished in the 7th century AD, is credited as having composed this text. It is written in prose and has details of the 8 sections of Ayurveda: internal medicine, surgery, gynaecology and paediatrics, rejuvenation therapy, aphrodisiac therapy, toxicology, psychiatry or spiritual healing, and ENT (ear, nose and throat). There are subsections on longevity, personal hygiene, causes of illness, influences of season and time on the human organism, types and classifications of medicine, the significance of the sense of taste, pregnancy and possible complications during birth, individual constitutions, and various aids for establishing a prognosis. There is also detailed information on five action-therapies (*pañcakarma*) including therapeutically induced vomiting, use of laxatives, enemas, and complications that might occur during such therapies and necessary medications.

**Ashtanga Hridaya**
This text was written in the 7th century AD and the translation of its name is Heart of Medicine. The text is written in a poetic manner and contains approximately 7120 poetic verses focusing on the 8 sections of Ayurveda and surgery. Social and preventive medicine also are included.

**Sharangdhara Samhita**
This text has 32 chapters and 2600 verses and can be broadly divided into the following 3 sections: 1) weights, measurement and time and place to collect herbs, and anatomy, physiology and diagnostics; 2) different herbal preparations, methods of preparation, precautions and purification of herbs and minerals; and 3) cleansing techniques, detoxification and treatment methods.

**Chakradattam**
This text was written in 1060 AD and contains extensive Ayurvedic formulations and is a comprehensive text on pharmacology. The purification of many minerals is described. In addition, cost effectiveness and simple single drug remedies are addressed.

**Sahasra Yogum**
This text is considered to be the 'practical prescriber' with more than 1000 Ayurvedic poly- herbal formulations and home remedies described.

**Yogamrutham**
This text provides information about different herbal and mineral formulations and their uses.

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