FACULDADE DE ODONTOLOGIA

APLICAÇÃO TÓPICA DE *ALOE VERA* E VITAMINA E EM ÚLCERAS INDUZIDAS NA LÍNGUA DE RATAS SUBMETIDAS À RADIOTERAPIA: AVALIAÇÃO CLÍNICA E HISTOLÓGICA

LETÍCIA DE FREITAS CUBA

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PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
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Orientadora: Maria Antonia Zancanaro de Figueiredo

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PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
Programa de Pós-graduação em Odontologia

Aplicação tópica de A*loe vera* e vitamina E em úlceras induzidas na língua de ratas submetidas à radioterapia: Avaliação clínica e histológica

Linha de Pesquisa: Enfermidades da Região Bucomaxilofacial: estudos clínicos, imunológicos e anatomopatológicos

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“Bom mesmo é ir à luta com determinação,abraçar a vida com paixão,

perder com classe e vencer com ousadia,

porque o mundo pertence a quem se atreve”

Augusto Branco

LISTA DE ABREVIATURAS

NMCP	Neoplasias malignas de cabeça e pescoço
HNC	Head and neck cancer
RT	Radioterapia/ Radiotherapy
QT	Quimioterapia
CT	Chemotherapy
MO	Mucosite oral
OM	Oral mucositis
EO	Estresse oxidativo
OS	Oxidative stress
ROS	Espécies reativas de oxigênio/ Reactive oxygen species
AOX	Antioxidantes/ Antioxidants
VE	Vitamina E
AV	Aloe vera
RL	Radicais livres
FR	Free radicals

RESUMO

RESUMO

As neoplasias malignas de cabeça e pescoço (NMCP) representam um problema de saúde pública mundial. Os carcinomas espinocelulares constituem cerca de 80% dessas neoplasias e tem como principais modalidades de tratamento a cirurgia, a radioterapia (RT) e a quimioterapia (QT) que podem ser utilizadas de forma isolada ou associadas. A RT age localmente destruindo as células tumorais, no entanto, não é seletiva, atingindo também células saudáveis de rápida renovação como as das glândulas salivares, mucosa oral e pele. Em consequência ao dano às células normais esta terapia gera efeitos deletérios importantes como, por exemplo, a mucosite oral (MO). Esta condição caracteriza-se pela presença de ulcerações dolorosas que podem evoluir para quadros tão graves que comprometam o curso do tratamento oncológico. Associada a etiologia da MO está o estresse oxidativo (EO) gerado pela RT, que seria capaz de induzir a produção de espécies reativas de oxigênio (ROS) responsáveis pelo dano celular e iniciação das lesões. Para limitar o EO existem substâncias chamadas antioxidantes (AOX), que tem como principal função eliminar as ROS. Estas podem ser produzidas naturalmente pelo organismo e também adquiridas através da dieta e suplementos vitamínicos. O objetivo deste estudo foi testar 2 tipos de antioxidantes, vitamina E(VE) e *Aloe vera*(AV), na prevenção e manejo da MO induzida por radiação em modelo murino, através da análise clínica e histológica Os animais foram divididos randomicamente em 2 grupos com 12 animais cada (VE 400mg; AV 70%) e 1 grupo com 11 animais (controle) e em 2 tempos experimentais (5 e 7 dias). Irradiou-se cada grupo com dose única de 30Gy e após 24h produziu-se uma lesão ulcerada no ventre lingual de cada animal medindo 6mm de comprimento e 3mm de largura. Os produtos estudados foram aplicados diariamente em seu respectivo grupo até a eutanásia programada. Durante a avaliação clínica, foram observados a presença de sinais inflamatórios, presença ou ausência da úlcera induzida e mensurado o tamanho das mesmas. Nesta fase do experimento foi possível constatar que as lesões foram mais frequentes nos animais dos grupos controle em ambos os tempos. O tamanho das úlceras foi maior nos grupos controle em comparação com os grupos VE e AV (5 dias: *p=0,006;* 7 dias*: p=0,002).* Na análise microscópica o grau de inflamação diferiu tanto nos grupos de estudo quanto nos tempos experimentais. Em 5 dias a diferença entre os grupos não foi estatisticamente significante. Já em 7 dias os animais do grupo controle apresentaram inflamação intensa, enquanto os que receberam VE e AV variaram entre leve e moderada (*p=0,002).* Diante da gravidade das lesões de MO e suas implicações ao paciente, é de suma importância que se encontre alternativas terapêuticas para prevenir ou amenizar suas manifestações clínicas. Os resultados deste estudo sugerem que os AOX presentes na VE e

no AV podem favorecer a redução da intensidade do processo inflamatório envolvido na MO, bem como a severidade das lesões.

Palavras chave: estomatologia; radioterapia; mucosite oral; antioxidantes; ratos.

ABSTRACT

ABSTRACT

Head and neck cancer (HNC) has been a worldwide public health problem. Squamous cell carcinomas account for about 80% of these neoplasms and the main treatment modalities are surgery, radiotherapy (RT), chemotherapy (CT) or a combination between them. RT acts locally destroying tumor cells. However, this therapy is not selective and also affects rapid renewal cell groups such as the salivary glands, oral mucosa and skin, resulting in a wide range of deleterious effects, being oral mucositis (OM) one of these. This condition is characterized by painful ulcerations that can progress to severe conditions that compromise the course of cancer treatment. The etiology of OM is oxidative stress (OS) generated by RT, that would be able to induce the production of reactive oxygen species (ROS) responsible for cellular damage and initiation of injuries. Antioxidants (AOX) are agents produced by humans or acquired by diet and vitamin supplements that eliminate ROS and minimize OS. The objective of this study was to assess the effect of 2 types of antioxidants, vitamin E (VE) and *Aloe vera* (AV) in prevention and management of radioinduced OM in a murine model by clinical and histological analysis. The animals were randomly divided into 3 groups of 12 animals each (400 mg VE, 70% AV and control) and 2 time periods (5 and 7 days). They were irradiated with a single dose of 30 Gy, and after 24h, a lesion was produced on the ventral tongue of each animal. The products were applied daily in their respective group until euthanasia. On clinical evaluation, it was observed the presence of inflammatory signs, presence or absence of induced ulcer and measurement of their size. Lesions were present more frequently in the control group animals in both periods of observation. The size of the ulcers was greater in the control group compared with the groups AV and VE (5 days: $p = 0.006$; 7 days: $p = 0.002$). Under microscopic analysis, the degree of inflammation differed between the study groups and experimental periods. At 5 days, the statistical difference was not significant amongst groups. However, after 7 day period, the animals in the control group displayed intense inflammation, while those in groups VE and AV exhibited mild to moderate inflammation ($p = 0.002$). Given the severity of OM injuries and their implications to the patient, it urges the search of alternative therapies to prevent or reduce clinical manifestations. The results of this study suggest that VE and AV may contribute to minimize inflammatory response and improve the healing of induced tongue lesions of rats submitted to radiation.

Keywords: stomatology; radiotherapy; oral mucositis; antioxidants; mice.

SUMÁRIO

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1. INTRODUÇÃO

O Brasil vem sofrendo expressivas mudanças no seu perfil demográfico, especialmente em virtude do processo de envelhecimento da população. Isto decorre, entre outros fatores, da urbanização, industrialização e dos avanços da ciência e tecnologia. Este fenômeno trouxe uma importante alteração no perfil de morbimortalidade do país, diminuindo a ocorrência das doenças infectocontagiosas e colocando as doenças crônico-degenerativas, como o câncer, no novo centro de atenção dos problemas de doença e morte da população brasileira (INCA, 2014).

As NMCP também fazem parte deste grupo de doenças ao qual a população está mais suscetível. Vincula-se a isso, não apenas o envelhecimento populacional e aumento da expectativa de vida, mas a prática de hábitos nocivos à saúde como o consumo de tabaco e álcool, exposição ao HPV e radiação solar (BHIDE, NUTING, 2010). Estima-se que no ano de 2014 ocorram 11.280 casos novos de câncer da cavidade oral em homens e 4.010 em mulheres no Brasil (INCA, 2014).

Dentre as modalidades terapêuticas usadas no manejo das NMCP destaca-se a radioterapia, amplamente utilizada de forma exclusiva ou concomitante a cirurgia e quimioterapia. A radiação age direta ou indiretamente na estrutura do DNA interferindo na sua duplicação. No entanto, não é seletiva às células tumorais, atingindo também os tecidos saudáveis da mucosa oral, sendo capaz de gerar efeitos deletérios que podem causar diversas complicações na área anatômica inserida no portal de radiação (DEBONI *et al*, 2012).

O termo mucosite surgiu em 1980 para descrever reações inflamatórias na mucosa bucal de pacientes submetidos à radio ou quimioterapia (PETERSON, CARRIELLO, 2004). A MO é o efeito agudo de maior frequência durante a radioterapia na região da cabeça e do pescoço. Hoje é considerada a mais severa complicação não hematológica da terapia do câncer, ocorrendo em praticamente todos os pacientes submetidos a esta modalidade terapêutica na referida região (TOLENTINO *et al*, 2011; BEY*et al*, 2012).

Caracteriza-se por eritema, seguido de ulcerações dolorosas na mucosa bucal que interferem no estado nutricional e na qualidade de vida dos pacientes, podendo até mesmo limitar ou interromper a terapia oncológica. Sua evolução é complexa, uma vez que é influenciada por outras complicações, como xerostomia, disgeusia, odinofagia e infecções oportunistas como a candidíase. Além disso, representa um fator de risco para infecções sistêmicas que podem requerer antibioticoterapia pesada e até mesmo

internação hospitalar, elevando significativamente os custos do tratamento (CITRIN *et al*, 2010; TOLENTINO *et al*, 2011; DEBONI *et al*, 2012).

Há evidências consideráveis de que os efeitos citotóxicos da radiação ionizante são decorrentes das reações físico-químicas que levam à produção de radicais livres (RL). Estes compostos são espécies reativas com um ou mais elétrons não pareados em sua última camada eletrônica, o que o torna altamente instável. Estas moléculas buscam estabilidade em outros elementos, como proteínas, lipídios e DNA causando a desestruturação das mesmas, gerando reações em cadeia que culminarão no que chamamos de EO. A formação dos RL e o consequente EO estariam fortemente associados a iniciação da MO (SONIS, 2004; SONIS *et al*, 2004; LALLA, SONIS, PETERSON, 2008; SONIS, 2010, AL DASOOQUI *et al*, 2013).

A mucosite tem sido foco de diversos estudos, pois sua prevenção e/ou tratamento efetivo permitiria doses terapêuticas mais agressivas para o tumor e provável aumento das taxas de sobrevida. Vários métodos são sugeridos na prevenção e manejo da MO, tais como manutenção da higiene bucal, uso de agentes antiinflamatórios, antimicrobianos, anestésicos tópicos, protetores de mucosa, laserterapia e fatores de crescimento, embora a maioria delas seja utilizada de forma paliativa (WORTHINGTON, CLARKSON, EDEN, 2006; WORTHINGTON *et al*, 2011; BEY *et al*, 2012; EPSTEIN *et al*, 2012).

Os consistentes relatos vinculados a formação de ROS pós exposição à RT, somados aos resultados de diversos estudos, demonstraram que as lesões de mucosa podem ser atenuadas e até mesmo prevenidas por agentes capazes de limitar o EO causado pelos RL. Os AOX são substâncias que atuam neutralizando as ROS através do bloqueio da sua formação ou eliminando-as do organismo (SONIS *et al*, 2004; CITRIN *et al*, 2010; URBAIN *et al*, 2012).

O Alfa-tocoferol é o principal constituinte da vitamina E sendo o mais importante antioxidante natural presente no organismo humano. Sua fundamental função é inativar os RL da membrana celular e, portanto, a VE pode ser considerada como um potencial protetor da mucosa (WADLEIGH *et al*, 1992; FELEMOVICIUS *et al*, 1995; MANZI *et al*, 2003; FERREIRA *et al*, 2004).

O AV é uma planta empiricamente utilizada como auxiliar na cicatrização de lesões ulceradas. Esta contém substâncias, como por exemplo os flavonoides, que são os principais constituintes polifenólicos do AV conferindo suas propriedades AOX. Estima-se que a quantidade de flavonoides presente nas folhas do AV seja de 0,24 a 0,34% e a capacidade antioxidante varie entre 85,7 a 94,9 µmol (TEAC)/g. Estudos *in vitro* e em animais sugerem que esta planta seja capaz de eliminar a produção de RL, influenciando

significativamente na iniciação e progressão de lesões inflamatórias orais (SU *et al*, 2004; AHMADI, 2012; VARONI *et al*, 2012).

As repercussões da MO radioinduzida na qualidade de vida dos pacientes, bem como as comorbidades vinculadas a esta patologia são capazes de, consequentemente, demandar modificações no plano de tratamento antineoplásico, assim como o uso de analgésicos, nutrição enteral ou parenteral, internação hospitalar e, até mesmo, a interrupção da terapia oncológica. Diante dos atuais achados em relação a patogenia da MO, estratégias de prevenção e manejo dos quadros clínicos dos portadores tem sido sugeridas. Porém, até o momento, poucos estudos demonstraram evidências científicas suficientes para recomendar diretrizes efetivas de tratamento. Levando em consideração a severidade da patologia em questão e suas implicações no paciente oncológico, a proposta deste estudo foi avaliar a resposta clínica e histológica do uso tópico da VE e do AV, na cicatrização de úlceras induzidas na língua de ratas submetidas à radioterapia.

2. ARTIGO I

O artigo a seguir intitula-se "**ANTIOXIDANT AGENTS: A FUTURE ALTERNATIVE APPROACH IN THE PREVENTION AND TREATMENT OF RADIOINDUCED ORAL MUCOSITIS?**" Foi aceito e publicado pelo periódico Alternative Therapies In Health and Medicine (Anexos A e B), o qual apresenta Fator de Impacto 1.143.

Antioxidant Agents: A Future Alternative Approach in the Prevention and Treatment of Radiation-induced Oral Mucositis?

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Running title: Antioxidant agents and oral mucositis

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ABSTRACT
Radiotherapy is a therapeutic modality frequently employed for patients with head and neck cancer (HNC). It destroys tumor cells, but it is not selective, also affecting healthy tissues and producing adverse effects. One that stands out is oral mucositis because of the morbidity that it is capable of causing. This lesion is characterized by the presence of erythema, ulcerations, pain, opportunistic infections, and weight loss. These side effects can lead to serious situations that require the interruption of the antineoplastic treatment and can result in hospitalization and even death. The complex mechanisms linked to the pathogenesis of oral mucositis were recently established, and since then, the control of oxidative stress (OS) has been tied to the prevention and management of this disease. The authors have carried out a review of the literature about the use of antioxidant agents in the prevention and treatment of radiation-induced oral mucositis, using the PUBMED database. This review has shown that the research on use of antioxidants (AOX) has proved insufficient to justify suggesting the products in treatment protocols. Results are promising, however, and AOX may represent a future alternative in the prevention and treatment of oral mucositis.

Key words: Head and neck cancer, ionizing radiation, oxidative stress, antioxidant, oral mucositis

Oral mucositis (OM) is one of the local effects most commonly found among patients subjected to radiotherapy (RT) in the head-and-neck region. It is a debilitating condition resulting from use of that therapeutic modality, resulting in cytotoxicity.[1-5]

Historically, it was believed that OM is an event triggered by the direct effect of radio and/or chemotherapy (CT) on the epithelial cells, consequently hampering their replication. Recently, it became possible to understand the true biological complexity of the pathogenesis of this disease.[6-8] Current studies indicate that lesions begin with damage to DNA bases and the formation of free radicals (FR).[9-11]

Consistent reports in various studies examining the link between mucositis and the formation of reactive oxygen species (ROS) after exposure to RT, have demonstrated that lesions of the mucosa can be attenuated and even prevented by agents capable of limiting the OS that it causes. Antioxidants (AOX) are substances that neutralize ROS by blocking their formation or eliminating them from the body.[6-8,10-12]

In view of the patterns of contemporary society and of the increase in life expectancy, it is estimated that a greater number of cases of chronic degenerative disease, and consequently, of head and neck cancer (HNC) will occur. Accordingly, strategies are needed that are aimed at preventing these lesions and/or attenuating the damage to healthy tissues that RT can cause.

RT is one of the therapeutic modalities most used in oncology, mainly for the elderly, whose frail bodies make surgery or CT not viable. Studies indicate that RT is one of the principal factors capable of negatively impacting the quality of life of patients with HNC.[13] The morbidity caused by OM makes it a principal object of study. Therefore, the authors conducted a review of the scientific literature covering the biological mechanisms of radiation-induced oral mucositis as well as the role of AOX in its prevention and treatment. Accordingly, the authors surveyed the literature related to this subject in the PUBMED database, using as a search criterion a requirement for complete articles published in English. They analyzed and selected current studies covering the pathogenesis, classification, clinical characteristics, and use of AOX as an alternative for the prevention and treatment of OM.

MUCOSITIS

RT of the head and neck is capable of inducing mucositis in the majority of the patients during receipt of that treatment alone or in combination with other therapeutic options. Episodes of severe OM occur in 30% to 70% of the patients undergoing RT, and when combined with CT, the incidence of these lesions tends to increase, varying from 50% to 100%.[2,4,14,15]

The first manifestations of OM are usually seen around the second week of RT. To control the deleterious effects of the treatment, the radiation doses are divided into daily fractions, ensuring a gradual course of therapy. With cumulative doses of approximately 15 Gy, erythema of the mucosa can be observed, which is considered the first sign of OM. In these cases, the patient may complain of a burning sensation when eating certain foods. On reaching doses of approximately 30 Gy, more severe cases can be observed, revealing the presence of irregular ulcerations that may be associated with the pseudomembrane and accompanied by pain and the inability to tolerate solid foods or liquids. The ulcerations usually persist for 2 to 4 weeks after the end of RT and spontaneously heal. The lesions typically involve the buccal mucosa, lips, ventrum, borders of the tongue, mouth floor, and soft palate. It is uncommon that keratinized mucosa is affected, such as the gingiva, dorsum of the tongue, and hard palate. When present in these areas, the lesions can be associated with an infection etiology.[4,15-17]

The pain present in the mouth is capable of limiting the intake of nutrients, fluids, and medications, resulting in weight loss, malnutrition, and a consequent need for enteral feeding. In addition, OM represents an increasing risk of systemic infections, such as pneumonia and candidiasis, that could require antibiotics, hospitalization, and

even the interruption of antineoplastic treatment. The symptoms of OM and its severe secondary complications cause a visible reduction in the quality of life of the patient. In addition, these complications can have a significant economic impact due to the costs associated with the control of pain and secondary infections, the use of dietary supplements, and requirement for parenteral or enteral nutrition as well as any necessary hospitalizations. Therefore, it is believed that the control of symptoms and other complications resulting from episodes of OM increases the costs of treatment, depending on the degree of mucositis presenting.[8,14,15]

To measure the severity of OM, a series of systems have been developed. However, the majority of them use clinical criteria and subjective information furnished by the patient, making it difficult to standardize the parameters. Currently, the most accepted classification is the one recommended by the World Health Organization (WHO), which combines evaluations of alterations in the mucosa, level of pain, and changes in functionality, measured by the ability to eat, in a single score. The ulcerations are characterized as absent or present, without considering their size. The severity of the lesions is determined by a score varying from zero to 4 (Table 1), where various factors can have an influence (Table 2).[2-4,16-19]

Table 1. Classification of OM according to WHO criteria

Severity	Characteristics
Degree 0:	No signs or symptoms
Degree 1:	Erythema and slight pain
Degree 2:	Presence of ulcers and pain, continued ability to eat
Degree 3:	Presence of ulcers and pain, inability to eat solid foods
Degree 4:	Presence of ulcers and inability to swallow, requirement for parenteral or enteral support

Abbreviations: OM, oral mucositis; WHO, World Health Organization

Table 2. Factors influencing development of radiation-induced OM[2-4,16-19]

Factors	Description
Patient	Advanced age, low body mass index (BMI), hyposialosis, poor oral-health conditions, loss of mucosal integrity, comorbidities, harmful habits—tobacco and alcohol, and genetic factors
Tumor	Type and degree of cell differentiation
Treatment	Type and dose of RT used, radiation portal, and concomitant use of other therapies

Abbreviations: OM, oral mucositis

BIOLOGICAL EVENTS OF OM

For many years, the pathogenesis of OM was based on the concept that the cytotoxicity of ionizing radiation acted in a nonspecific way, affecting rapidly growing cells, such as those of the oral mucosa, and consequently causing the loss of renewal capacity in those tissues.[3,6-8,15]

Lately, notable advances have occurred in the understanding of the biopathology of OM, not only as a result of its direct cellular damage but also as a series of complex biological events in the cells and tissues of the submucosa. Sonis et al described the development of OM in 5 phases.[6-8,14,15,20-22]

Phase 1. The main characteristic of the first is the formation of one type of ROS, free radicals (FR), which are unstable molecules because they have one or more unpaired electrons in their outer shells. This instability leads them to obtain the missing electrons from other molecules, such as DNA, RNA, and lipids and to reestablish stability by damaging molecules vital for cells. This phenomenon is called OS and plays a causative role in the initiation of mucositis.

Phase 2. These events lead to the second phase, known as the response to the primary damage, in which it is still not possible to observe any clinical alteration in the oral mucosa of the radiated patient. FR induce new DNA breaks, causing cell death, with marked intercellular signaling in the connective tissue, endothelium, and submucosal infiltrate. These mechanisms are capable of activating a series of transcription factors, of which NF-kB is probably the most studied. As a result of these reactions, a substantial number of genes are expressed, including those that control the production of pro-inflammatory cytokines. The increase in these proteins in the mucosa causes damage to the connective tissue and endothelium, reduction in epithelial oxygenation, and death of or damage to basal cells of the epithelium.

Phase 3. In the signaling-and-amplification phase, regulation of pro-inflammatory cytokines occurs, particularly of tumor necrosis factor-α (TNF- α), interleukin-β (IL-1β) and interleukin-6 (IL-6). These cytokines damage the cells of the mucosa and activate molecular pathways that worsen the lesions.

Phase 4. The fourth phase, the most clinically significant one, occurs in response to the chain of apoptotic events induced by RT. As a result, a thinning of the mucosa occurs, culminating in ulceration. This step is characterized by a rich inflammatory infiltrate that contains macrophages, neutrophils, and mastocytes. Bacterial colonization is also a common characteristic, which is markedly increased after the formation of ulcers. The colonizing bacteria are not quiescent, and the products released from the cell wall of these organisms can penetrate the mucosa, stimulating macrophages through pathways of the innate immune system to produce other pro-inflammatory cytokines.

Phase 5. In the final phase of mucositis, the process of wound healing occurs, with cell differentiation and tissue regrowth, with restored integrity. Epithelization begins at the margins of the healing wound of the ulcer and is generally completed within 4 weeks of the last dose of radiation.

Considering this theory, researchers have come to understand that radiotherapy in a fractionated regime results in the overlap of cellular events, which culminate in tissue damage. This model favors comprehension of the process and prompts further investigations of pharmacological treatments and identification of predictive biomarkers.[6-8,14,15,20,21]

ANTIOXIDANTS

Study of the risks and benefits of the use of antioxidant agents during antineoplastic therapy has been of utmost importance to oncologists, radiotherapists, and other healthcare professionals involved with this subject. Simone et al conducted a search of studies related to AOX supplementation in the Medline and Cancerlit databases from 1965 to 2003.[23,24]The researchers found 280 studies, including randomized clinical trials and observational studies. On analyzing these studies, the authors reported that supplementation with AOX reduced adverse effects and protected

normal tissues. Block et al carried out a systematic review of the literature in healthcare databases and found 845 studies involving AOX and antineoplastic therapy.[25]Of these studies, 19 filled rigorous inclusion criteria, and despite the studies' low statistical power, the researchers concluded that AOX supplementation increased the survival of patients as well as the response of the tumor to the therapy.

Even before the start of oncological treatment, the reduction in AOX levels and increase in OS can be detected in patients with cancer.[26] Sharma et al evaluated patients with carcinoma of the tongue and observed that the levels of plasma lipid peroxides, which are markers of OS, were significantly increased in patients with cancer when compared to controls ($P = 0.001$).[27] However, the levels of glutathione, superoxide dismutase, and vitamins C and E were reduced in these patients. The researchers concluded that an increase in the levels of OS markers and a decrease in AOX levels existed in the group with carcinoma of the tongue. In a review of the literature, Moos reported that RT caused a decrease in plasma AOX levels, in addition to inducing ROS production.[9] This finding reflects the body's lack of defense against OS, making it more susceptible to the cytotoxicity of the treatment. Moos concluded that indications exist for the use of AOX, aimed at reversing some of the adverse effects of the RT.

In this context, Urbain et al investigated the plasma AOX levels of patients receiving cancer chemotherapy, comparing them with the incidence and severity of mucositis episodes.[11] The researchers concluded that patients who showed normal levels of AOX tended not to need parenteral nutrition, considering that need to be a common marker for OM. Accordingly, an exhaustive discussion has occurred in oral medicine on the potential benefits of using AOX for the purpose of preventing and attenuating the deleterious effects of RT.

Despite its clinical and economic consequences, no effective therapy for OM has been developed. Some interventions are used successfully, although the majority in a palliative manner. Patients should be encouraged to maintain good oral hygiene and to use anti-inflammatory and antimicrobial agents, topical anesthetics, and protective agents for the mucosa. Low-intensity laser therapy and the administration of epithelial growth factors are also some available resources that can be considered in preventive interventions.[1,2,4,5,28]

Low-energy Laser

Although it is not a classic antioxidant, the low-energy laser is capable of reducing the formation of FR.[1,2,4,5,28] In addition, microscopic and molecular findings have demonstrated its ability to induce increased cell division, modification of nerve transmission, and tissue regeneration. It achieves these results by stimulating the growth of fibroblasts, which have an active role in epithelial repair and cytoprotection. These events should delay an increase in the latency of the lesions, attenuation of the severity of the peak of mucositis, a shorter duration for the ulcers, and relief of pain.

The efficacy of laser therapy is evident, but the establishment of standardized protocols for prevention and treatment presents a great obstacle, as demonstrated in studies.[2, 28] In addition, the procedure involved demands great complexity and cost, making it difficult for low-income patients to access.[28]

Epithelial Growth Factors

Epithelial growth factors have been widely investigated, demonstrating excellent results in the prevention of OM.[1,2,4,5,29] They are capable of stimulating cell proliferation, differentiation, and maturation. Palifermin is part of this group, and it is one of the substances currently most studied. However, despite promising results, its

clinical indication depends on further studies guaranteeing that the product does not stimulate tumor growth and that its cost is compatible with its use in treatment of patients.

Antioxidants

In view of the model proposed by Sonis et al, in which the release of ROS with consequent OS is considered the principal factor for activation of the numerous events responsible for the development of OM, control of ROS has been the subject of studies of chemoprotective intervention. Accordingly, AOX are potential agents for this aim, capable of impeding the formation of ROS and even eliminating them from the body.[2,3,11,12]

Many AOX and FR scavengers can limit OS. Superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase are some examples of AOX that are capable of naturally protecting against damage caused by FR. Antioxidant defense can also be provided by agents with a low molecular weight that are proton donors, such as ascorbic acid, tocopherols, polyphenols, and thiols. However, none of the AOX shows radioprotective potential. This dichotomy may be due to the reactivity of the ROS induced by radiation, compared with those generated under conditions of OS in general.[12]

Thus, thiols, such as amifostine; recently developed nitroxides; and well-known natural AOX, such as vitamin E, have been widely studied and considered as possible radioprotectors for the prevention of mucositis.[1,3,9,11,12]

ANTIOXIDANTS AGAINST OM

Amifostine

Amifostine (ethyol) is a potent, synthetic antioxidant that eliminates 3 types of FR: superoxide, hydroxyl, and lipoperoxyl. It is an analogue of cysteine and cysteamine and is classified as a phosphorylated aminothiol, which exerts its effects as a selective cytoprotective agent of normal tissues against the toxicity of antineoplastic treatments.[9,12,18]

Amifostine has been tested by various investigators for the prevention of OM. Bourhis et al administered 150 mg/m^2 of amifostine to patients with stage IV HNC at 15 to 30 minutes before each RT session.[30]Despite the small sample, the researchers observed a reduction in the severity and duration of OM. In another study, Antonadou et al evaluated 50 patients subjected to RT and CT for HNC, using doses of 300 mg/m^2 of amifostine.[31]The researchers concluded that the product was effective in reducing mucositis and dysphagia. However, in a study carried out by Haddad et al that evaluated the effects of amifostine for 58 patients with advanced-stage HNC who were receiving CT and RT, a dose of 500 mg did not appear to alter the occurrence of mucositis.[32]

Saavedra et al investigated the effects of amifostine on apoptosis that was induced by radiation in peripheral blood lymphocytes of patients with HNC.[33]The researchers concluded that a significant reduction in cellular apoptosis occurred after the administration of the drug. They suggested that this effect can depend on individual characteristics and that further studies were needed with larger groups of patients. Taken together, the results of these studies provided new information on the biological actions of amifostine in vivo.

The use of amifostine in the prevention of mucositis continues to be investigated. However, controlled, randomized clinical trials that have administered the drug by different routes showed divergent results.[3,18,29,34] The studies have not provided sufficient evidence to include the drug in the guidelines recommended by the American

Society of Clinical Oncology (ASCO) for the prevention of OM. In addition, the adverse effects common with its use should be considered, such as nausea, vomiting, hypotension, and allergic reactions.

Vitamin E

Alpha-tocopherol, the main constituent of vitamin E, is the most important natural antioxidant present in the human body, and its principal function is to eliminate FR. A variety of studies on vitamin E as a radioprotector have presented favorable results.[1,3,9,35]

The efficacy of vitamin E in the treatment of OM was evaluated by Wadleigh et al, and an experimental group was compared to a placebo group.[36] The researchers observed that the patients in the experimental group did not show adverse effects and had a more rapid resolution clinical features or lesions of OM, as shown, compared with the placebo group.

Vitamin E was also tested as a radioprotector of the intestinal mucosa of rats that received supplementation orally for 10 days and topically for 30 minutes before radiation, with a total dose of 1100 cGy. The results demonstrated tissue protection, reducing cases of enteritis due to radiation.[37]

In a clinical study by Ferreira et al, 54 patients with HNC, who were undergoing RT at doses varying from 50 to 70 Gy, were evaluated.[38]The investigators used mouthwash containing vitamin E and observed a reduction in the incidence and symptomatology of OM lesions. However, studies exist in the literature with results showing no beneficial effects in the prevention of OM with vitamin E supplementation.[10]

Polyphenols

For centuries, the use of plants and teas in the treatment of numerous diseases has been widely discussed. The protective role of diets rich in the polyphenols of fruits and vegetables in the prevention of some types of cancer and of chronic degenerative and inflammatory diseases is well-established and accepted by the scientific community. However, little is known about the role of these substances in the prevention of oral disease. Flavonoids are constituents of polyphenols and are present in various plants, such as aloe vera (AV), camomile and calendula, providing them with AOX properties.[39]

Studies examining the value of AV in the prevention and treatment of OM diverge in their results. Two phase 2 studies evaluated the efficacy of formulations based on AV in the management of OM and did not present positive results.[40,41] However, a review of the literature concerned with the antioxidant, anti-inflammatory, and wound-healing properties of the plant suggests that AV can be an alternative in the treatment of OM.[42]

Some studies based on the use of the tea of camomile flowers for the treatment of OM reported positive results. Despite the fact that these better designed, current experiments tested camomile mouthwash against OM from CT,it is believed that it is possible to extend the positive results to cases of radiation-induced OM, since OM from CT and RT show the same biopathology.[39,43,44]

The flowers of *Calendula officinalis* have also been included in some studies. Two used gel extracted from the flower, one treating OM in animals and the other in human patients, and both demonstrated that the gel was effective in reducing the intensity of thelesions and accelerating wound healing for radiation-induced OM.[45,46]

The use of substances extracted from plants, including polyphenols, is of extreme importance in the prevention of OM, mainly because of their antioxidant activities, which make them capable of countering OS, but also because of their low rate of adverse effects.[39]

Zinc

A deficiency of serum zinc in patients with HNC has been reported in various studies. Investigators have demonstrated that zinc is a cofactor for more than 300 enzymes and a structural constituent of many proteins, and it prevents the formation of FR. Ertekin et alconducted a randomized and placebo-controlled study that included 30 patients with HNC who were treated, on average, with 6400 cGy of radiation.[47] The patients in the experimental group received supplementation with 150 mg/day of zinc sulfate. The results indicated that zinc supplementation was beneficial, reducing the discomfort and severity of OM.

Another study investigated the use of a zinc-containing L-carnosine as a mouthwash for patients with HNC who were subjected to RT, either alone or in combination with CT.[48] Compared with the control group, the zinc group showed a lower incidence of OM, reduced pain, and a lower need for analgesics.

A broader study that was double-blinded and placebo-controlled also tested systemic supplementation with zinc for 100 patients who had HNC and who were subjected to RT or chemoradiotherapy.[49]The authors found that supplementation with zinc delayed the appearance the lesions and decreased the severity of the OM episodes.

In a recent systematic review of the literature conducted by the Mucositis Study Group of the Multinational Association of Supportive Care in Cancer, 99 publications evaluating the use of natural agents in the management of OM were analyzed.[50] Given the consistently positive results found in randomized clinical trials testing zinc, the researchers suggested that systemic supplementation with this substance, when it is administered orally, can be considered beneficial in the prevention of OM in patients with HNC who are undergoing RT alone or in combination with CT.

RK-0202

The promising results of the previously mentioned studies for the use of AOX, whether synthetic or natural, as an alternative in the prevention of OM, encourage the testing of new substances with antioxidant properties.

RK-0202 is a combination of the thiol antioxidant N-acetylcysteine with a proprietary vehicle for intra-oral application. A randomized, phase 2 study demonstrated that RK-0202 reduced the incidence of OM, in comparison with the controls, justifying a phase 3 trial to confirm its efficacy.[12,14,16]

Nitroxide

In-vitro and *in-vivo* preclinical studies have indicated that the oxidized form of nitroxide can act as a radioprotector, reducing the damage to salivary glands and alopecia.[12] On the basis of these findings, investigators tested the efficacy of tempol- 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl -for the prevention of radiation-induced OM in rats.[35] The researchers considered its use as beneficial in the reduction of severe episodes of mucositis.

Conclusions

Given the satisfactory responses observed in the different studies, at this moment RK 0202, tempol, and many other substances that are being tested systematically,

represent the possibility of beneficial future treatments. Current studies are still incipient, and the literature shows a scarcity with regard to the relationship of these substances to the prevention and treatment of OM.

FINAL CONSIDERATIONS:

The lack of effective preventive strategies for OM, considering the serious consequences of this disease, has prompted research into new therapeutic targets. Low-energy LASER, palifermin, and amifostine are among the therapeutic choices that contribute to the prevention and treatment of OM. Their high costs, procedural complexities, and adverse effects, however, restrict their clinical applicability.

Considering their low cost and risk, natural antioxidants are alternatives for the treatment of OM, favoring patients' adherence to a regimen. The most striking results are reported with vitamin E and zinc, although herbal substances such as marigold and chamomile stand out for preventive measures for OM. Studies relating the role of the most varied types of antioxidant agents in the prevention of OM represent an important part of current research. Connected to this research is the new understanding of the biopathology of OM and its initiation due to the production of FR.

New studies should focus on the development of an understanding of the performance of each antioxidant against radiation-induced ROS, supporting AOX as standard treatments against OS that is implicated with the development of OM.

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3. ARTIGO II

O artigo a seguir intitula-se "**TOPICAL APPLICATION OF *ALOE VERA* AND VITAMIN E ON INDUCED ULCERS OF THE TONGUE IN RATS SUBJECTED TO RADIATION THERAPY: CLINICAL AND HISTOLOGICAL EVALUATION**" foi formatado e submetido de acordo com as normas do periódico Supportive Care in Cancer (Anexos C e D), o qual possui Fator de Impacto 2.495.

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Topical application of *Aloe vera* and vitamin E on induced ulcers of the tongue in rats subjected to radiation: clinical and histological evaluation

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Abstract:

Objective: To assess the effect of 2 types of antioxidants, vitamin E (VE) and *Aloe vera* (AV), on healing of induced oral lesions after radiation in a murine model by clinical and histological analysis.

Methods: The animals were randomly divided into 2 groups of 12 animals each (400 mg VE, 70% AV) and 1 group of 11 animals (control) and 2 time periods (5 and 7 days). They were irradiated with a single dose of 30 Gy, and after 24h, a lesion was produced on the ventral tongue of each animal. The products were applied daily in their respective group until euthanasia.

Results: On clinical analysis, there was a higher frequency of lesions in the animals of the control group at both periods. The area of the lesions was also greater in the control group compared with the groups AV and VE (5 days: $p = 0.006$; 7 days: $p = 0.002$). On microscopic analysis, the degree of inflammation differed between the study groups and experimental periods. At 5 days, the statistical difference was not significant among the groups evaluated, but at 7 days, animals in the control group showed intense

inflammation, while those in groups VE and AV exhibited mild to moderate inflammation ($p = 0.002$).

Conclusion: The results suggest that VE and AV contributed to the decrease in inflammatory response and healing of the lesions induced on the tongue of rats subjected to radiation.

Keywords: oral medicine; radiotherapy; oral mucositis; antioxidants; mice

Introduction

Radiotherapy (RT) is a therapeutic modality widely used in the treatment of head and neck cancer (HNC). RT consists in the use of ionizing radiation capable of interacting with the tissues through chemical and biological reactions that prevent the replication of tumor cells. However, ionizing radiation is not selective and also acts on healthy tissue, causing deleterious effects that may cause various oral complications [1-3].

Oral mucositis (OM) is the most frequent acute local effect in patients treated with RT. It is a debilitating condition that begins around the third week of treatment with radiation doses of 10 and 30 Gy [4]. It presents clinically as an inflammatory response, with areas of mucosal ulceration in varying degrees of severity. It is accompanied by pain and eating difficulties and may result in weight loss and malnutrition and susceptibility to opportunistic infections. It affects the quality of life of patients and can become a dose-limiting factor in treatment [1, 5-8].

There is considerable evidence that the cytotoxic effects of ionizing radiation are due to physical-chemical reactions that lead to the production of free radicals (FR). These compounds would also be related to mediators in oral lesions induced by radiation. On the basis of the model postulated by Sonis *et al*., in which the release of reactive oxygen species (ROS) with consequent oxidative stress (OS) is considered the main activation factor of numerous events responsible for the development of OM. Its control has been the subject of studies on cytoprotective interventions. Accordingly, antioxidants (AOX) are potential agents capable of preventing the formation of ROS and even eliminating them from the body [9-14].

There are several types of AOX and FR quenchers that can limit OS. Some enzymes present in the body are naturally able to protect tissues against damage caused by FR, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione

reductase. Antioxidant defense may also be provided by low molecular weight agents such as ascorbic acid, tocopherols, polyphenols and thiols. Thus, the well-known natural AOX such as alpha-tocopherol, the main constituent of VE, and flavonoids present in plants, including AV, empirically used as an aid in healing ulcers, have been widely studied and suggested as possible radioprotective agents for the prevention of mucositis [1-3, 15-24].

The major impact of radioinduced OM on the quality of life of patients, as well as comorbidities caused by this ulceration, often requires changes in anticancer treatment regimen and the use of opioid analgesics, enteral or parenteral nutrition, hospitalization and even the interruption of cancer therapy. On the basis of the pathogenesis of OM, strategies have been proposed for the prevention and management of clinical manifestations of patients. However, to date, few studies have shown enough scientific evidence to recommend effective treatment guidelines [6, 8, 12]. Considering the severity of the condition in question and its implications in cancer patients, the aim of this study was to evaluate the clinical and histological response of the topical application of VE and AV, in the healing of induced tongue lesions in rats subjected to radiation.

Methods:

Animals

The sample consisted of 35 female Wistar rats, about 90 days old, weighing 200-300 g, obtained from the animal facility of the Pontifical Catholic University of Rio Grande do Sul (PUCRS). The animals were housed in the Center for Experimental Biological Models CeMB/PUCRS. They were kept in plastic boxes identified in accordance with the subgroup, which were lined with autoclaved wood shavings, placed in a micro isolation chamber at 23 ± 1°C, with a light-dark cycle of 12 h. During the experimental period, the animals were given food and filtered water *ad libitum*. This research was conducted in accordance with the ethical principles applicable to the use of laboratory animals established by the National Board of Animal Experimentation Control, and the study protocol was approved by the Scientific and Ethics Committee of the Dental School, PUCRS and by the Ethics Committee for the Use of Animals of PUCRS.

Experimental design

The animals were randomly selected and numbered on their tails to form each of the 3 groups according to the treatment to be received [AV group (n = 12): 70% AV gel; VE group (n = 12): 400 mg VE gel; C group (n = 11): hydroxymethylcellulose] and 2 time periods [5 and 7 days]. Afterwards, they were immobilized with the aid of retainer and positioned vertically so that the head was exposed to radiation. The irradiation protocol was established and carried out at the Radiotherapy Department of São Lucas Hospital, using a Phoenix teletherapy apparatus with Cobalt-60 source, 30 x 30 cm irradiation field, source to surface distance of 76 cm and dose of 58.97 cGy/min for a total single dose of 30 Gy.

Twenty-four hours after irradiation, the animals were sedated and anesthetized by isoflurane inhalation at 4V% until the loss of protective reflexes. A lesion was immediately produced in the medium third of the ventral tongue of each animal, up to 3 mm from the tip to standardize the assessment criteria. The lesions were made using two contiguous incisions with a 3 mm diameter disposable punch, producing a lesion 6 mm long, 3 mm wide and 1mm deep. Analgesia was provided throughout the experiment with the use of dipyrone at 150 mg/kg/day. Immediately after producing the lesions, animals started receiving the designated treatment for each group.

Topical application of 1 ml of the substance was performed under restraint every 24 h until the established period for each experimental group. Feed and water were removed 30 min after application, avoiding their consumption and subsequent removal of the product. The animals of the study groups were euthanized at the designated times by deep isoflurane anesthesia at 6 and 8 days after irradiation.

Treatments

• 70% *Aloe vera* gel (70% glycolic extract of *Aloe vera*, 10% purified water, 1% preservative solution of methylparaben and propylparaben and 19% hydroxyethylcellulose), prepared in the University Pharmacy Panvel, PUCRS.

• Vitamin E gel (400 mg alpha-tocopherol acetate, purified water, glycerol, soybean oil, methylparaben, propylparaben and gelatin powder): obtained drug from Ephynal® 400 mg (Bayer HealthCare).

• Hydroxymethylcellulose (placebo substancein gel form): prepared in University Pharmacy Panvel, PUCRS

Clinical and histological evaluation

After euthanasia, each animal was immediately weighed, and evaluated clinically the ventral region of the tongue subjected to trauma, to determine the absence or presence of induced lesion and local inflammatory signs. The lesions were measured using a periodontal millimeter probe.

After the clinical analysis was performed, the tongue of each animal was surgically removed. These were fixed in 10% formalin for 24 h, and a longitudinal portion was taken of the center of the lesion area. The specimens were embedded in paraffin, and two 3-μm thick sections were made of each specimen. The slides were prepared and stained with hematoxylin and eosin (HE).

They slices were examined with an Olympus binocular microscope (model BX50). A calibrated and blinded examiner evaluated all sections obtained. Intra-examiner calibration was done by reanalysis of 20 slides with 7 days between observations (Kappa = 0.889 ± 0.061, $p<0.001$). Next, the field showing the most intense inflammatory response was chosen (cells and blood vessels) that determined the score, according to the criteria mentioned in the diagram below [25-27]:

0. Absent: absence of inflammation
1. Slight: sparse mononuclear cells
2. Moderate: mononuclear infiltrate and/or sparse neutrophils and eosinophils
3. Intense: polymorphonuclear infiltrate of neutrophils and eosinophils

Statistical analysis

SPSS 17.0 software was used. The Fisher exact test was used for comparisons regarding the presence and absence of lesion and loss of weight, considering the differences with a significance level of 5% ($p<0.05$). The Kruskal-Wallis test was used for comparative analysis between groups for inflammatory response as well as the size

of the lesion. In the comparative analysis of the inflammatory response with respect to times, we used the Mann-Whitney test, with a significance level of 5%.

Results

During the experiment, 2 animals died. Thus, 33 rats were included in the study with the following distribution: AV5 (n=5), AV7 (n=6); VE5 (n=6), VE7 (n=5); C5 (n=5), C7 (n=6). In the 5-day experimental period, there was no weight loss in any animal. However, in the groups evaluated at 7 days, all animals showed weight loss, ranging from 50 to 100 g. There was no statistical difference in weight loss between treatments (p=0.221) but rather over time after irradiation of animals (p=0.001).

Clinical evaluation

Signs of inflammation, such as erythema and edema, were observed in all animals at both experimental times, but with no statistical difference between the groups. The animals evaluated at 7 days showed visible fibrosis in tongue structure. This was not detected in groups evaluated at 5 days.

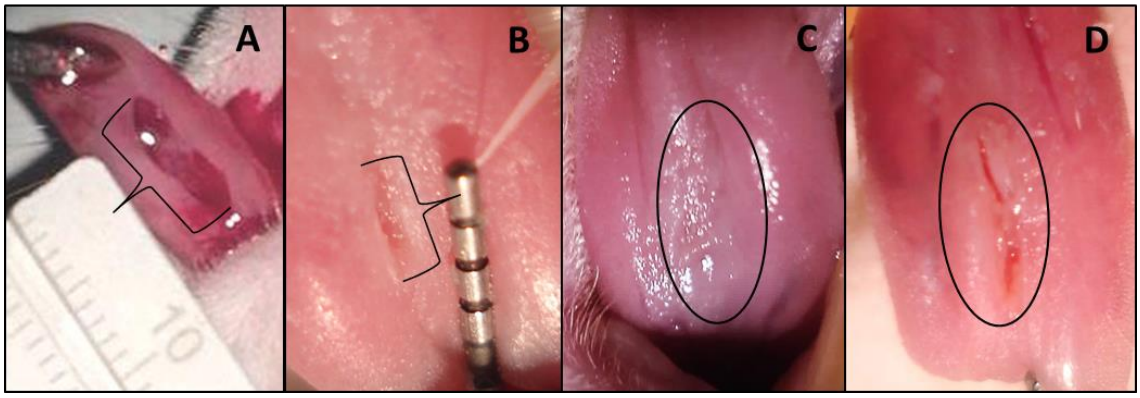
In clinical examination at 5 days, all animals in the control group showed lesions in the tongue, whereas in groups AV and VE, this was observed respectively in 3 and 2 animals (Figure 1). However, there was no statistically significant difference between groups ($p = 0.06$; $p = 0.44$). In the 7-day experimental period, complete healing of the lesions was observed in 100% of animals in the VE group and of 5 animals in the AV group. In the control group, all animals remained with some degree of ulceration (Figure 2). In this experimental time there was statistically significant difference between groups ($p = 0.002$; $p = 0.015$). The average size of these lesions is described in Table 1. It was found that in both experimental periods, the animals of the control group had larger lesions compared to the groups AV and VE (5 days: $p = 0.006$; 7 days: $p = 0.002$)

Table 1. Comparison of the size of induced lesions in relation to time and study groups.

Size of lesion (mm^2)	**TIME**					
	5 days			**7 days**		
	AV (*n*=5)	VE (*n*=6)	C (*n*=5)	AV (*n*=6)	VE (*n*=5)	C (*n*=6)
Median	0	0	8	0	0	4
Minimum	0	0	4	0	0	1
Maximum	4	2.5	90	3	0	30
P value	0.006			0.002		

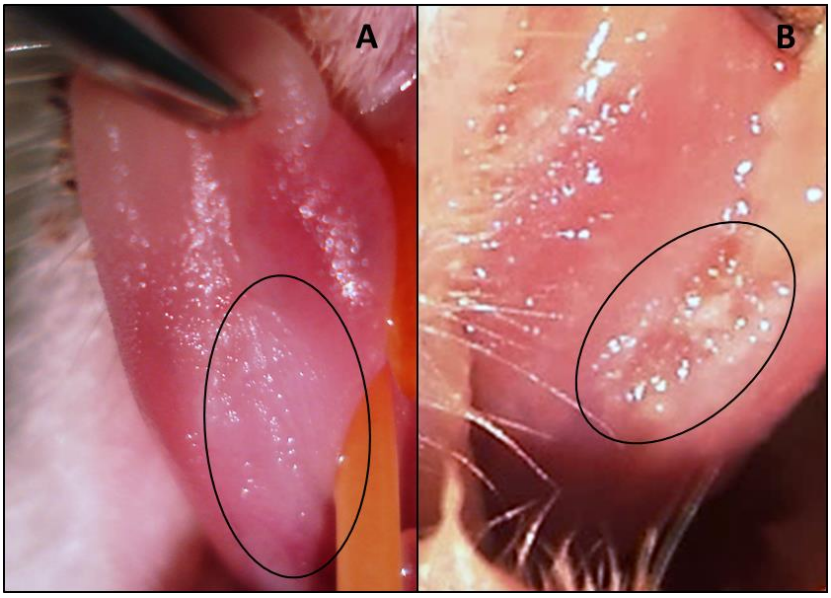
*Kruskal-Wallis test

Figure 1. Lesion induced on ventral tongue of rats after irradiation: clinical evaluation at 5 days.



A: Immediately after induction of lesion measuring 6 x 3 x 1mm. **B**: Animal of AV5 group showing a discrete lesion. **C:** Animal of VE5 group with total healing of lesion. **D**: Animal of C5 group demonstrating persistence of lesion.

Figure 2. Lesion induced on ventral tongue of rats after irradiation: clinical evaluation at 7 days.



A: Animal of VE7 group showing total healing of induced lesion. **B:** Animal of C7 group with persistent lesion.

Histological analysis

The degree of inflammation differed in the study groups as well as experimental times. In animals in the AV5 and VE5 groups, the intensity of the inflammatory process ranged from mild to moderate, while in the C5 group, all animals had moderate intensity. However, there was no statistical difference between the groups compared to the control. On the other hand, in the animals in the VE7 and AV7 groups, the intensity of inflammation remained between mild and moderate, while all animals in the C7 group developed an intense degree of response (Figure 3).

In relation to the performance of each treatment at the two study times, it was observed that AV showed better results at 5 days, predominantly mild inflammation progressing to moderate on the seventh day. VE showed better performance at 7 days where inflammation decreased from moderate to mild, but there was no significant difference in these results. However, in group C, the inflammatory process progressed, going from moderate at 5 days to intense at 7 days (Table 2).

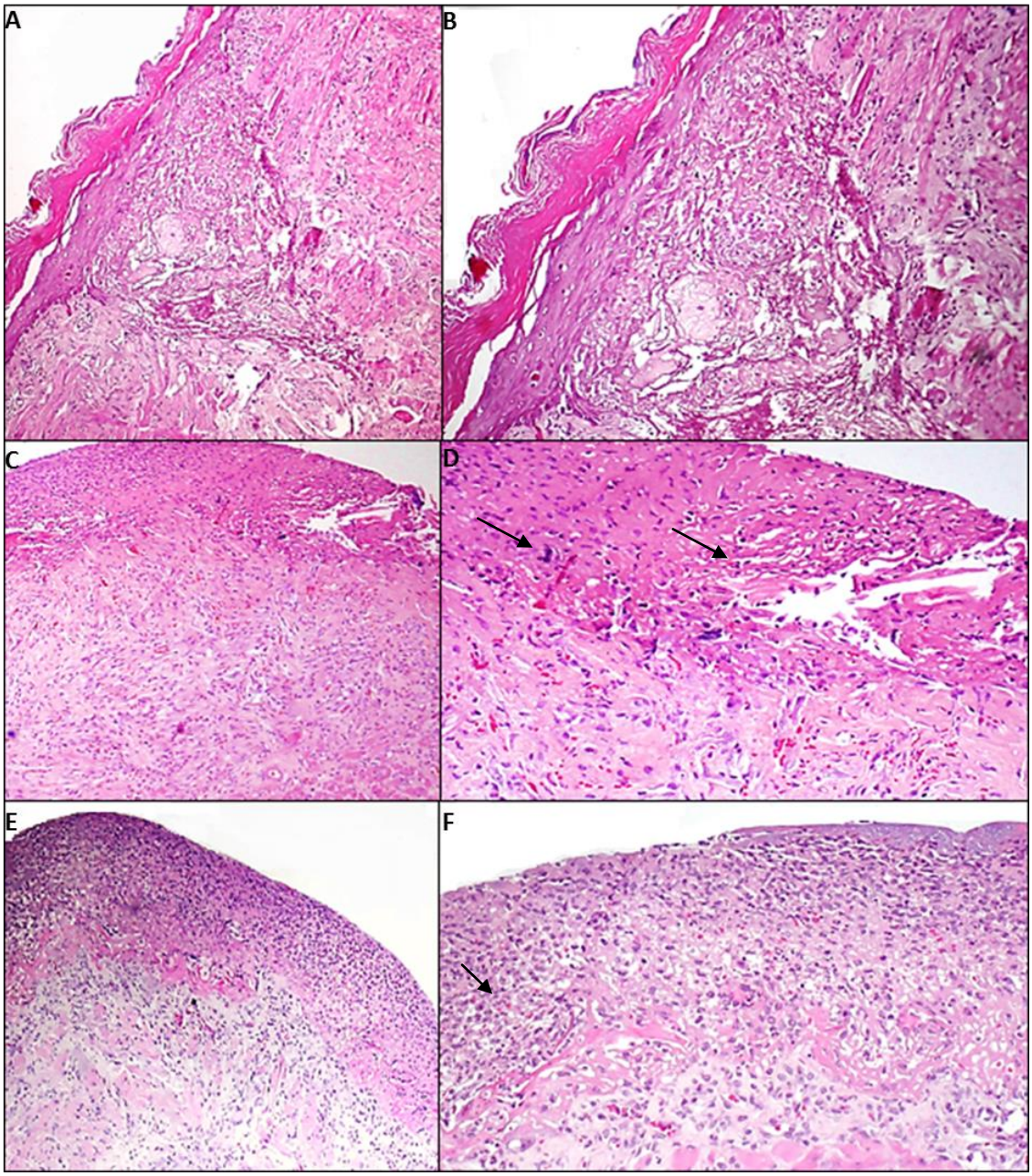
Table 2. Comparison of inflammatory response in relation to time and groups.

Inflammatory response	**TIME**								
	5 days			**7 days**			***P*** **value ***		
	AV (*n*=5)	VE (*n*=6)	C (*n*=5)	AV (*n*=6)	VE (*n*=5)	C (*n*=6)	AV	VE	C
Slight	3	1	0	2	2	0	0.53	0.53	0.004
Moderate	2	5	5	4	3	0			
Intense	0	0	0	0	0	6			
P value**	0.089			0.002					

*comparison between times (Mann-Whitney test)

**comparison between treatments in relation to control (Kruskal-Wallis test)

Figure 3. Histological characteristics observed in tongue of irradiated rats (Photomicrographs, HE staining).



A: Sparse mononuclear cells featuring mild inflammation (approximate magnification: 40x). **B**: Mild inflammation (approximate magnification: 100x). **C**: Inflammatory infiltration of mononuclear cells with scattered neutrophils and eosinophils featuring moderate inflammation (approximate magnification: 40x). **D**: Moderate inflammation (approximate magnification: 100x). **E**: Inflammatory infiltrate of polymorphonuclear cells featuring intense inflammation (approximate magnification: 40x). **F**: Intense inflammation (approximate magnification: 100x).

Discussion

Despite technological advances in RT, acute complications such as OM are still part of the routine of patients with HNC treated with this therapy. In these patients, the incidence of OM can be more than 70% and may be exacerbated by combination with chemotherapy. The OM-induced radiation is accompanied by dry mouth, opportunistic infections, changes in taste, pain, loss of appetite and, in severe cases, loss of nutritional status and need for interruption of anticancer treatment [9-11]. To minimize OM, some actions have been successfully applied, although most of them being palliative. Patients can be advised to maintain oral hygiene and to use anti-inflammatory agents, antimicrobials, topical anesthetics and mucosal protection. Low-intensity laser therapy and the administration of epithelial growth factors and, more recently, substances with antioxidant capacity are also some resources that may be considered preventive interventions [1, 4, 5, 8, 24]

AOX have held a prominent position in the strategies of prevention and treatment of OM from the understanding of the complexity of biological events involved in its pathogenesis. The model described by Sonis *et al.* postulates that the formation of ROS and subsequent OS induced by RT play a key role in the initiation of OM [2, 9-11, 13-16, 28]. Thus, AOX have been widely studied and have shown promising results, as they represent a likely therapeutic alternative with low cost and risk, where patients have easy access to treatment [2, 28].

The main constituent of VE is alpha-tocopherol, an antioxidant capable of reacting with FR, eliminating them from the body and hence controlling OS. Studies involving VE as a radioprotective have shown favorable results [1, 5, 29]. AV, a plant historically used as an aid in wound healing, is rich in flavonoids, constituents of polyphenols. The protective role of diets rich in polyphenols of fruits and vegetables in the prevention of some cancers and chronic degenerative and inflammatory diseases is well established and accepted by the scientific community [23]. Therefore, we chose to test these 2 types of AOX as viable substances for intraoral use at the highest possible concentration of the main ingredient.

The choice of the animal model and the methods used in this study was based on the need for a strict standardization of analysis criteria. Thus, it was appropriate to use single-dose radiation to induce an aggressive response of the mucosa, since dose fractionating is used in attempt to reduce the damage of treatment. In addition, we opted

for the induction of a traumatic ulcer, because mucosal lesions could appear in different mucosal sites of the animal and in different sizes and intensities across the same dose, compromising the standardization of clinical analysis.

Mucositis induction in an animal model is technically difficult since the protocols already described in the literature vary with the species of animal, the site of exposure, total radiation dose (whether single or fractionated) and the radiation source. The development time of the injury and its intensity depend directly on the protocol used, which can hinder the analysis criteria. Thus, based on previously published works, which met standards similar to those desired for this study, we opted for an irradiation protocol with a single dose (total 30 Gy). The experimental times were also determined from the literature, in which there are reports that, with this radiation dose, the beginning of OM lesions would be observed at 5 days, peaking in severity between 7 and 8 days [7].

The response to topical application of VE and AV in the management of mucositis showed satisfactory results in this study. OM classical signs such as erythema and edema were observed in all groups, but their intensity was not measured in this analysis. In both experimental periods, the control animals exhibited an exacerbated clinical feature when compared to VE and AV groups, and this difference was more statistically relevant at 7 days. Regarding the intensity of the inflammatory process, AV and EV also showed greater ability to control inflammation than did the placebo, showing better performance at 7 days.

These findings suggest that after 5 days of treatment, the test substances exhibited a slight advantage compared to group C. However, during treatment, both products showed clear clinical improvement and inflammatory process, with VE showing a slight superiority in both the physical and histological examinations. Thus, we can infer that both substances were able to hamper the progression of lesions to their peak severity.

Similar results were found in other studies conducted in rodents, in which authors found significant evidence that VE had a radioprotective effect in both oral and intestinal mucosa [18, 30]. In the study of Uçuncu *et al*., besides the clinical and histological examinations, metabolic aspects were also evaluated, where the antioxidant capacity of EV was reinforced, since there was a decrease in OS and increased plasma AOX levels [30].

Human studies have also been performed demonstrating favorable results, but they were only considered clinical, since the histological analysis was not feasible for ethical reasons. A study with daily doses of 400 mg VE found that patients in the experimental group showed no adverse effects and had faster resolution of OM [17]. In the clinical study conducted by Ferreira *et al*., 54 HNC patients undergoing RT with doses ranging from 50 to 70 Gy were evaluated. The authors used oral rinses containing VE and observed a reduction in the incidence and symptoms of OM lesions [20]. The data suggested that intestinal absorption of VE did not seem significant and that the protective action on the mucosa was due to a local effect. However, studies have not had beneficial effects in the prevention of OM with VE supplementation. Santos found that supplementation with 400 mg VE/day was not effective in the prevention of OM. However, this was an evaluation in humans with heterogeneous sample in which patients received different doses of radiation and had different cancers of the digestive tract. The authors suggested that the time of treatment and the dose used was insufficient and that negative results may have been influenced by these factors [31]. In view of these findings, we believe that the results obtained in our study can also be reproduced in humans, but detailed and tightly controlled methods should be used to avoid possible biases interfering with the results.

Studies of AV efficacy in the prevention and treatment of OM differ in their results. In a literature review of the antioxidant, anti-inflammatory and healing properties of the plant, the authors suggest that this may be an alternative treatment for OM [22]. However, two phase 2 studies tested the efficacy of formulations based on AV in the management of OM and showed no positive results [21, 32]. The present study contradicts the results of the works described, since our findings showed that AV was beneficial in regard to both lesion severity and wound healing as compared with the control group. The effects of AV on other pathologies of the oral cavity, such as lichen planus, aphthous stomatitis, candidiasis, burning mouth syndrome and even caries and periodontitis are promising [23]. However, products containing significant concentrations of the plant that can be used on the oral mucosa are not easily found on the market, since it naturally has an unpleasant taste and the stability of their properties can be compromised when linked to a carrier. Our aim of obtaining an AV gel with the highest concentration possible while maintaining stability of the antioxidant property of the plant, which could be used on the oral mucosa was to promote greater contact of the

drug with the lesion and might have been decisive for its favorable action. We did not find comparative studies between EV and AV in the literature.

Despite the favorable results of these products, in a recent systematic review of the literature published by the Mucositis Study Group of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology evaluated several studies on natural agents in the management of OM. The use of vitamin E was cited in 4 clinical studies in humans applied topically or systemically in patients receiving RT or chemotherapy, and in 3 of them, the authors reported beneficial effects. But AV was found in one clinical trial showing no effective contribution. For both products the authors concluded that there was insufficient scientific evidence to include them in the guidelines for OM management [33].

Other clinical signs linked to OM were observed in this study. Eating difficulties and weight loss are common in irradiated patients and can be related to pain caused by OM. In this work, we observed weight loss exclusively in 7-day groups. In these animals, there was also tongue paralysis, which was not seen in the animals evaluated at 5 days. The reduction in the range and movement of the tongue has also been reported in human studies and is strongly associated with weight loss, as it reduces the efficiency and safety of swallowing [34,35]. This finding suggests that the fibrosis of the tongue was crucial for weight loss, and this was not influenced by the presence or absence of lesion or even use of the test products, but by the time after irradiation. That represented an adversity, because it made clinical analysis and image capture difficult in these groups.

Based on these results and comparing them with previous studies, it is observed that the AOX tested showed radioprotective potential. However, it is known that the reactivity of ROS induced by radiation, comparing them with those generated under oxidative stress conditions, is generally more intense, where not all AOX are able to have a protective effect. Understanding the performance of each antioxidant substance, given the complexity and aggressiveness of radiation-induced OS, still seems to be a challenge [2, 15, 16].

Conclusion

The results of this study suggest that VE and AV contribute to reducing the inflammatory process and severity of lesions and favor tissue repair of induced lesions

on irradiated mucosa. Future investigations can be based on the use of VE and AV as alternative prevention and treatment of OM. Despite the animal studies done, well-designed clinical studies with robust methods are needed to include these AOX in the protocols for OM management.

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4. DISCUSSÃO COMPLEMENTAR

A excisão cirúrgica com margem de segurança é considerada o padrão ouro no tratamento do carcinoma espinocelular, a neoplasia de maior prevalência na cavidade bucal. No entanto, a dimensão do tumor, sua localização ou ainda, as condições clínicas do paciente podem contra indicar este procedimento ou exigir a complementação do mesmo. Nesses casos, é comum lançar-se mão de outras modalidades terapêuticas, tais como a RT e/ou QT.

A RT é uma alternativa amplamente empregada em associação com a cirurgia ou de forma exclusiva. Inúmeras complicações orais estão fortemente vinculadas ao seu mecanismo de ação, como por exemplo a xerostomia, o trismo, fibrose e atrofia da mucosa, alteração do paladar, disfagia, osteorradionecrose, entre outras. Dentre elas destaca-se a MO, lesão de origem inflamatória que resulta do dano tecidual causado pela RT e/ou QT bem como por sua capacidade de influenciar negativamente o curso do tratamento antineoplásico (EPSTEIN *et al*, 2012).

Recentes avanços nas terapias contra o câncer, com o desenvolvimento de tecnologias visando reduzir a toxicidade do tratamento aos tecidos normais, como radioterapia conformacional, de intensidade modulada, feixe de prótons e guiada por imagem, levaram a mudanças na incidência, natureza e severidade desta complicação. No entanto, ainda permanece como uma entidade patológica frequente e de difícil manejo (BHIDE, NUTTING, 2010; CITRIN *et al*, 2010; COTRIM, YOSHIKAWA, SUNSHINE, 2012).

Sonis *et al*, descreveram o desenvolvimento da MO em 5 fases, nas quais o EO induzido pela radiação é descrito como o principal responsável por desencadear uma cascata de reações de culminam no rompimento do tecido epitelial. A partir dessa hipótese, passou-se a investigar substâncias capazes de impedir ou modular o EO, atuando como protetores da mucosa (SONIS *et al*, 2004; SONIS, 2010; AL DASOOQUI *et al*, 2013)

Existem muitos AOX e removedores de RL que podem limitar o EO. A superóxido dismutase, catalase, glutationa peroxidase e glutationa redutase são alguns exemplos de AOX capazes de proteger contra os danos causados naturalmente pelos RL. A defesa antioxidante também pode ser fornecida por agentes de baixo peso molecular, que são doadores de um átomo de hidrogênio, tais como ácido ascórbico, tocoferóis, polifenóis e tióis. Entretanto, nem todos os AOX apresentam um potencial

radioprotetor, uma vez que os RL induzidos pela radiação podem ser mais reativos do que aqueles gerados sob condições gerais de EO (CITRIN *et al*, 2010).

Sabe-se ainda que pacientes acometidos de NMCP apresentam elevados níveis de peroxidação lipídica acompanhada por depleção de AOX, fatores intimamente relacionados a carcinogênese (SHARMA *et al*, 2009; MARAKALA, MALATHI, SHIVASHANKARA, 2012). Isso reflete em uma importante falha na defesa orgânica contra o EO. Dessa forma supõe-se que, mesmo antes do início da RT, os pacientes estejam mais suscetíveis à citotoxicidade do tratamento. Sendo assim, na busca constante por novas alternativas terapêuticas para a prevenção e manejo da MO necessita-se de contínuas investigações e esclarecimentos que permitam aos profissionais e pacientes utilizarem com segurança os tratamentos indicados, proporcionando uma melhor qualidade de vida durante e após a QT e RT.

Através deste experimento procurou-se avaliar as respostas clínicas e histológicas do AV e da VE. Ambas as substâncias foram descritas por outros autores como possíveis protetores de mucosa tendo em vista o seu potencial antioxidante. Por suas características naturais estes produtos seriam capazes de conter ou limitar o EO sem causar efeitos colaterais aos usuários (VARONI *et al*, 2012).

Os materiais foram aplicados topicamente na forma de gel sobre a lesão (AV 70% e VE 400mg), buscando verificar e comparar possíveis variações no grau de resposta inflamatória tecidual a partir do emprego dos produtos.

Li *et al*, em 2011, descreveram a indução de MO em animais de laboratório em um experimento no qual o dorso da língua de ratos foi irradiado com dose de 30Gy. Já Galleta (2006) e Lee *et al* (2007) haviam irradiado toda a cabeça dos animais, avaliando respectivamente mucosa labial e dorso da língua. Em nosso estudo optamos por irradiar a cabeça dos animais, uma vez que o posicionamento dos mesmos, para incluir no portal estruturas anatômicas específicas, exigiria anestesia durante a irradiação. Submetendo toda sua cabeça, os animais puderam ser posicionados através de contensores artificiais o que permitiu a realização da radioterapia sem anestesia, reduzindo o risco de morte e consequente perda de espécimes.

Os modelos de indução da MO consagrados na literatura variam de acordo com a espécie de animal, local de exposição, dose total de irradiação (seja fracionada ou única) e fonte de radiação. A dose aplicada neste experimento baseou-se nos trabalhos que cumpriam padrões semelhantes aos desejados para este estudo. Optou-se por um protocolo de irradiação em dose única (totalizando30Gy), uma vez que o objetivo do fracionamento é proteger os tecidos normais. Neste experimento visou-se induzir a MO de

forma realmente agressiva sem minimizar o efeito da radiação sobre os tecidos (GALLETA, 2006; LEE *et al*, 2007; LI *et al*, 2011).

Apesar dos consagrados protocolos de indução da MO por RT, a análise clínica das lesões bem como a comparação de sua intensidade entre os indivíduos é tarefa difícil. Sabe-se que, mesmo com dose e portal de radiação idênticos, a resposta é individual, podendo-se observar lesões de distintas intensidades em diferentes sítios anatômicos comprometendo a padronização da análise (RODRIGUEZ-CABALLERO *et al*, 2012). O ventre de língua em sua porção central foi o local eleito para a indução da úlcera, pelo fato dessa região estar anatomicamente mais protegida de traumas e livre de vasos calibrosos. Dessa forma reduziu-se o risco de sangramento intenso no trans e pós operatório, visto que são animais roedores que ficariam alojados em grupos, na mesma gaiola. A mucosa jugal seria uma localização anatômica com maior facilidade de acesso para a confecção da úlcera. Contudo, é mais vulnerável a traumatismo (como mordidas) e, possivelmente, a intensificar o processo inflamatório (CAVALCANTE *et al*, 2011). Uma vez que se pretendia verificar a presença ou ausência de resposta inflamatória, sem que houvesse qualquer interferência, optou-se pelo ventre lingual, sítio anatômico mais protegido, favorecendo a fidelidade dos resultados.

A indução da úlcera após a RT cumpriu o propósito de produzir a lesão em um tecido já lesado e com resposta celular ao dano. Por questões logísticas e para padronização da metodologia em todo o período do experimento, uma vez que os grupos foram irradiados em diferentes momentos (conforme a disponibilidade do serviço de radioterapia), determinou-se o intervalo de 24h após a irradiação para a indução das lesões. A utilização de duas incisões consecutivas com *punch* de 3mm ao invés do emprego de um instrumento de maior diâmetro, objetivou que a lesão apresentasse maior comprimento do que largura restringindo-a à porção central da língua. Os lados esquerdo e direito do ventre lingual são irrigados por vasos calibrosos e, além disso, são mais vulneráveis a traumatismos (VERLI *et al* 2008).

Os tempos experimentais foram determinados a partir dos estudos de Galleta (2006), Lee (2007) e Li (2011) onde em torno do 6° dia após a irradiação (5 dias de tratamento) foi possível observar os primeiros sinais clínicos da MO e do 8° dia após a irradiação (7 dias de tratamento) o pico de severidade das lesões. Portanto, determinou-se esses mesmos períodos para a avaliação do curso da úlcera induzida no tecido irradiado.

Na análise clínica de 5 dias, toda a amostra do grupo controle apresentou a lesão ulcerada na língua, enquanto nos grupos AV e VE a mesma foi observada respectivamente em 3 e 2 animais. Neste

período os animais apresentaram discreto eritema da mucosa sem fibrose ou perda de peso. Já nos animais dos grupos de 7 dias verificou-se ainda eritema distribuído em mucosa labial e jugal, sangramento ocular e nasal, halitose e umedecimento dos pelos da região perioral, além de perda das papilas do dorso da língua. Nesta fase apresentaram morbidade notável, tornando-se necessária a intensificação da analgesia nas últimas 12h antes da eutanásia. No entanto, nesse mesmo período (7 dias), foi observada a completa cicatrização das lesões em todos os animais do grupo VE e em 5 animais do grupo AV. No grupo controle, a totalidade dos animais permaneceu com algum grau de ulceração.Sabe-se que as taxas de proliferação da mucosa oral são maiores em modelos murinos, se comparados aos humanos e que o tempo de latência de úlceras, assim como sua duração, são menores. Por outro lado, a radiosensibilidade dentro de cada espécie não varia com a dose total de irradiação. Em outros experimentos em modelos animais a reepitelização completa das lesões foi verificada em torno de 14 dias o que leva a crer que as substâncias estudadas apresentaram desfechos favoráveis (GALLETA, 2006; LEE *et al*, 2007; LI *et al*, 2011).

O uso do AV e da VE demonstrou resultados promissores tanto na análise clínica como histológica, uma vez que o grau de inflamação diferiu tanto nos grupos de estudo quanto nos tempos experimentais. Estes achados levam a crer que o uso tópico do AV e VE acelerou a cicatrização das lesões e o reparo tecidual na mucosa oral sem interferir sobre no quadro geral dos animais, que piorou com o decorrer do tempo, independente do grupo de tratamento. Ainda, os animais dos grupos C5 e C7 exibiram quadros clínicos mais exacerbados quando comparados aos grupos AV e VE, sendo esta diferença de maior relevância estatística em 7 dias. Em relação à intensidade do processo inflamatório, o AV e a VE também manifestaram maior capacidade de controle da inflamação do que a substância placebo.

Resultados semelhantes foram encontrados no estudo de Uçüncü *et al* (2006). Em modelo animal, os autores realizaram a análise clínica, histológica e dos aspectos metabólicos os quais reforçaram a capacidade antioxidante da VE, uma vez que constataram um decréscimo dos níveis plasmáticos de EO e aumento dos níveis de AOX. Clínica e microscopicamente os pesquisadores também observaram maior velocidade de cicatrização e menor intensidade do processo inflamatório no grupo tratado com VE.

Estudos em humanos também apresentaram evidências significativas do efeito protetor da VE na mucosa irradiada. Em 2 diferentes trabalhos, o primeiro utilizando doses diárias por via oral e o segundo aplicando bochechos, ambos com doses de 400mg, os pesquisadores observaram que os pacientes do grupo experimental não apresentaram efeitos adversos, tiveram a resolução mais rápida dos quadros de

MO, além da redução na incidência e sintomatologia das lesões de MO. Apesar da forma de administração distinta, os resultados benéficos foram semelhantes (WADLEIGH *et al*, 1992; FERREIRA *et al*, 2004).

Ferreira *et al* (2004), sugeriram que a absorção intestinal da VE não parece significativa e que a ação protetora na mucosa decorre de um efeito local. Assim, é importante salientar que neste experimento buscou-se a aplicação tópica dos produtos estudados visando observar seu efeito local na mucosa. Para tanto foi definida a medida de 1ml do gel por dose. É possível que pequenas quantidades tenham sido ingeridas pelos animais, contudo, assim como no trabalho citado, não parecem ter desempenhado qualquer efeito nocivo sistêmico, uma vez que o quadro geral dos animais foi semelhante, independente do grupo de estudo. Para futuras pesquisas, a análise dos níveis séricos destes AOX pode ser empregada como um indicador da absorção sistêmica dos mesmos a partir da aplicação tópica.

Na literatura investigada, exclusivamente um estudo não apresentou resultados favoráveis com o uso da VE. Santos, em 2009, testou a suplementação com 400mg/dia em uma amostra heterogênea de pacientes em tratamento para neoplasias malignas do trato aerodigestivo. De acordo com seu achado o autore sugere que o tempo de tratamento e a dose empregada podem ter sido insuficientes para apresentar benefícios em relação a MO.

Já no que diz respeito ao desempenho do AV na prevenção e tratamento da MO os resultados são divergentes. Dois estudos de fase 2 testaram a eficácia de formulações (solução e creme) a base de AV na forma tópica sem apresentarem resultados positivos (SU *et al*, 2004; DOOR *et al*, 2005). Já em um experimento realizado em humanos, os resultados clínicos do uso de bochechos a base de AV foram comparados com a aplicação da já consagrada benzidamina. O AV apresentou resultados tão satisfatórios quanto o fármaco citado. Assim sendo, os autores sugeriram que este possa ser uma boa alternativa terapêutica para a MO (SAHEBJAMEE *et al*, 2014). Em nossos achados o AV, quando comparado ao grupo controle, foi considerado benéfico tanto na redução da severidade quanto no reparo tecidual. A ação do AV em outras lesões ulceradas da cavidade bucal, tais como liquen plano, estomatite aftosa, candidíase, síndrome da ardência bucal e até mesmo, cáries e periodontites tem demonstrado resultados promissores (VARONI *et al*, 2012). O que difererenciou a metodologia deste estudo dos demais que utilizaram bochechos de AV foi a obtenção de um gel da planta, permitindo seu uso intraoral em alta concentração (70%). Foi possível manter a estabilidade das propriedades antioxidantes da mesma e

buscou-se promover um maior contato da droga com a lesão, o que pode ter sido decisivo para sua ação favorável.

Em uma revisão sistemática da literatura realizada pelo Mucositis Study Group of the Multinational Association of Supportive Care in Cancer, foram analisadas 99 publicações avaliando o uso de agentes naturais no manejo da MO. Os autores sugeriram que, apesar dos resultados favoráveis apresentados nos estudos que testaram AV e VE, as evidências científicas ainda são insuficientes para incluí-los nos protocolos de prevenção e tratamento da MO (YAROM *et al*, 2013). A partir dos achados obtidos em nosso experimento, bem como os relatados na literatura, presume-se que os mesmos possam ser reproduzidos também em humanos. No entanto uma metodologia criteriosa e rigidamente controlada deve ser empregada para que possíveis viéses não interfiram nos resultados. Acredita-se que dessa forma, os produtos estudados poderão ser disponibilizados como mais uma opção no manejo da MO.

As graves consequências da mucosite nos tecidos bucais estimulam os pesquisadores a investigarem novos alvos terapêuticos. O entendimento de sua biopatologia e iniciação a partir da formação de RL promoveu um crescente interesse na realização de estudos relacionando o papel dos mais variados tipos de agentes antioxidantes na prevenção da MO. Deve-se dar destaque ao uso de AOX naturais por representarem uma provável terapêutica de baixo custo e risco que favoreceria o acesso dos pacientes ao tratamento.

Os resultados deste estudo sugerem a possibilidade de uso desses agentes como uma alternativa futura para a prevenção e tratamento da MO. Busca-se também fomentar novas investigações por meio de estudos padronizados e com evidências científicas sólidas que possam embasar a inclusão dessas substâncias nos *guidelines* para MO.

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ANEXOS

Alternative Therapies in Health and Medicine

INFO FOR AUTHORS

Alternative Therapies in Health and Medicine (ATHM) is an international scientific forum for the dissemination of peer-reviewed information indexed in the National Library of Medicine to healthcare professionals regarding the use of complementary and alternative therapies in promoting health and healing.

Topics on this page include the following. Click on a link below to scroll to that section.

WRITING FOR *ALTERNATIVE THERAPIES IN HEALTH AND MEDICINE*

The editors of *Alternative Therapies in Health and Medicine* invite authors to submit original papers for consideration. Papers most likely to be published are those that present authoritative information and important new ideas on emerging therapies in health and medicine and their integration into the healthcare system for the promotion of health and wellness as well as the prevention and treatment of illness. Our readers are primarily physicians and other licensed healthcare practitioners. When submitting a manuscript to *ATHM,* please consider this audience. One way to determine if your paper is likely to be published is to show a draft of your manuscript to members of the medical community whose area of expertise is discussed in your paper. If they have difficulty understanding your paper, so, in all probability, will our editors and our readers. Emerging therapies in health and medicine include topics such as the following:

- Acupressure
- Anthroposophy
- Ayurveda
- Bioelectromagnetic therapy
- Biofeedback
- Chiropractic
- Craniosacral therapies
- Creative therapies
- Diet and nutrition
- Environmental medicine
- Health promotion
- Herbal medicine/phytotherapy
- Homeopathy
- Hypnotherapy
- Imagery
- Indigenous medical practices
- Massage/manual therapies
- Meditation
- Medical acupuncture
- Mind-body therapies
- Naturopathy
- Oriental medicine
- Osteopathic medicine
- Psychoneuroimmunology
- Psychotherapy
- Reflexology
- Reiki
- Relaxation/stress reduction
- Spiritual healing
- Tibetan medicine
- Traditional Chinese medicine
- Unani
- Yoga

TYPES OF MANUSCRIPTS TO SUBMIT

Following is an overview of the types of articles *ATHM* publishes.

PEER-REVIEWED SUBMISSIONS

ORIGINAL RESEARCH MANUSCRIPTS

Original Research—Original research is often but not always a randomized clinical trial (RCT). Intervention studies, cohort studies, case-control studies, epidemiologic assessments, observational studies reported according to the STROBE guidelines (www.strobe-statement.org), and surveys are other examples of original research. A clinical trial is a study that prospectively (and often randomly)

assigns human participants to intervention or comparison groups to evaluate the cause-and-effect relationship between an intervention and an outcome. All clinical trials must be registered before submission of a manuscript based on the trial, and the registration information should be included along with the submission. Trial registries include but are not limited to the following: http://www.actr.org.au/; http://www.clinicaltrials.gov; http://isrctn.org/; and http://www.trialregister.nl/trialreg/index.asp. All randomized clinical trials should include a CONSORT flow diagram and checklist (available at http://www.consort-statement.org/index.aspx?o=1030). Original research manuscripts should include an abstract that states one or more study objectives; the study setting, participant information with inclusion and exclusion criteria; the key features of any intervention(s); the primary outcome measures; the study results; discussion (including limitations) placing the results in context with the published literature; and conclusions. Data included in research reports must be original and should be as timely as possible. A structured abstract is required. See instructions for preparing structured abstracts below. Recommended length: 3000 to 5000 words (not including abstract, tables, figures, and references).

Brief Reports and Pilot Studies—These are short reports of original studies or evaluations or unique reports of case series. A structured abstract is required. Recommended length is between 750 and 2000 words (not including abstract, tables, figures, and references). They should include approximately 10 to 20 references and no more than 4 tables/figures. Authors should follow all requirements for original research manuscripts (see above) when submitting brief reports or pilot studies, including the understanding that they have not been published or submitted elsewhere.

REVIEW MANUSCRIPTS

Narrative Reviews—Narrative reviews usually address broad topic areas rather than a few tightly formulated questions. Narrative reviews often do not have a Methods section to describe the process of selecting the studies that the author discusses in the text. Narrative reviews tend to lecture about a topic and may suggest future research.

Systematic Review (Including Meta-analysis)—Systematic reviews educate by describing evidence to the reader. They are critical assessments of research literature pertaining to clinical topics, emphasizing factors such as cause, diagnosis, treatment, and therapy. Articles or data sources should be systematically reviewed according to clear criteria, preferably with a protocol written in advance. The data sources should be current and a structured abstract is required. If a meta-analysis is done it should follow QUORUM (www.consort-statement.org/mod_product/uploads/QUOROM%20checklist%20and%20flow%20diagram%201999.pdf) or MOOSE (www.consort-statement.org/mod_product/uploads/MOOSE%20Statement%202000.pdf) guidelines.

CASE REPORTS

Case reports generally use one (or more) specific case(s) to illustrate an interesting outcome, most commonly with a unique or innovative treatment. Case reports are designed to inform and offer innovative therapeutic approaches. Practitioners should present a clear diagnostic situation whenever possible and then explain the treatment. The case report should include the history, examination, investigations, case management, and outcome. The discussion section that follows should educate the reader about the treatment that was used. Case reports usually range from 600 to 1500 words and contain between 5 and 10 references. Pictures or graphs may be helpful. Consent for publication must be obtained from the patient.

RESEARCH LETTERS

Research letters reporting original research should not exceed 900 words of text and 10 references and may include a table or figure. They are not required to include an abstract but should follow the guidelines listed above for clinical trials. These research letters are peer reviewed.

HYPOTHESES
Critical assessments of emerging therapies discussing potential mechanisms of action and implications for the practice of medicine and the integration of emerging therapies into the healthcare delivery system are encouraged. Manuscripts usually contain between 3000 and 6000 words and are accepted for consideration with the understanding that they have not been published or submitted elsewhere. These manuscripts are peer reviewed.

NON–PEER REVIEWED SUBMISSIONS

LETTERS TO THE EDITOR
Letters discussing a recent article will be considered if they are received within 6 weeks of the article's publication. Letters may have no more than 5 authors and should not exceed 500 words of text and 7 references unless approved by the editor in advance. The letter should include the names and academic degrees (if any) for all authors, as well as the e-mail address for the corresponding author. Letters will be published at the discretion of the editors and shortened or edited for style and content.

AUTHOR REPLY LETTERS
Replies by authors should not exceed 600 words of text and 8 references and should be submitted by one of the original authors of the manuscript.

BOOK REVIEWS
Book reviews are written by request, are generally from 300 to 400 words, and follow the structured format outlined below.

1. **Audience:** In a phrase or two, describe the field of medicine to which the book applies (eg, clinical research, oncology, cardiology) and the types of health professionals who would most benefit from reading the book.
2. **Purpose:** A brief overview indicating the author's goal in writing the book.
3. **Overview/Highlights:** A general summary of the contents of the book, focusing on where the book succeeds in relating new and valuable information to readers.
4. **Limitations:** A brief summary of the book's weaknesses, such as information that should have been covered, unsubstantiated claims, cumbersome writing style.
5. **Commentary:** Final thoughts on the book, perhaps including other books of interest on the same topic and a discussion of how this book compares to those. Information already addressed in the book review should not be repeated here.

MANUSCRIPT FORMATTING AND OTHER DETAILS
Manuscript Content
Your manuscript should be formatted according to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (www.icmje.org). You may also find the Consolidated Standards of Reporting Trials (CONSORT) statement helpful for describing a randomized, controlled trial (www.consort-statement.org).

Authorship
Disputes over authorship, and multiauthorship in particular, are better resolved early on, preferably before a study begins. The author who is designated as "corresponding author" when the manuscript is submitted will be asked to approve editorial changes to the article on behalf of all authors prior to publication of the article.

Ethics
When human experimentation is being reported, include a statement to confirm that the work was done in accordance with the appropriate institutional review body and carried out with the ethical standards set forth in the Helsinki Declaration of 1975. When laboratory animals are used, there should be a statement that the work was carried out according to the National Research Council's protocol for, or any national law on, the care and use of laboratory animals.

Abstracts
Abstracts should be approximately 250 to 300 words for original research and reviews and are used to summarize the paper. Abstracts are not required for hypotheses, research letters, case reports, editorials, columns or commentaries, book reviews, or other intermittent special publications. Abstracts for original research and reviews should include the following headings whenever possible: Background/Context, Objective, Methods/Design, Setting, Participants, Interventions, Primary Outcome Measures, Results, Conclusions, and Trial Registry information. The structure for abstracts is as follows:

- **Background:** One or two sentences explaining why this study is necessary and important.
- **Primary Study Objective:** What are the primary study objectives?
- **Methods/Design:** Outline the key elements of the study design, including a sample size calculation.
- **Setting:** Where the study was done (how many sites, what kind of sites, etc).
- **Participants:** Who participated in the study as well as their key demographic characteristics, the dropout rate, adverse events, etc.
- **Intervention:** The key features of the intervention must be described. Trademarked product names should not be used.
- **Primary Outcome Measures:** What outcome measures were used to measure the primary study objectives. These should be specified in advance (in the trial registry, for example).
- **Results:** The results of the primary outcomes of the study, such as risk, confidence intervals, numbers needed to treat, or *P*-values, should be quantified and reported.
- **Conclusion**: Conclusions supported by the results should be discussed as well as the clinical implications.

References
Start references on a separate page following the text, and number them consecutively in the text by order of appearance. In the text, designate reference numbers either as superscript or on the line in parentheses. (Do not use the footnote or endnote function in Word.) Abbreviate journal titles according to Index Medicus. If in doubt, cite complete journal name. Follow the format and punctuation set forth in the *AMA Manual of Style,* 10th ed, as illustrated in the following examples. Do not use periods in abbreviations of journal titles. List all authors, but if the number exceeds 6, list the first 3 names followed by “et al.”

Journal article
Pert CB, Dreher HE, Ruff MR. The psychosomatic network: foundations of mind-body medicine. *Altern Ther Health Med.* 1998;4(4):30-41.

Book chapter
Schiffman JD. Immunology of influenza. In: Cane MB, ed. *Viruses and Influenza.* Orlando, FL: Academic Press; 1990:191-196.

Book
Avery GB. *Neonatology: Pathophysiology and Management of the Neonate.* 3rd ed. Philadelphia, PA: JB Lippincott; 1987.

Tables
Number and title tables consecutively in the order in which they are mentioned in the text. Each column within a table should have a heading. Define abbreviations in the legend.

Figures
If you are unable to submit figures electronically, submit 1 copy by post. On the back of the copy note the figure number, last name of the primary author, and orientation (top/left/right). Include the name of the photographer or illustrator, if applicable. In clinical photographs in which the patient can be recognized, include a release signed by the patient or guardian granting permission to publish the photograph. If permission is not obtained, the photograph will be edited to ensure anonymity.

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Proprietary Interest
Authors with financial or proprietary interest in the subject matter or materials discussed (eg, employment, stock ownership, honoraria, etc) will be asked to submit a statement for publication on the first page of the article.

Drug Names
Use full generic names only, including inactive moiety. The trade name of a drug may be cited in parentheses the first time the generic name appears.

Abbreviations and Symbols
With the exception of standard units of measurements, avoid abbreviations. Do not use abbreviations in the title or abstract. When using a large number of abbreviations, list them in a table.

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Upon publication, authors will receive 2 complimentary copies of the issue in which their article appears. If you wish to purchase additional copies or reprints, notify the managing editor when you grant final approval of your edited article.

SUBMITTING MANUSCRIPTS/MANUSCRIPT PROCESSING
All manuscripts must be submitted electronically to athmsubmissions@innovisionhm.com. They are usually acknowledged and assigned a manuscript number within a week of receipt in our office. The manuscript number should be used in all future communications with InnoVision Health Media.

Include your mailing address, phone number, and fax number in your e-mail message as well as an electronic version (Microsoft Word preferred) of each item listed in the checklist below. Tables and figures should be included as attachments if possible. If they cannot be sent as attachments, please send a high-quality hard copy by post.

Manuscripts should be submitted as a series of files including a cover letter, the manuscript (including title page, the abstract, manuscript text, and references), and all tables, figures, and legends. Please submit a signed copy of the copyright transfer form. Most submissions are subject to peer review (see details below). Presentation of data at scientific meetings does not preclude submission.

Peer Review
The majority of manuscripts submitted to us are put through peer review. The time from receipt of initial submission to final editorial decision takes an average of 3 to 6 months. Manuscripts that our editors believe warrant rapid publication (most commonly original research) will be peer-reviewed as quickly as possible, with a goal of publication of within 2 months after receipt of the manuscript. We follow the International Committee for Medical Journal Editors (www.icmje.org) on publication guidelines and encourage authors to follow their recommendations if possible.

One of the journal's editorial staff will read your paper to assess the validity, originality, and significance of the work presented. Our acceptance rate is low; an important feature of our selection process is that many papers are turned away on the basis of in-house evaluation alone. That decision will be communicated quickly. Positive in-house reviews by the editorial staff are followed by peer review. If the manuscript is sent out for peer review, you will be informed by the editorial coordinator. Reviews are blinded; that is, authors and reviewers are not identified by name during the review process. After the manuscript has been reviewed, you will be informed whether it has been accepted for publication, rejected, or requires revision.

Revisions
Revisions may be requested for submissions that pass the initial review stages. This does not constitute acceptance for publication but is an invitation to strengthen your paper for further scrutiny. After revision, your paper may again be subjected to a full peer review, usually by the same reviewers. The reviewers' comments must be answered or rebutted in the text of the manuscript (where applicable) and in a separate, accompanying letter to facilitate the review of your revised manuscript. Some of the comments will be technical and some substantive; all should be addressed.

Decision
You will be notified via e-mail of the final decision about your submission.

Accepted Manuscripts
At the time your paper is accepted for publication, you may refer to it as being "in press." No publication date will be set at this time; an edited version of your manuscript will be sent to you for approval, and you will be notified when a publication date has been established. We increasingly publish articles online ahead of print publication. You will be informed at least a week in advance of the online publication dates. The online article is identical to the print version and is citable by the digital object identifier (DOI).

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Sometimes we make mistakes in rejecting a manuscript, and if you think we have, we would like to hear an appeal from you for us to reconsider our decision. In your appeal, please tell us why you think our decision to reject your manuscript was mistaken and set out your specific responses to comments you feel are the main reason for your manuscript being rejected.

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Revised May 2009

CHECKLIST FOR AUTHORS

Cover Letter
With each manuscript submitted, include a cover letter that explains why you think this journal in particular should publish your paper. The paper may be longer than requested; please take this opportunity to say what concessions you might be prepared to make (eg, omission of a table or shortening of the methods).

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- Abstract, on a separate page, including title, structured abstracts up to 250 words, unstructured up to 150 words
- Text, starting on a new page, printed on one side of each page only
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- Tables, including title and legend, if applicable
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- Permissions (eg, for personal communications or reproduced figures)
- Acknowledgments (obtain written permission from each person listed in this section)

ANEXO B

De: Katie Tholkes [ktholkes@innovisionhm.com]
Enviado: terça-feira, 16 de setembro de 2014 12:30
Para: Maria Antonia Z de Figueiredo
Assunto: ATHM 5209: Revision Accepted

Dear Dr Figueiredo:

I am pleased to inform you that your revised manuscript, "Antioxidant agents: a future alternative approach in the prevention and treatment of radioinduced oral mucositis?" has been accepted by our editor in chief for publication in *Alternative Therapies in Health and Medicine.* Thank you for your extra efforts in revising your paper.

The journal staff will decide at a future editorial meeting when your article will be published. We will be in touch with you when we begin to prepare your manuscript for publication. Please note it may be several months before your article is published, as we have many manuscripts in line and only three or four are published per issue.

Thank you again for your fine contribution to *ATHM*. Please let me know if you have any questions.

Sincerely,

Katie Tholkes

Editorial Assistant

Alternative Therapies in Health and Medicine

ANEXO C

Instructions for Authors

Close

• Original Articles – body text is limited to 3500 words. There may be 45 references and no more than six figures/tables.

Review Articles – generally solicited by the editors but unsolicited proposals containing an abstract and outline can be sent to the editors for consideration. The word limit for Review Articles is up to 4,000 words for body text (excludes figures, charts, references, abstract).

Letter to the Editor – SCC occasionally accepts letters to the editor pertaining to articles published in the Journal. These should not exceed 1000 words body text and will be passed to the authors of the article to which the comment applies to solicit a response. There may be up to 10 references.

Commentary – articles should be on innovative areas or opportunities for further research. The body text is limited to 1,000 words. There may be up to 20 references, and one figure or chart.

Review procedure

All manuscripts undergo strict peer review. Manuscripts are initially considered by the Editor-in-Chief. Any manuscript that does not meet the general certain criteria of the journal, e.g.

• relevance to the aims of the journal with the topic being of overall general interest

• sufficiently original and contributing to the advancement of the field,

• clearly written with appropriate study methods, well-supported data and conclusions which are supported by the data

will be returned to the author without review.

All other submitted manuscripts are assigned to an Associate Editor who will manage the external peer review process and editorial decision. The Journal encourages authors to recommend individuals who could be considered as reviewers, providing the editorial office with full names and contact details. Authors are also given the opportunity to request the exclusion of a specific reviewer. In this case, authors should provide justification for their request.

Each manuscript is reviewed by a minimum of two expert referees who will provide unbiased, critical and independent assessment of the submission. The (corresponding) author is notified by email of the editorial decision, which will include any applicable criticisms and comments from the reviewers and managing editor. The decision to accept with/without revision or otherwise, will be made by the Editor-in-Chief based on the critical assessments of the experts.

Manuscripts which are returned to the authors for minor or major modifications should be resubmitted online within one or three months, respectively; otherwise, they will be considered withdrawn. Normally, revised manuscripts are reassessed by the same reviewers to determine if the authors have satisfactorily addressed their criticisms and comments. Depending upon this evaluation, the manuscript may be accepted or rejected. Any questions or concerns regarding the editorial decision on a manuscript must be submitted directly to the editorial office within 3 months.

Confidentiality

All manuscripts are treated by the assigned reviewers as privileged and confidential information. Reviewers may request advice from another party, subject to the general principles of confidentiality and permission of the managing editor. Reviewers' comments are not published or made available publicly except with the prior written permission of the reviewer, author and editor. However, reviewers' comments are shared with the other reviewers of the same paper, and reviewers will be notified of the editor's decision. The reviewers' identity remains anonymous. All reviewers are asked to disclose any potential conflict that could influence their opinions of manuscripts, prior to review of manuscript.

Manuscript preparation

We urge authors to follow the guidelines for authors to speed up the review and publication process.
All manuscripts are subject to copyediting upon acceptance, however, authors are asked to ensure that manuscripts from non-native English language speakers should have the language and grammar checked by a native speaker or a professional agency. Poorly written articles cannot be reviewed and will be returned to the authors.

- Authorship Criteria and Contributions

All listed authors should have seen and approved the final version of the manuscript.
All authors of accepted articles must sign an authorship form affirming that they have met all three of the following criteria for authorship, thereby accepting public responsibility for appropriate portions of the content:
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2. drafting the article or revising it critically for important intellectual content;
3. approval of the version to be published and all subsequent versions.
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Other group members who are not authors should be listed in the Acknowledgment section of the manuscript as participating investigators.
Individuals who do not meet the criteria for authorship but who have made substantial, direct contributions to the work (e.g., purely technical help, writing assistance, general or financial or material support) should be acknowledged in the
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Copies of such material should be included with the submitted paper as a supplemental file.
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Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well

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Title page

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusions

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Structured Abstract

Authors are asked to state the relevance of their manuscript to inform research, policies and/or programs.

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

- LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

Scientific style

- Please always use internationally accepted signs and symbols for units (SI units).
- Genus and species names should be in italics.
- Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

The entries in the list should be numbered consecutively.

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance.N Engl J Med 965:325–329

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- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

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- ISSN.org LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

- EndNote style (zip, 2 kB)

Authors preparing their manuscript in LaTeX can use the bibtex file spbasic.bst which is included in Springer's LaTeX macro package.

Tables

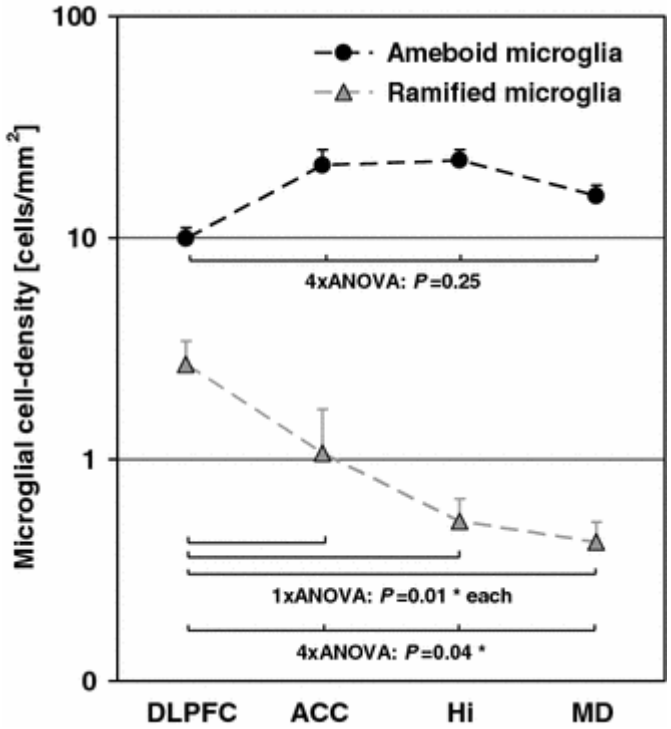
- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

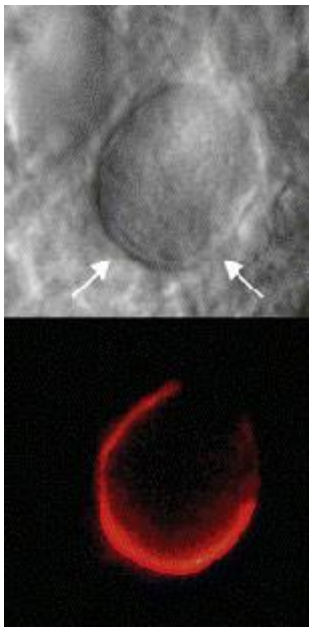
- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



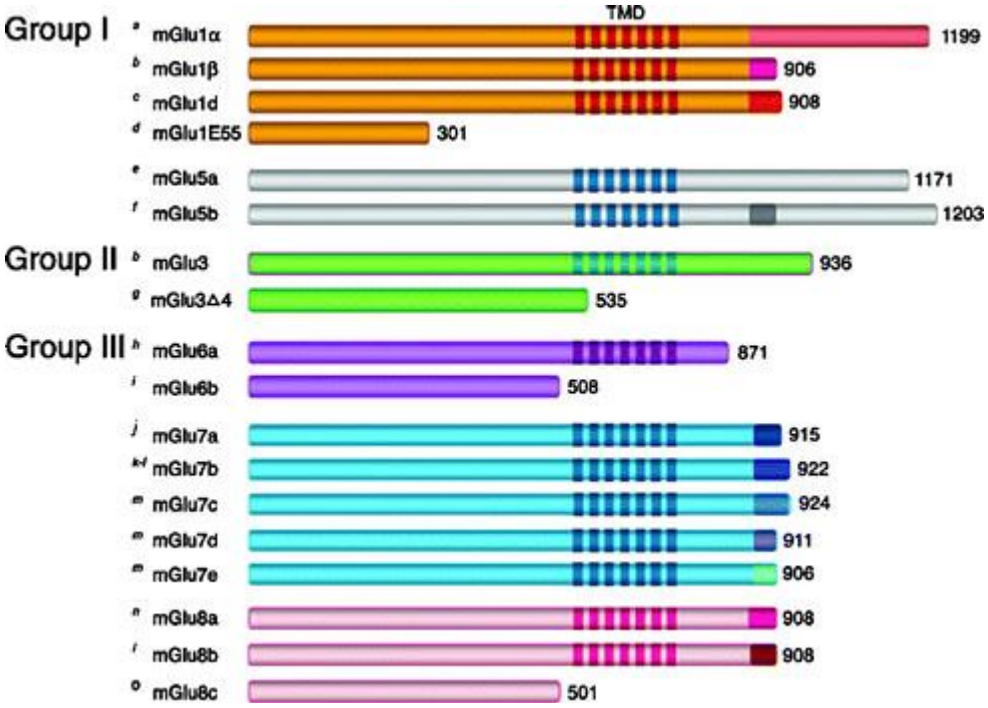
- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art



- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- When preparing your figures, size figures to fit in the column width.
- For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.
- For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

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Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

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Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

- Always use MPEG-1 (.mpg) format.

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

Spreadsheets

- Spreadsheets should be converted to PDF if no interaction with the data is intended.
- If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
- Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

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Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" before the References when submitting a paper:

- Disclosure of potential conflicts of interest
- Research involving Human Participants and/or Animals
- Informed consent

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If no conflict exists, the authors should state:

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

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When reporting studies that involve human participants, authors should include a statement that the studies have been approved by the appropriate institutional and/or national research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.
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The following statements should be included in the text before the References section:
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For retrospective studies, please add the following sentence:
"For this type of study formal consent is not required."

2) Statement on the welfare of animals

The welfare of animals used for research must be respected. When reporting experiments on animals, authors should indicate whether the international, national, and/or institutional guidelines for the care and use of animals have been followed, and that the studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted (where such a committee exists).
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"This article does not contain any studies with human participants performed by any of the authors."
"This article does not contain any studies with animals performed by any of the authors."
"This article does not contain any studies with human participants or animals performed by any of the authors."

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All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. Hence it is important that all participants gave their informed consent in writing prior to inclusion in the study. Identifying details (names, dates of birth, identity numbers and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scientific purposes and the participant (or parent or guardian if the participant is incapable) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort scientific meaning.
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If identifying information about participants is available in the article, the following statement should be included:
"Additional informed consent was obtained from all individual participants for whom identifying information is included in this article."

ANEXO D

ENC: JSCC: Submission Confirmation for Topical application of Aloe vera and vitamin E on induced ulcers of the tongue in rats subjected to radiation: clinical and histological evaluation

Dear Dr Figueiredo,

Your submission entitled "Topical application of Aloe vera and vitamin E on induced ulcers of the tongue in rats subjected to radiation: clinical and histological evaluation" has been received by journal Supportive Care in Cancer.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is http://jscc.edmgr.com/.

Circumstances may vary, but the review process can usually take 6-8 weeks to be completed after reviewers have agreed to evaluate a manuscript. You can follow the progress of your paper through our online system. If you have not received a decision from the Editor-in-Chief by 12 weeks from the date you submitted your paper, you may also inquire regarding its status by clicking on the CONTACT US link in Editorial Manager.

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to this journal.

Kind regards,

Lori Fleming
Editorial Office
Supportive Care in Cancer

ANEXO E

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 23 de outubro de 2013

O Projeto de: Dissertação

Protocolado sob nº:	0051/13
Intitulado:	Aplicação tópica de aloe vera e vitamina E em úlceras induzidas em língua de ratas submetidas à radioterapia: Avaliação clínica e histológica.
Pesquisador Responsável:	Profa. Dra. Maria Antonia Z. de Figueiredo
Pesquisadores Associados:	Letícia de Freitas Cuba
Nível:	Dissertação / Mestrado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *23 de outubro de 2013*

Este projeto deverá ser imediatamente encaminhado ao CEUA/PUCRS.

Profa. Dra. Luciane Macedo de Menezes
Coordenadora da Comissão Científica e de Ética da
Faculdade de Odontologia da PUCRS

Av. Ipiranga, 6681, Prédio 06 sala 210
Porto Alegre /RS – Brasil – Cx. Postal:1429
90619-900
Fone/Fax: (51) 3320-3538
e-mail: odontologia-pg@pucrs.br

ANEXO F

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA, INOVAÇÃO E DESENVOLVIMENTO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Ofício 13/14 - CEUA

Porto Alegre, 12 de março de 2014.

Prezado Sr(a). Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou seu Protocolo de Pesquisa, registro CEUA 13/00376, intitulado **"Aplicação tópica de Aloe Vera e vitamina E em úlceras induzidas em língua de ratas submetidas à radioterapia: Avaliação clínica e histológica".**

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está **autorizada** a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Nº de Animais	Espécie	Duração do Projeto
36	Rattus norvegicus	03/2014 – 12/2014

Atenciosamente,

Prof. Dr. João Batista Blessmann Weber
Coordenador da CEUA/PUCRS

Ilma. Sra.
Profa. Maria Antônia Zancanaro de Figueiredo
FO
Nesta Universidade

PUCRS
Campus Central
Av. Ipiranga, 6681 – P. 99 – Portal Tecnopuc – sala 1512
CEP: 90619-900 – Porto Alegre/RS
Fone: (51) 3353-6365
E-mail: ceua@pucrs.br

APÊNDICES

APÊNDICE A

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA
FICHA DE AVALIAÇÃO CLÍNICA

IDENTIFICAÇÃO

Rata nº: __ **Peso inicial:** _____Kg **Peso final:** _____Kg

Tratamento:

□ Grupo 1 (produto 1)
□ Grupo 2 (produto 2)
□ Grupo 3(produto 3)

Tempo:

□ Subgrupo A (6 dias)
□ Subgrupo B (8 dias)

AVALIAÇÃO CLÍNICA LOCAL

□ Presença de úlcera: ( ) Sim ( ) Não

□ Tamanho da ulceração: _____

□ Outras áreas de ulceração: □ Sim □ Não

Localização: _____

Sinais inflamatórios: ( ) Eritema ( ) Edema

Sinais secundários: □ Sim □ Não

Especifique (sangramento, supuração, abscesso...):

_____
_____
_____
_____
_____
_____
_____
_____

Fotos: _____

Data da avaliação: __/__/____.

APÊNDICE B

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA
FICHA DE AVALIAÇÃO HISTOLÓGICA

IDENTIFICAÇÃO

Rata nº: ___ Lâmina nº:_____

Tratamento **Tempo**

□ Grupo 1 (produto1) □ Subgrupo A (6 dias)
□ Grupo 2 (produto2) □ Subgrupo B (8 dias)
□ Grupo3 (produto3)

AVALIAÇÃO HISTOLÓGICA (HE) DA ÁREA COM MAIOR RESPOSTA CELULAR

Variável/Resposta	SIM	NÃO
EDEMA		
HIPEREMIA		
PRESENÇA DE CÉLULAS INFLAMATÓRIAS (linfócitos, plasmócitos, macrófagos, neutrófilos, eosinófilos e células gigantes)		
FIBROPLASIA		

Escore:

□ 0 – Ausente: Ausência de inflamação

□ 1 – Leve: Células mononucleares esparsas

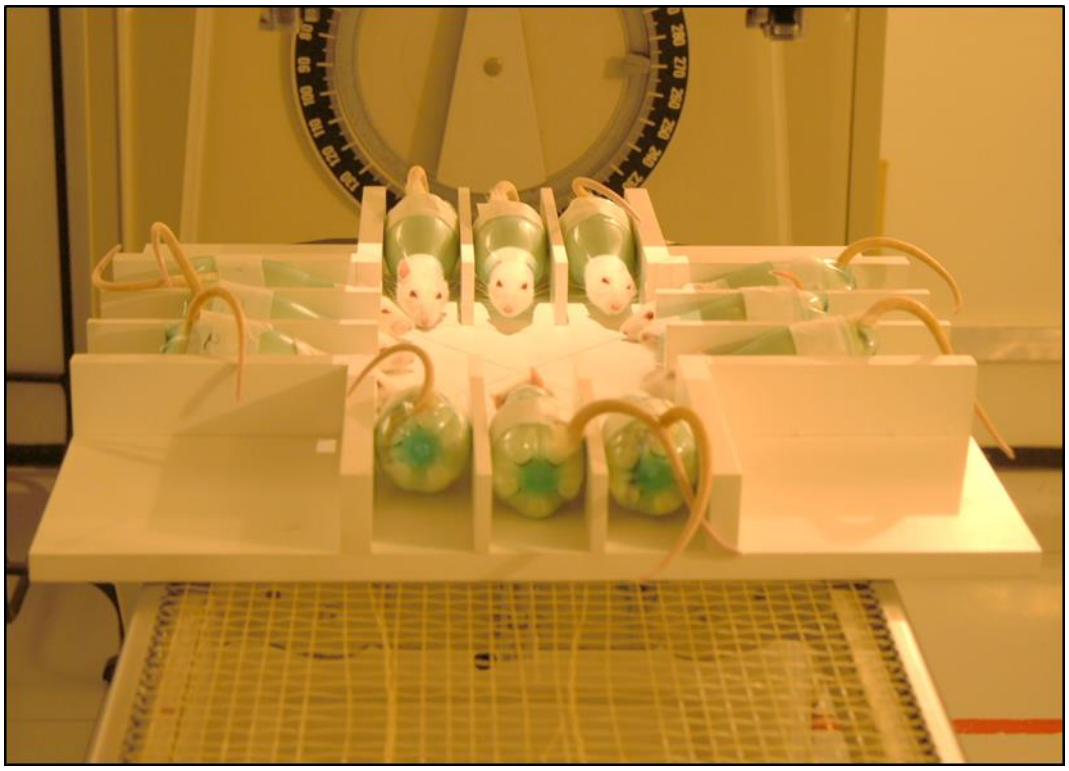
□ 2 – Moderada: Infiltrado mononuclear e/ou neutrófilos e eosinófilos esparsos

□ 3 – Intensa: Infiltrado polimorfonuclear de neutrófilos e eosinófilos

Observações:_____

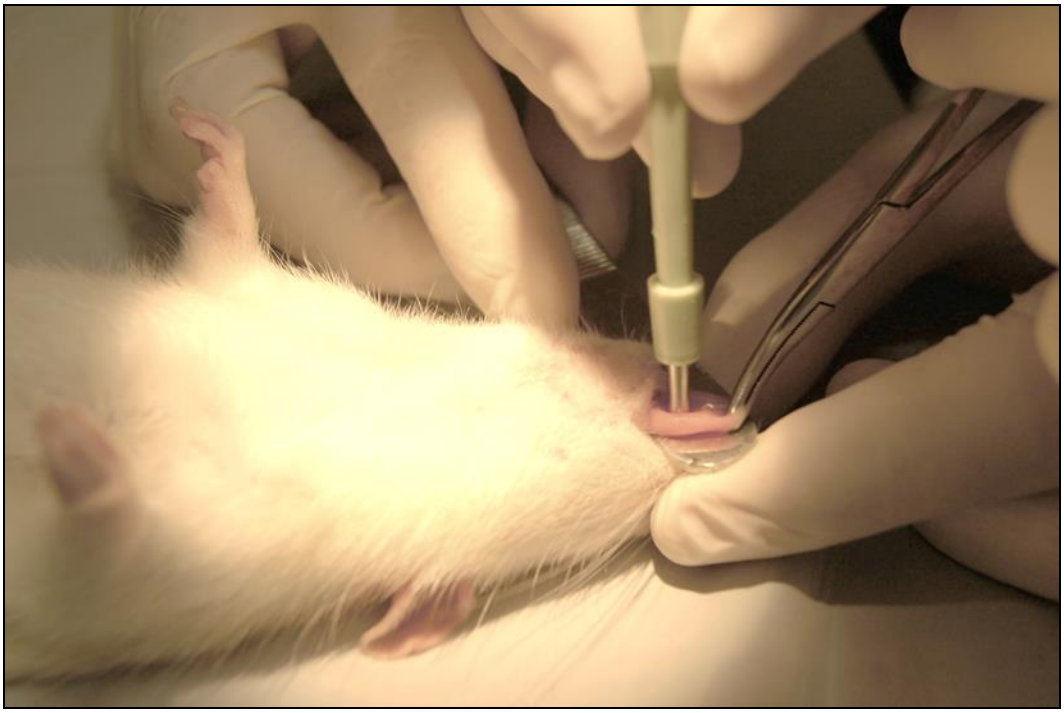
Fotos:_____Data da avaliação:___/___/___

APÊNDICE C



Contenção e posicionamentodos animais para a irradiação, destacando o portal empregado.

APÊNDICE D

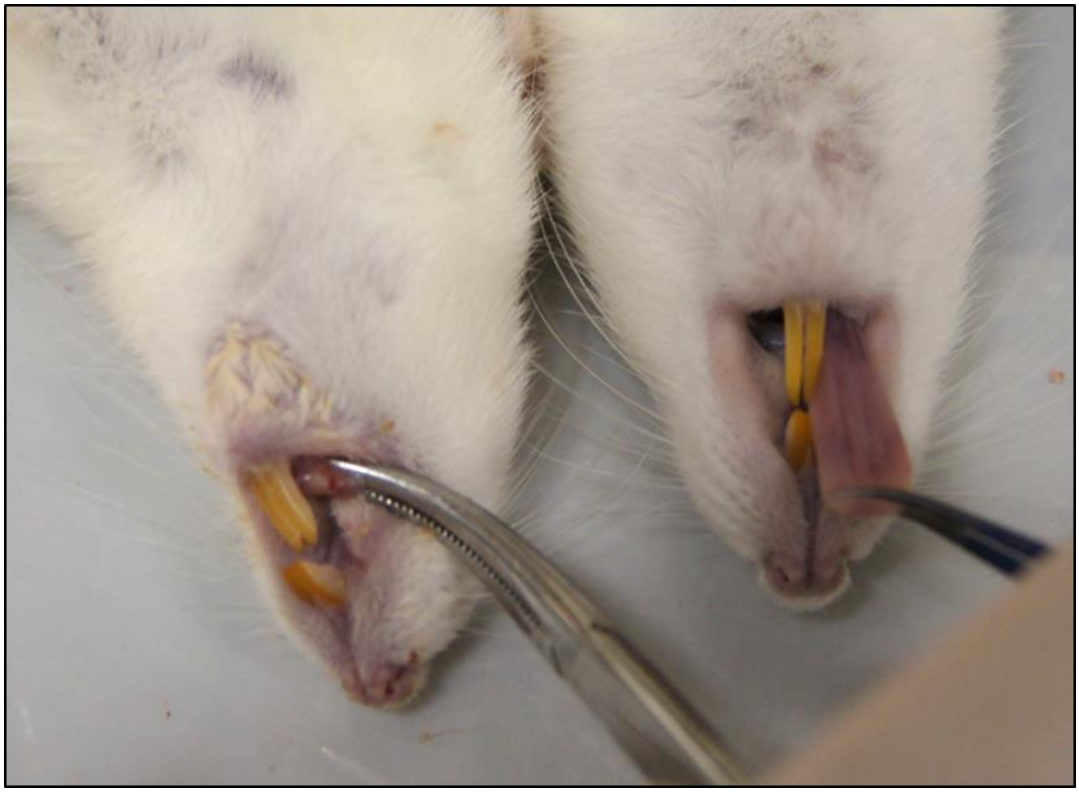


Indução da úlcera na porção central do ventre da língua utilizando *punch* de 3mm.

Procedimento evidenciando a aplicação tópica do produto de acordo com os respectivos grupos.

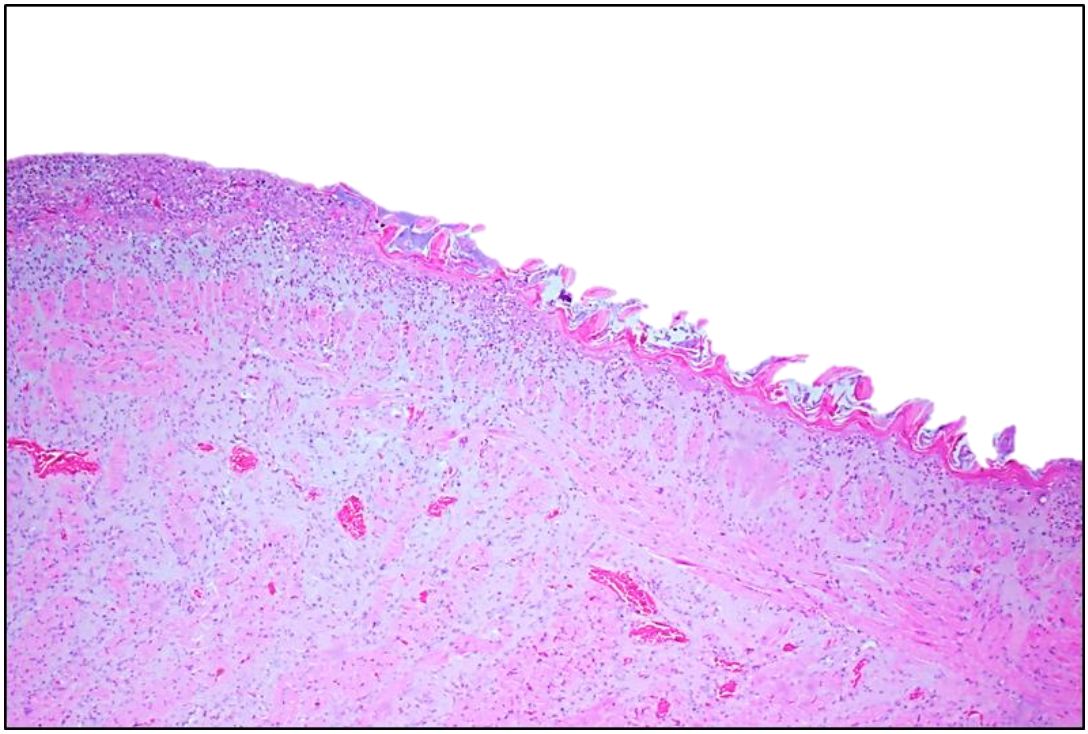
APÊNDICE F

Umedecimento e perda de pelos da região perioral de animal submetido a radioterapia.



Comparação da capacidade de tração da língua de animal irradiado (grupo C7) em relação a um não irradiado demonstrando fibrose tecidual.

APÊNCICE G



Fotomicrografia evidenciando atrofia e perda das papilas do dorso da língua (HE - aumento aproximado:100x).