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ORIGINAL ARTICLE

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

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**Abstract**
*Objective* The aim of this study is to assess the effect of two 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 three groups of 12 animals each (400 mg VE, 70 % AV and control) and two time periods (5 and 7 days). They were irradiated with a single dose of 30 Gy, and after 24 h, 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

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## Introduction

Oral mucositis (OM) is the common acute local effect in patients treated with RT to the head and neck. It is a debilitating condition that typically begins around the third week of treatment (cumulative dose of 30 Gy), but can also occur sooner [1]. 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 [2–8].

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 vitamin E (VE), and flavonoids present in plants, including *Aloe vera* (AV), empirically used as an aid in healing ulcers, have been widely studied and suggested as possible radioprotective agents for the prevention of mucositis [2–4,15–24].

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 [5, 6, 8, 12, 25]. 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, weighed on a Urano balance (model IDU 2500/0.5). 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 three 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 two time periods [5 and 7 days]. Afterwards, they were immobilized with the aid of retainer and positioned vertically so that only 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 × 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.

After 24 h of irradiation, the animals were sedated and anesthetized by isoflurane inhalation at 4 V% 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 by a calibrated and blinded examiner 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 1 mm 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 (Fig. 1).

### Treatments

- Seventy percent Alo*e 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 substance in gel form): prepared in University Pharmacy Panvel, PUCRS

### Clinical and histological evaluation

After euthanasia, each animal was immediately weighed, and we 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.

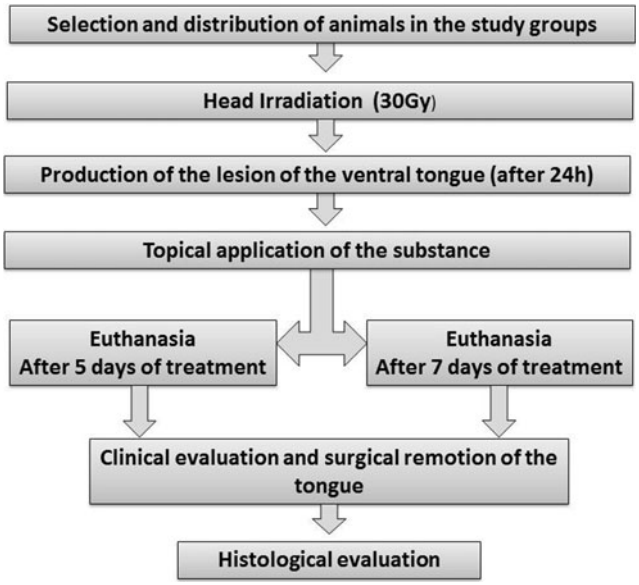


**Fig. 1** Study design

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).

The slides 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 below [26–28]:

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

To the best of the authors' knowledge, this histopathologic method was not tested in oral mucositis so far. It is proposed by the authors based on their experience with this method evaluating inflammatory response to various dental materials and oral agents.

### 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, two 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 limited motion 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 three and two animals (Fig. 2). 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 five animals in the AV group. In the control group, all animals remained with some degree of ulceration (Fig. 3). 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 $p = 0.002$)

### 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

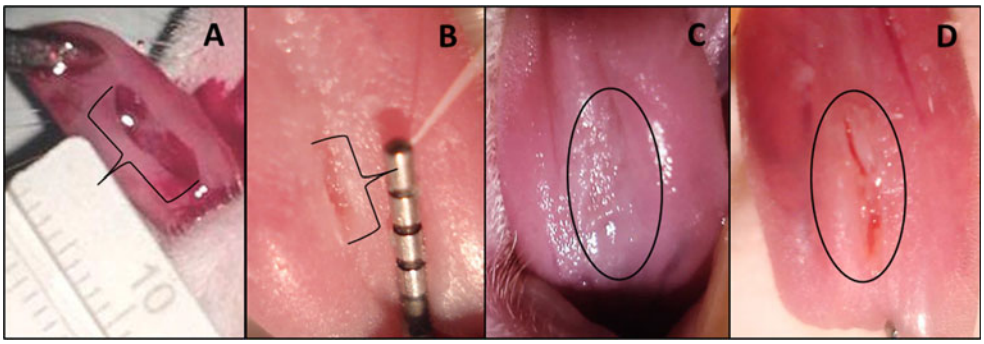


**Fig. 2** Lesion induced on ventral tongue of rats after irradiation: clinical evaluation at 5 days. **a** Immediately after induction of lesion measuring 6 × 3 × 1 mm. **b** Animal of AV5 group showing a discrete lesion. **c** Animal of VE5 group with total healing of lesion. **d** Animal of C5 group demonstrating persistence of lesion

between mild and moderate, while all animals in the C7 group developed an intense degree of response (Fig. 4).

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).

## 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, 2, 5, 8].

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 plays a key role in the initiation of OM [3, 9–11, 13–16, 29]. 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 [3, 29].

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 [2, 5, 30]. 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 two

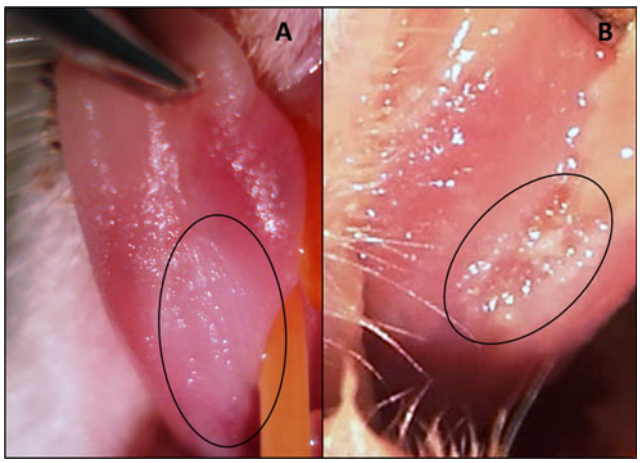


**Fig. 3** Lesion induced on ventral tongue of rats after irradiation: clinical evaluation at 7 days. **a** Animal of VE7 group showing total healing of induced lesion. **b** Animal of C7 group with persistent lesion

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

| Size of lesion | 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 $mm^2$ | 0 | 0 | 4 $mm^2$ |
| Minimum | 0 | 0 | 4 $mm^2$ | 0 | 0 | 1 $mm^2$ |
| Maximum | 4 $mm^2$ | 2.5 $mm^2$ | 90 $mm^2$ | 3 $mm^2$ | 0 | 30 $mm^2$ |
| *P* value | 0.006 | | | 0.002 | | |

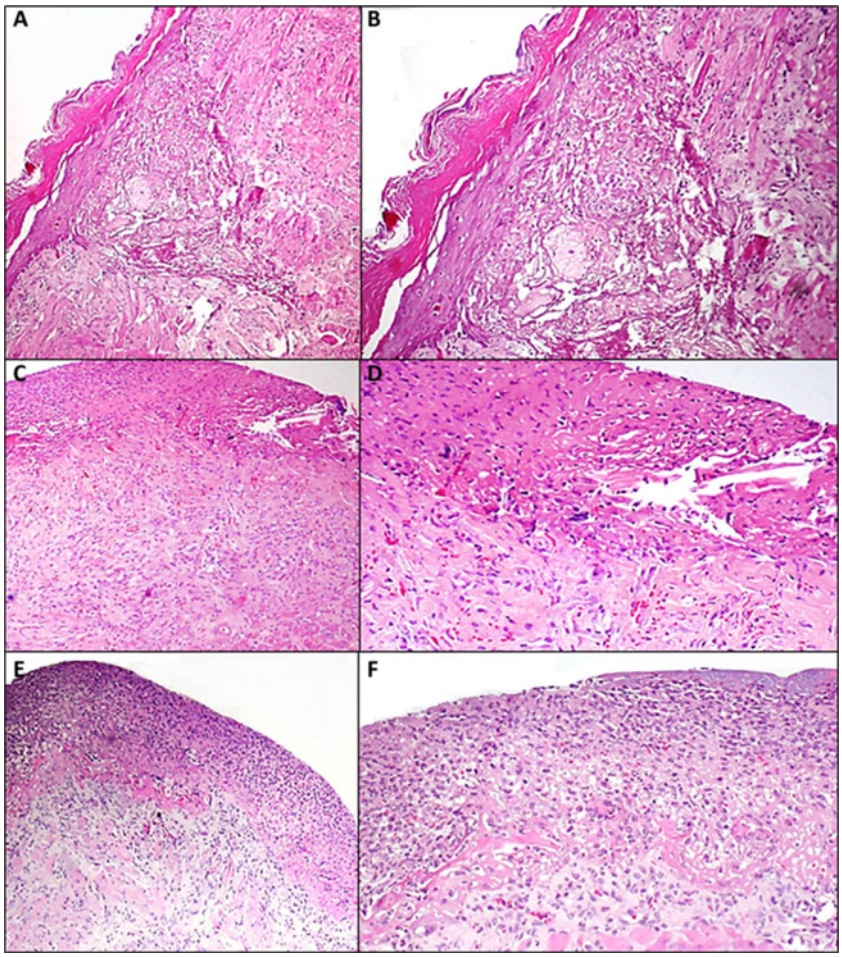


**Fig. 4** Histological characteristics observed in tongue of irradiated rats (photomicrographs, HE staining). **a** Sparse mononuclear cells featuring mild inflammation (approximate magnification ×40). **b** Mild inflammation (approximate magnification ×100). **c** Inflammatory infiltration of mononuclear cells with scattered neutrophils and eosinophils featuring moderate inflammation (approximate magnification ×40). **d** Moderate inflammation (approximate magnification: ×100). **e** Inflammatory infiltrate of polymorphonuclear cells featuring intense inflammation (approximate magnification ×40). **f** Intense inflammation (approximate magnification: ×100)

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.

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

| Inflammatory response | TIME | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 days | | | 7 days | | | *P* value[a] | | |
| | AV ($n=5$) | VE ($n=6$) | C ($n=5$) | AV ($n=6$) | VE ($n=5$) | C ($n=6$) | AV | VE | C |
| Moderate | 2 | 5 | 5 | 4 | 3 | 0 | | | |
| Intense | 0 | 0 | 0 | 0 | 0 | 6 | | | |
| *P* value[b] | 0.089 | | | 0.002 | | | | | |
| 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[b] | 0.089 | | | 0.002 | | | | | |

[a] Comparison between times

[b] Comparison between treatments in relation to control

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].

During clinical analysis it was not possible to keep the examiner blinded; this represents a limitation of the study. However, the results found in the histological analysis, which was blinded, corroborated with the clinical findings.

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 VE 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 VE was reinforced, since there was a decrease in oxidative stress and increased plasma AOX levels [31].

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 [32]. 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, a clinical patient trial and an animal model study tested the efficacy of formulations based on AV in the management of OM and showed no positive results [21, 33]. 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. 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 VE and AV in the literature.

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 four clinical studies in humans applied topically or systemically in patients receiving RT or chemotherapy, and in three 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 [34].

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 [35, 36]. In this work, we observed weight loss exclusively in 7-

day groups. In these animals, there was also tongue-limited movement, which was not seen in the animals evaluated at 5 days. This finding suggests that situation 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.

Based on these results and comparing them with previous studies, it is observed that the AOX tested showed regenerative 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 oxidative stress, still seems to be a challenge [3, 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.

**Compliance with ethical standards** 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.

**Conflict of Interest** The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

The authors have full control of all primary data and agree to allow the journal to review their data if requested.

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