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Evaluation of radioprotective effect of aloe vera and zinc/copper compounds against salivary dysfunction in irradiated rats

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Abstract: The aim of this study was to evaluate the radioprotective and reparative effects of compounds based on aloe vera, zinc, and copper against salivary gland dysfunction in Wistar rats. A total of 150 Wistar rats were randomly divided into 12 groups, in which the animals received aloe vera and/or zinc and copper. In eight of these groups the animals were also subjected to irradiation before or after administration of the substances. After 27 days, sialometry tests were performed. Data were analyzed using ANOVA and the Tukey test ($P < 0.05$). Rats that had been administered aloe vera before or after irradiation showed a significantly higher salivary flow rate than rats that had been simply irradiated. When both substances were administered, a statistically significant difference in the salivary flow rate was observed in comparison with the irradiation alone group seven days after irradiation. The present results suggest that aloe vera exerts positive protective and reparative effects, and can be considered a potential radioprotective substance. (J Oral Sci 56, 191-194, 2014)

Keywords: radiotherapy; Wistar rat; aloe; zinc; copper.

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Introduction

The deleterious effects of ionizing radiation are mediated by the formation of free radicals, which are highly reactive. They remove hydrogen atoms from acids, thereby causing lipid peroxidation and consequently cell death (1). The chemical changes caused by radiation can affect a cell in several ways, and can result in premature death, retardation of cell division or even permanent modifications which are passed on to the cells of later generations (2).

Xerostomia caused by interruption or reduction of salivary flow in patients who have undergone radiation therapy is one of the most common and major treatment-related toxicities in the head and neck region. In many cases, xerostomia can lead to tooth loss, opportunistic infections and discomfort for affected patients (3).

Considering these factors, efforts to preserve the salivary glands are crucial, and can increase the quality of life (4). Several previous studies have attempted to find effective radioprotective agents (5-7). A number of different therapeutic approaches have yielded favorable results, including stabilization of the basement membrane using lidocaine hydrochloride, which protects the ultrastructure of the gland and maintains the salivary flow (5-7). Antioxidants have also been tested for their protective effects against damage caused by free radicals. However, synthetic substances have a number of limitations, which include adverse effects such as toxicity, which limit their clinical use (8,9).

Aloe vera is a naturally occurring substance that accelerates the healing of wounds and increases the tensile

Table 1 The different experimental groups used in this study

Groups	No. of animals	Substance administered	Study design
1	10	Water	Sacrificed 27 days after start
2	10	Water	Sacrificed 7 days after irradiation
3	10	Water	Sacrificed 27 days after irradiation
4	10	Aloe vera 225 mg/kg	Aloe vera (20 days) + interval (7 days) => sacrificed
5	10	Aloe vera 225 mg/kg	Aloe vera (20 days) => irradiation + interval (7 days) => sacrificed
6	10	Aloe vera 225 mg/kg	Irradiation => Aloe vera (20 days) + interval (7 days) => sacrificed
7	10	Zinc (9 mg/kg) + Copper (7 mg/kg)	Zinc and copper (20 days) + interval (7 days) => sacrificed
8	10	Zinc (9 mg/kg) + Copper (7 mg/kg)	Zinc and copper (20 days) => irradiation + interval (7 days) => sacrificed
9	10	Zinc (9 mg/kg) + Copper (7 mg/kg)	Irradiation => Zinc and copper (20 days) + interval (7 days) => sacrificed
10	10	Aloe vera (225 mg/kg) + Zinc (9 mg/kg) + Copper (7 mg/kg)	Aloe vera + Zinc and copper (20 days) + interval (7 days) => sacrificed
11	10	Aloe vera (225 mg/kg) + Zinc (9 mg/kg) + Copper (7 mg/kg)	Aloe vera + Zinc and copper (20 days) => irradiation + interval (7 days) => sacrificed
12	10	Aloe vera (225 mg/kg) + Zinc (9 mg/kg) + Copper (7 mg/kg)	Irradiation => Aloe vera + Zinc and copper (20 days) + interval (7 days) => sacrificed

strength of scar tissue (10). In addition, it has antimicrobial (11), anti-tumor/anti-oxidant (9,12), antirheumatic (13) and antidiabetic properties (14). Aloe vera extract has also proven to be effective for the treatment of burns induced by radiation (15).

Zinc stimulates the production of proteins that capture carcinogenic substances, such as cadmium (16). It also helps to scavenge free radicals as part of the enzyme superoxide dismutase, which disrupts the molecules responsible for the formation of these radicals that are harmful to living tissue. The enzyme also takes up copper, allowing it to disable free radicals and thus boost the immune system (16).

Little is known about the radioprotective effects of aloe vera and zinc/copper on the salivary glands. Therefore, the aim of the present study was to evaluate, through measurement of salivary secretion, whether administration of these substances could ameliorate radiation-induced damage to salivary glands in rats.

Materials and Methods

This study was started after receiving approval from the Animal Research Ethics Committee at the Faculty of Dentistry of Piracicaba (UNICAMP), registered as protocol 2675-1. A total of 150, 3-month-old male rats (*Rattus norvegicus, Albinus Wistar*), weighing approximately 300 g were used. Throughout, the animals were kept in polycarbonate cages, in an environment where both temperature and humidity were controlled and there was a cycle of alternating 12-h days and nights. The food provided consisted of standard balanced feed and the control groups had *ad libitum* access to water. They were divided randomly into the following experimental groups: 1) Control group; 2) Irradiated control 1; 3) Irradiated control 2; 4) Aloe vera; 5) Aloe vera before irradiation; 6) Aloe vera after irradiation; 7) Zinc and copper control; 8) Zinc and copper before irradiation; 9) Zinc and copper after irradiation: 10) Aloe vera + zinc + copper control; 11) Aloe vera + zinc + copper before irradiation; and, 12) Aloe vera + zinc + copper after irradiation (Table 1).

All animals were anesthetized intramuscularly with 80 mg/kg ketamine hydrochloride (Dopalen Agribrands of Brazil Ltd., Paulínia, SP, Brazil) and 8 mg/kg xylazine hydrochloride (Rompum, Bayer SA, São Paulo, SP, Brazil), and then exposed to a single 15-Gy dose of radiation. The irradiation was carried out using a Varian linear accelerator, Clinic 6/100 model, with a focal length of 100 cm and a 15 × 20-cm collimation field, so that only the head and neck were irradiated. For the experimental groups (groups 4-12), all substances were administered by oral gavage for 20 consecutive days. Before sacrifice, sialometry was performed, for which the animals were anesthetized again according to the protocol described above. Afterwards, pilocarpine (0.1 g of pilocarpine hydrochloride/10 mL distilled water) was administered intramuscularly to stimulate salivary flow. To collect the saliva, all animals were placed in cages so that their heads were turned towards a previously weighed, 30 mL plastic container. As soon as flow was noted, whole saliva was collected for 30 min into pre-weighed tubes. The containers were weighed again, and the final saliva volume was estimated from the weight of the saliva secreted, assuming that the specific gravity of saliva is 1.0 g/cm^3 (Fig. 1). After the sialometry test, all the

Fig. 1 Sialometry procedure.

Table 2 Mean ± SD of pilocarpine-stimulated salivary flow in milligrams (mg)

Experimental groups	Salivary flow (Grams)
1	2.9 ± 1.18 [A]
2	1.5 ± 0.29 [C]
3	1.0 ± 0.84 [C]
4	2.9 ± 0.97 [A]
5	2.6 ± 0.22 [A]
6	2.1 ± 0.56 [A]
7	2.3 ± 0.79 [A]
8	1.6 ± 0.66 [C]
9	1.2 ± 0.76 [C]
10	2.3 ± 0.33 [A]
11	2.2 ± 0.16 [AB]
12	1.2 ± 1.11 [C]

Different superscript letters indicate statistically significant differences ($P < 0.05$).

animals were sacrificed using an overdose of anesthetic. For statistical analysis, data were initially evaluated using ANOVA. The significance of differences at $P < 0.05$ was determined using the Tukey test.

Results

Data analysis by ANOVA showed that there was a statistically significant difference ($P < 0.05$) in salivary flow between Control group 1 and Irradiated control group 2, and between Control group 1 and Irradiated control group 3, whereas the differences between Irradiated control groups 2 and 3 were not significant. Comparison of the control groups revealed no significant differences in salivary flow between Control group 1 and the control groups for each of the substances studied, i.e. groups 4, 7, and 10, suggesting that they exerted no toxic effects on the tissues.

On separately analyzing the study groups that had been given aloe vera, there was a statistically significant difference ($P < 0.05$) between groups 5 and 6 and the Irradiated control groups 2 and 3. However, there were no significant differences between zinc and copper groups 8 and 9 with the irradiated control groups 2 and 3. Analysis of the groups that had received all of the substances studied (aloe vera and zinc in conjunction with copper) revealed a significant difference ($P < 0.05$) between group 11 and irradiated control group 2. However, there was no significant difference in salivary flow between groups 12 and 3.

Analysis of the data demonstrated no significant differences ($P > 0.05$) between the lag phases in any of the groups studied.

Discussion

Despite the widespread use of radiation therapy for certain malignancies of the head and neck, it is known that, in certain cases, even when the salivary glands are not the target organ, irradiation in this region leads to severe alterations in their structure and function. The damage caused is directly proportional to the radiation dose and results in hyposalivation, sometimes almost total reduction of salivary flow (80%) being observed (4). In the present study, we compared control groups with irradiated control groups, and observed a 50% and 66% reduction in salivary flow after 7 days and 27 days, respectively. This suggested that changes had probably occurred at the cellular level, resulting in a greater reduction of salivary flow with time.

Maintenance of salivary flow is critical for the preservation of oral health, and xerostomia is associated with side effects such as changes to the oral mucosa, difficulty with mastication and deglutition, oral infections, and tooth decay, all of which are exacerbated in patients who have undergone radiotherapy (2,6). Therefore, the search for natural substances that can protect against damage due to ionizing radiation has intensified, as this is considered a more cautious strategy than the use of synthetic substances. Earlier studies have demonstrated the protective efficacy of substances such as vitamin E (1) and plant extracts (9). In the present study, aloe vera, zinc and copper were tested, as they are proven antioxidants unassociated with any adverse effects (9,17).

In order to test the protective action of these substances, a high dose (15 Gy) of X-radiation was applied 20 days after the test substances had been administered, as recommended previously (1). After an interval of seven days, salivary flow was maintained in the animals that had been given aloe vera, thus highlighting its significantly protec-

tive effect. When the Irradiated aloe vera group 5 and Irradiated control group 2 were compared, both of which were sacrificed at 7 days after irradiation, the radioprotective effect of aloe was confirmed, in accordance with the findings of a previous study (9). Conversely, the use of zinc or copper alone did not provide effective protection, since the animals' salivary flows were not maintained. However, when aloe vera + zinc + copper were used in combination, there was an increase in salivary flow, once again confirming the radioprotective effect of aloe vera.

As the effect of radiation continues over the long term and damage remains ongoing even after discontinuation of irradiation, the same radiation dose was used in all the groups and the test substances were administered later in order to test their reparative effect. As the extent of the damage is directly proportional to the time elapsed, this reparative effect is very valuable. In the present study, rats that were sacrificed 7 days after irradiation (Group 2) showed less damage to salivatory function than those sacrificed at 27 days (Group 3). Similarly in Group 6, where the animals were also sacrificed at 27 days after irradiation, the salivary flow was maintained, and although lower than that in Control group 1, it was higher than that in Irradiated group 3. These results may have been attributable to the action of aloe vera.

When observing the association between aloe vera + zinc + copper, no significant difference in salivary flow was evident between Control groups 1 and 10. In these groups, no toxicity was observed. However, in Groups 11 and 12 (where the animals received the test substances and were then irradiated), no reparative effect was evident, since in Group 12, the amount of salivary flow was similar to that in Irradiated groups 2 and 3.

Within the limitations of this study, it can be concluded that aloe vera is effective as a radioprotective agent or for preservation of glandular tissue, as it helped to maintain salivary function.

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