

# The Effect of Alcohol Consumption on the Development of Periapical Lesion Induced in Rats: a Micro-CT Analysis

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• **Conflicts of interest:** none declared.

## ABSTRACT

**Objective:** the aim of this study was to evaluate the influence of alcohol consumption on the increase of periapical bone destruction in rats. **Material and Methods:** the sample included 12 Wistar male rats, randomly assigned into a control group and an alcohol group (n=6). Rats in the alcohol group were submitted to self-administration of a 25% pure alcoholic solution. The control group received only filtered water throughout the study. After 5 weeks of adaptation to the alcohol dose, all animals were anesthetized and the pulps of their mandibular left first molar were exposed to the oral cavity to induce periapical lesion. Twenty-eight days after the pulp exposure, those rats were euthanized due to overdose of anesthesia and their mandibles were removed and sectioned to obtain a micro-computed tomographic (micro-CT) scan. The rats' left hemimandibles were fixed and scanned on the SkyScan 1173 (Bruker, Konitch, Belgium) microtomograph. The size of the periradicular lesions was measured from the images obtained on the micro-CT and the surface area and volume were calculated. It was also evaluated the weight gain rate and the ingestion of solid/liquid of both groups. Data were analyzed by the Student's t-test (p<0.05). **Results:** the control group showed higher rates of weight gain and ingested more solid and liquid than the alcohol group (p<0.05). Periapical lesions found in the alcohol group had higher volume and surface area than the ones of the control group (p<0.05). **Conclusion:** the chronic consumption of alcohol contributed to the increase of periapical bone destruction in cases of apical periodontitis.

**Keywords:** Alcoholism; Micro-computed tomography; Osteoclastogenesis; Periapical periodontitis.

## Introduction

Alcohol is a widely consumed psychoactive substance with properties that can cause addiction.<sup>1</sup> Its harmful use has a great influence in the development of health problems, such as mental and behavioral disorders, liver cirrhosis, some cancer types and cardiovascular diseases, as well as injuries resulting from violence and traffic accidents.<sup>2</sup> The World Health Organization (WHO) estimates that the total alcohol per capita consumption worldwide by people over 15 years of age has risen from 5.5 liters of pure alcohol in 2005 to 6.4 liters in 2010.<sup>3</sup>

Alcohol consumption is associated with harmful effects on different organs of the human body. The effects on bone tissue are dose-dependent and the long-term excessive consumption can lead to a decrease in bone mass and bone mineral density and also lead to a poor bone healing.<sup>4</sup> The direct effects of alcohol on bone tissues are related to a decrease in the activity and differentiation of osteoblasts, apoptosis of osteocytes, and an increase in bone resorption by osteoclasts.<sup>5</sup>

Literature evidence supports the hypothesis that periodontal disease can be exacerbated by chronic alcohol consumption, resulting in an increased alveolar bone loss.<sup>6,7,8</sup> As the process of bone resorption in periodontal inflammation occurs similar to the mechanism of development of apical periodontitis, it is believed that excessive alcohol consumption can influence the increase of

the periapical lesion. However, there are few studies assessing the influence of alcohol consumption on increased periapical bone destruction.<sup>9,10</sup>

Recent studies reported an increase in the inflammatory response and osteoclastic activity and a decrease in bone mineral density in periapical lesions of rats submitted to alcohol consumption.<sup>9,10,11</sup> However, to date little is known about this bone loss using non-destructive and three-dimensional imaging methods, such as, for example, the microcomputed tomography. Therefore, the aim of this study was to evaluate the effects of alcohol consumption on the size of periapical lesions induced in rats, using microcomputed tomographic images. The null hypothesis tested was that there would be no difference in the size of the periapical lesions between rats submitted or not to chronic alcohol consumption.

## Material and Methods

### Ethical aspects

This study was conducted in accordance with the ethical principles of animal experimentation of the Local College of Animal Experimentation (COBEA), and the guidelines for the practice of euthanasia of the National Council for Control of Animal Experimentation (CONCEA). It was obtained the approval of the Ethics Committee of Use and Care of Experimental Animals (CEUA) of the Instituto de Biologia Roberto Alcantara Gomes (IBRAG, UERJ) (ID: 049/2018).

### Selection and preparation of the animals

Twelve male Wistar rats (*Rattus norvegicus*, Wistar) from the bioterium of the Laboratory of Experimental Surgery (LCE/UERJ), weighing 250g to 350g each, were selected. The animals were kept inside plastic boxes in a controlled environment (temperature of 21°C- 25°C, 12-hour light/ dark cycles), receiving balanced feed and *ad libitum* water throughout the experiment. The rats had their weight measured once a week during the study.

### Alcohol consumption

Six rats were submitted to daily self-administration of an alcoholic solution containing increasing concentrations of absolute alcohol (Ethanol – Química Moderna, São Paulo, SP, Brazil). The alcoholic solution was prepared with the dilution of absolute ethylic alcohol (ethanol) in the filtered water of consumption for the animals. The alcohol dosages were gradually introduced through weekly increases of 5% in the concentration of the solution until it reaches a final concentration of 25%. The other six rats received only filtered water, being the control group.

After five weeks, the animals were anesthetized with intramuscular administration of 0.1ml of 10% ketamine hydrochloride (Syntec, Cotia, SP, Brazil) associated with 0.05ml of 2% xylazine hydrochloride (Syntec) per 100g of the animal's body weight.

After anesthesia, the animals had the pulp of their lower left first molar exposed using a carbide bur size ¼ in the mesial fossa of the teeth occlusal, in a depth equivalent to the diameter of the bur to avoid furcation perforation, and a

file size 10 was introduced to verify the access cavity.

The pulps were exposed to the oral environment for 28 days, during which the alcohol administration schedule was continued, as illustrated in the Figure 1.

### Bone loss evaluation

Twenty-eight days after the pulp access, the rats were euthanized by overdose of anesthesia, and had their mandibles removed and stored in a 10% buffered formaldehyde solution. To evaluate the size of the periapical lesions, the mandibles were sectioned in half and the left hemimandibles were scanned in the SkyScan 1173 microtomograph (Bruker, Kontich, Belgium). The parameters for image acquisition were as follows: 114 mA e 70Kv, 1120x1120 matrix, isotropic resolution at 17 µm. The images were reconstructed using the NRecon software (v1.6.1.0; Bruker Micro-CT, Kontich, Belgium) and the imaging process and analysis in axial view were performed using the CTan software (v1.6.6.0, Bruker Micro-CT) with a segmentation process of the images, in order to visualize and differentiate the periapical lesion, the root canals and the dentin. The region of interest (ROI) was manually selected (personalized ROI), including the area of the periapical lesion and periodontal ligament around all roots of the left mandibular first molar, and non-including the space of the root canals and periodontal ligament of adjacent teeth. The size of the periapical lesions was measured, and the surface area and volume of the lesions were calculated.

The CTvox software (v1.6.6.0, Bruker Micro-CT) was used for tridimensional reconstruction of the models for better visualization (Figure 2).

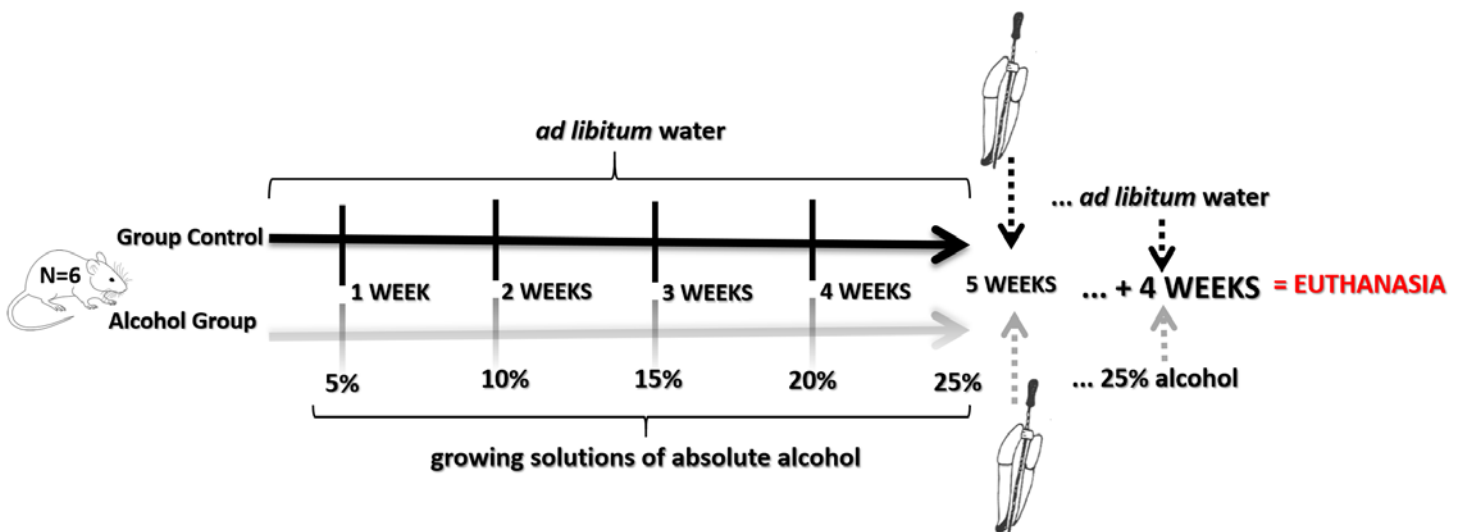


Figure 1. Representative diagram of the experimental study design. N = number of animals per group.

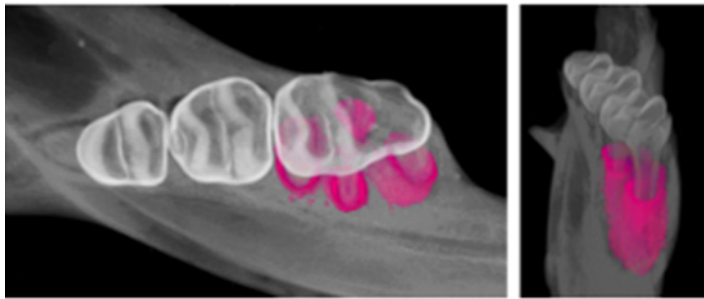


Figure 2. Representative images of the 3D visualization.

### Statistical analysis

The collected data were analyzed by the Bioestat® software (Instituto Mamirauá, Tefé, AM, Brazil) using the Student's *t*-test. The level of significance was set at 5%.

## Results

### Body weight gain and solid/liquid consumption

The animals of the control group had higher percentual rates of body weight gain ( $p < 0.05$ ). The control group also presented a higher percentual rate of both solid and liquid consumption, compared with the alcohol group ( $p < 0.05$ ). The mean values and standard deviation of the body weight and solid/liquid consumption are shown in Table 1.

Table 1. Mean  $\pm$  SD of solid consumption, liquid consumption, and weight gain.

Group	Diary solid intake/ animal (g)	Diary liquid intake/ animal (mL)	Weight gain (%)
Control	19.9 $\pm$ 0.78 <sup>a</sup>	43.44 $\pm$ 3.92 <sup>a</sup>	35.16 $\pm$ 6.14 <sup>a</sup>
Alcohol	11.16 $\pm$ 1.12 <sup>b</sup>	30.77 $\pm$ 3.04 <sup>b</sup>	1.37 $\pm$ 8.95 <sup>b</sup>

Different superscript letters indicate significant statistical differences ( $p < 0.05$ ).

Abbreviation: Standard Deviation (SD)

### Micro-CT evaluation of the periapical lesions

All animals of both groups developed periapical lesion after pulp exposure. It was observed that in the animals of the alcohol group, the periapical lesion presented significantly greater volume and surface area ( $p < 0.05$ ). The mean values and standard deviation of the volume and area of the periapical lesions are shown in Table 2. The Figure 3 shows micro-CT images exhibiting the periapical lesions.

Table 2. Mean  $\pm$  SD of the area and volume of the periradicular lesions.

Group	Area (mm <sup>2</sup> )	Volume (mm <sup>3</sup> )
Control	58.89 $\pm$ 6.35 <sup>a</sup>	4.22 $\pm$ 0.53 <sup>a</sup>
Alcohol	76.77 $\pm$ 8.74 <sup>b</sup>	6.17 $\pm$ 0.98 <sup>b</sup>

Different superscript letters indicate significant statistical differences ( $p < 0.05$ ). Abbreviation: Standard Deviation (SD)

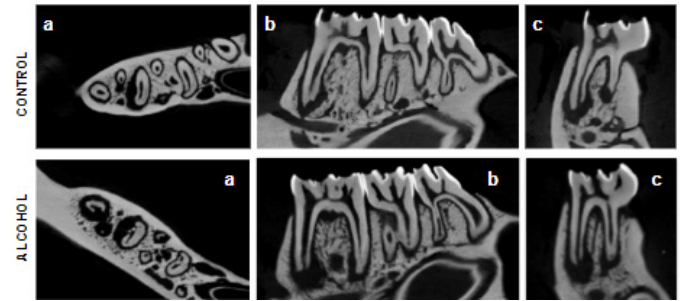


Figure 3. Microtomographic images of the periapical lesions of the control and alcohol groups. a) axial view; b) sagittal view; c) coronal view.

## Discussion

The present study was drafted to evaluate the influence of alcohol consumption on the increase of periapical bone destruction in rats. The rats are used in several experiments to mimic human conditions.<sup>12</sup> Despite some differences and limitations, the use of rats in experiments similar to this is considered satisfactory and provides a greater understanding of the harmful effects of excessive alcohol consumption.<sup>12</sup>

The methodology applied in this study had as main concern to maintain fidelity to the style of alcohol consumption as occurs in humans. It was chosen the method of self-administration of alcohol, since methods such as intraperitoneal injections or gavage would cause excessive stress to the animals in addition to not mimicking the human consumption profile.<sup>13</sup>

Different concentrations of alcoholic solutions have been used in several studies to simulate the alcoholic beverages consumed by humans. Wagner *et al.* (2016)<sup>14</sup> used different concentrations of alcohol, defining low concentration as 5%, intermediate concentration as 13%, and high concentration as 20%. Another study also used 20% of alcohol as chronic consumption dose.<sup>11</sup> Vasconcelos *et al.* (2013)<sup>15</sup> used a 5% weekly increase in the concentration of alcohol dosage, until reaching a final concentration of 25%. In the present study, the same methodology was used for the alcohol consumption by the animals. The gradual increase in the concentration was performed to better adapt the animals to the alcohol, reducing the effects caused by the beginning of the consumption.<sup>16</sup>

The length of time of alcohol intake is widely variable in the studies. Liberman *et al.* (2011)<sup>17</sup> administered alcoholic solution to the animals for 9 weeks. In other studies, the alcohol was offered to the rats for 8 weeks.<sup>9,11,18</sup> In the present study, the administration of the alcoholic solution was gradually increased over 5 weeks and thereafter maintained for 4 weeks, resulting in 9 weeks of consumption. It is believed that this length of time of administration can be compared to the chronic consumption of alcohol by humans, considering the reduced life span of rats.<sup>19</sup>

After the five weeks of adaptation to the alcohol dosage, the animals' left first mandibular molar was accessed exposing

the pulp to the oral cavity, allowing its infection and therefore, the formation of the periapical lesion. The pulp cavity was exposed for 28 days. This period has been used in several other studies and is safe for the formation and evaluation of periapical lesion.<sup>20,21,22</sup>

The strength of the present study was the use of microcomputed tomographic (micro-CT) images in the evaluation of the periapical lesions. A greater amount of information about the lesions total size can be obtained using micro-CT, such as volume and surface area. Moreover, the macro aspect of the lesion can be assessed through three-dimensional reconstruction, allowing a better visualization of the growth pattern of bone destruction at the site.<sup>23</sup> To date, there is limited information in the literature about a standard protocol for acquisition, reconstruction and analysis of periapical lesions in rats using micro-CT.

This lack of standardization leads to inappropriate measures, compromising its scientific impact.<sup>24</sup> The main factor to be considered in the analysis of periapical lesions using micro-CT is the selection of the region of interest (ROI) to be evaluated. It is common the use of automatic or elliptical selections in the studies,<sup>25,26,27</sup> however, the use of non-personalized ROIs results in the inclusion of areas that are not part of the periapical lesion, such as the root canal space and periodontal ligament of adjacent teeth.<sup>24</sup> In the present study, the ROI was demarcated manually in a personalized way, allowing measurements to be precisely acquired.

The results obtained in this study revealed that the group that received alcohol had a reduced consumption of both liquid and solids, and consequently had a less weight gain than the control group ( $p < 0.05$ ). It is believed that due to the high caloric content of alcohol, the rats that ingested the alcoholic solution felt satisfied.<sup>19</sup> As a result, the rats ingested less feed and consequently stopped gaining weight throughout the experiment, characterizing a process of malnutrition that can interfere in the bone loss.<sup>28</sup>

It was observed in the present study that the control group developed periapical lesion, which was already expected due to the pulp exposure. However, in the group of alcohol consumption, there was a significant increase in bone destruction ( $p < 0.05$ ). Therefore, the null hypothesis that there would be no difference in the size of the periapical lesions

between groups was rejected. The values presented in Table 2 show the statistical difference in the volume and total surface area of the lesions between the groups ( $p < 0.05$ ), showing that alcohol consumption has a direct effect on increasing osteoclastic activity in the site of the lesion.

In the current literature, there are several studies evaluating the relationship between chronic alcohol consumption and increased alveolar bone loss in periodontitis induced in rats. Most of these studies observed that alcohol consumption increases the alveolar bone resorption.<sup>7,14</sup> However, there are few studies evaluating this relationship in periapical lesions. The results of the present study corroborate the findings of Dal-Fabbro *et al.* (2019a)<sup>9</sup>, that assessed the influence of chronic alcohol consumption on periapical lesions using histological and immunohistochemical methods, and observed an increase in periapical lesions due to an exacerbation of the local inflammatory process, with increased osteoclastic activity due to an imbalance in the RANKL/OPG (Receptor activator of nuclear factor kappa-B ligand/Osteoprotegerin) ratio.<sup>9</sup> In another study, Dal-Fabbro *et al.* (2019b)<sup>10</sup> observed that the consumption of alcohol in lower concentrations (below 10%) did not interfere in the development of periapical lesions, however, higher concentrations (above 15%) directly affected the degree of inflammation and increase in the periapical lesions.

Further studies with standardized methodologies are necessary to assess and fully understand the complex mechanism of exacerbation of periapical bone resorption, the increase in inflammation response and the systemic repercussions related to the chronic alcohol consumption.

## Conclusion

Chronic alcohol consumption contributed to the increase in periapical bone destruction, leading to an increase in the volume and area of the periapical lesion. Further studies are still necessary to understand the mechanisms involved in the exacerbation of bone resorption by alcohol consumption.

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