



UNIVERSIDADE FEDERAL DE UBERLÂNDIA
FACULDADE DE ODONTOLOGIA



ANA CAROLINA DE PAULA MENDONÇA

**ANÁLISE DE ESPAÇOS SINOSÓIDES E
FIBROSE EM FÍGADOS DE RATOS COM
PERIODONTITE APICAL CRÔNICA
ASSOCIADO A INALAÇÃO PASSIVA DE
FUMAÇA DE CIGARRO E AO CONSUMO
CRÔNICO DE ÁLCOOL**

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Trabalho de conclusão de curso
apresentado a Faculdade de
Odontologia da UFU, como requisito
parcial para obtenção do título de
Graduado em Odontologia

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UBERLÂNDIA

2024

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RESUMO

Este estudo teve como objetivo avaliar o impacto do consumo crônico de álcool e da inalação passiva de fumaça de tabaco, isoladamente ou combinados, na saúde hepática de ratos com periodontite apical (PA), em comparação com ratos sem PA. Trinta e cinco ratas adultas da linhagem Wistar foram divididas em cinco grupos: controle (sem PA, sem exposição ao álcool ou fumaça), controle-PA (PA induzida apenas), ShS-PA (PA com exposição passiva à fumaça), A-PA (PA com consumo de álcool) e A+ShS-PA (PA com exposição tanto ao álcool quanto à fumaça passiva). Amostras de fígado foram analisadas histologicamente quanto à fibrose e quantificação do espaço sinusoidal. Embora a PA e os hábitos deletérios não tenham induzido fibrose significativa, o grupo controle apresentou espaços sinusoidais menores em comparação com todos os outros grupos ($p < 0,05$). Os grupos com PA induzida apresentaram percentagens semelhantes de dilatação sinusoidal ($p > 0,05$). Em geral, a combinação de PA com consumo de álcool e inalação passiva de fumaça de tabaco pode aumentar significativamente os espaços sinusoidais no fígado de ratos. No entanto, não há efeito na fibrose hepática.

Palavras-chave: Periodontite apical; Hábitos prejudiciais; Espaços sinusoidais; Fibrose hepática.

ABSTRACT

This study aimed to assess the impact of chronic alcohol consumption and passive tobacco smoke inhalation, alone or combined, on hepatic health in rats with apical periodontitis (AP), compared to rats without AP. Thirty-five female adult Wistar rats were divided into five groups: control (no AP, no alcohol or smoke exposure), control-AP (induced AP only), ShS-AP (AP with passive smoke exposure), A-AP (AP with alcohol consumption), and A+ShS-AP (AP with both alcohol and passive smoke exposure). Liver samples were histologically analyzed for fibrosis and sinusoidal space quantification. While AP and deleterious habits did not induce significant fibrosis, the control group exhibited smaller sinusoidal spaces compared to all other groups ($p < 0.05$). Induced AP groups showed similar sinusoidal dilation percentages ($p > 0.05$). Overall, AP combined with alcohol consumption and passive smoke inhalation could significantly increase sinusoidal spaces in rats' liver. However, there's no effect in hepatic fibrosis.

Key-words: Apical periodontitis; Deleterious habits; Sinusoidal spaces; Hepatic fibrosis

1. MANUSCRITO

Analysis of sinusoidal spaces and fibrosis in rat livers with chronic apical periodontitis associated with passive cigarette smoke inhalation and chronic alcohol consumption

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Acknowledgments

The present study was supported by the National Council for Scientific and Technological Development – CNPq grant INCT Saúde Oral e Odontologia 406840/2022-9; 406261/2023-7; and by the Research Support Foundation of the State of Minas Gerais – FAPEMIG grant numbers APQ-02105-18 and APQ-00927-23.

Authors' Contributions

Moura CCG, Ferraz DC: conception and design study, acquisition, analysis and interpretation of data, drafting the article, and critical review of important intellectual

content; Mendonça ACP: acquisition, analysis and interpretation of data and article draft; Rende SGS, Reis CCM: acquisition, analysis and interpretation of data. All authors have read and approved the final version of the manuscript

Ethics Approval

The experimental protocol was approved by the Ethics Committee for Animal Use at UNIUBE, Uberaba, Brazil (Protocol nº 001/2021).

Conflict of interest

There is no conflict of interest.

Analysis of sinusoidal spaces and fibrosis in rat livers with chronic apical periodontitis associated with passive cigarette smoke inhalation and chronic alcohol consumption

Abstract

This study aimed to assess the impact of chronic alcohol consumption and passive tobacco smoke inhalation, alone or combined, on hepatic health in rats with apical periodontitis (AP), compared to rats without AP. Thirty-five female adult Wistar rats were divided into five groups: control (no AP, no alcohol or smoke exposure), control-AP (induced AP only), ShS-AP (AP with passive smoke exposure), A-AP (AP with alcohol consumption), and A+ShS-AP (AP with both alcohol and passive smoke exposure). Liver samples were histologically analyzed for fibrosis and sinusoidal space quantification. While AP and deleterious habits did not induce significant fibrosis, the control group exhibited smaller sinusoidal spaces compared to all other groups ($p < 0.05$). Induced AP groups showed similar sinusoidal dilation percentages ($p > 0.05$). Overall, AP combined with alcohol consumption and passive smoke inhalation could significantly increase sinusoidal spaces in rats' liver. However, there's no effect in hepatic fibrosis.

Keywords

Apical periodontitis; Deleterious habits; Sinusoidal spaces; Hepatic fibrosis

Introduction

The consumption of alcohol and tobacco has significant impacts on systemic health, extending beyond the obvious consequences for oral health (Doll et al., 2004). Excessive alcohol consumption and tobacco is responsible for damage to various organs and systems of the human body, including the liver and its important functions (Piano, 2017). Moreover, studies involving animal models indicate that the consumption of alcohol and tobacco may negatively impact alveolar bone, leading to increased bone loss and reduced bone density (Dal-Fabbro et al., 2019).

In addition, apical periodontitis (AP) is an inflammatory condition affecting the periodontal tissues surrounding the tooth root, resulting from persistent bacterial infection in the apical region of the tooth (Nair et al., 2011). Despite local inflammatory response, AP is also related with systemic diseases (Kojima et al., 2017; Huang et al., 2016; Haapasalo et al., 2016). Studies suggest that AP may play a role in the progression of systemic diseases such as diabetes, cardiovascular diseases, and respiratory complications (Sanz et al., 2020). The main mechanism related to that include bacterial dissemination through the bloodstream, triggering systemic inflammatory responses (Siqueira et al., 2013). Although further research is needed to fully elucidate these associations, understanding the interrelation between AP and systemic diseases is crucial for an integrated approach in managing both oral and overall systemic health (Preshaw et al., 2021).

Additionally, epidemiological studies have demonstrated a significant association between the prevalence of apical periodontitis and the consumption of alcohol and tobacco (Vasques et al., 2023; Ríos-Orsorio et al., 2022). Individuals who excessively consume alcohol and smoke have a substantially increased risk of developing apical periodontitis compared to non-users (Pinto et al., 2020). Alcohol and tobacco are known to compromise the immune system and trigger chronic inflammatory responses in the body, rendering periodontal tissues more susceptible to bacterial infection and disease progression (Leite et al., 2018 Dal-Fabbro et al., 2019). Moreover, alcohol may increase the permeability of the oral mucosa, facilitating the entry of bacteria into the apical region of the teeth (Pereira et al., 2013). Similarly, the toxic chemicals present in tobacco can compromise the vascularization of periodontal tissues, impairing their ability to defend against infections (Aminoshariae et al., 2020; Dal-Fabbro et al., 2019).

In addition to their direct effects on oral health, the association between alcohol consumption, tobacco use, and apical periodontitis can have significant repercussions on the liver (Bandiera et al., 2020). Excessive alcohol consumption is a leading cause of liver disease, including fatty liver, alcoholic hepatitis, and cirrhosis (Cantiga -Silva et al., 2021). These hepatic conditions can further compromise the body's immune system, rendering it less capable of combating infections, including those affecting periodontal tissues (Dal-Fabbro et al., 2019). Moreover, chronic inflammation associated with alcohol consumption can exacerbate the inflammatory response in the oral cavity, worsening apical periodontitis (Pinto et al., 2020). Similarly, the toxic chemicals present in cigarettes can adversely affect liver function, increasing the risk of developing liver diseases (Cantiga-Silva et al., 2021).

Thus, the aim of the present study was to evaluate the effects of chronic alcohol consumption and passive inhalation of tobacco smoke on the hepatic health of rats with induced AP. The null hypothesis is that rats with induced AP associated or no with deleterious habits don't play a synergistic effect on sinusoidal dilatation and liver fibrosis.

Methods

Animals

The experimental protocol was approved by the Ethics Committee for Animal Use at UNIUBE, Uberaba, Brazil (Protocol No. 001/2021). All procedures adhered to the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) guidelines (Nagendrababu et al., 2021). Thirty-five female adult Wistar rats weighing approximately 150g were utilized. Sample size calculation was based on data from a previous study (Justino et al., 2017), considering a minimum treatment difference of 1.53 and a standard deviation of 0.61. The calculation indicated a total of 7 rats per group with a test power of 95% and a significance level of 5%.

The rats were housed in polypropylene cages in a temperature-controlled environment (22–25°C), under 12-hour light and dark cycles, with air humidity maintained at approximately 55%, and provided with ad libitum access to food and water. Weekly weight measurements were conducted using a semi-analytical balance. Rats not exposed to tobacco smoke inhalation or an alcoholic diet and without apical periodontitis (control) were used as a reference for comparison of weight gain curves.

Alcohol consumption and smoke inhalation

The animals were distributed randomly into one of five experimental groups (n=7 per group): (1) rats not exposed to secondhand smoke (ShS) or an alcoholic diet and without apical periodontitis (control); (2) rats with induced apical periodontitis (control -AP); (3) rats exposed to ShS and induced apical periodontitis (ShS-AP); (4) rats exposed to an alcoholic diet and induced apical periodontitis (A-AP); and (5) rats exposed to an alcoholic diet plus ShS and induced apical periodontitis (A+ShS-AP).

Exposure to cigarette smoke, aiming to simulate conditions consistent with secondhand smoke, followed the methodology established by Santiago et al. (2017). A transparent acrylic box containing four cylindrical inhalation chambers (7.0 cm in diameter and 23.0 cm in length, with a total volume of 885 cm³) was utilized. One end of the chamber was accessible for animal handling, while the opposite funnel-shaped end was connected to a peristaltic pump. This pump drew in the smoke and distributed it into the animal chambers. During exposure, four animals were positioned with their noses directed toward the cylindrical end, securely restrained in place. Marlboro cigarettes (Phillips Morris, Santa Cruz do Sul, RS, Brazil) served as the smoke source. Each cigarette, as indicated on the product label, contains 0.8 mg of nicotine, 10 mg of tar, and 10 mg of carbon monoxide.

Animals were introduced to the cylindrical chambers without cigarette smoke for one week to acclimate. In the second week, smoke inhalation commenced by burning one cigarette twice daily (morning and afternoon, with a 6-hour interval). By the third week, exposure increased to four cigarettes per day (two in the morning and two in the afternoon, with a 6-hour interval). At this juncture, the baseline was established, and the exposure regimen persisted until euthanasia. Animals not exposed to smoke were housed in smoke-free chambers to ensure equivalent stress conditions.

Alcohol consumption was gradually initiated ad libitum. In the first week, a 5% alcoholic solution was administered, followed by 10% in the second week, and 20% in the third week. Subsequently, a baseline was established, and alcohol administration continued until euthanasia, maintaining a 20% alcoholic solution concentration. Rats in group A+ShS-AP were initially administered alcohol as described above. Cigarette smoke exposure commenced in the third week, with inhalation of two cigarettes per day, gradually increasing following the aforementioned protocol to ensure equivalent total

exposure time. In the eighth week, all animals underwent apical periodontitis induction. Further details are summarized in Figure 1.

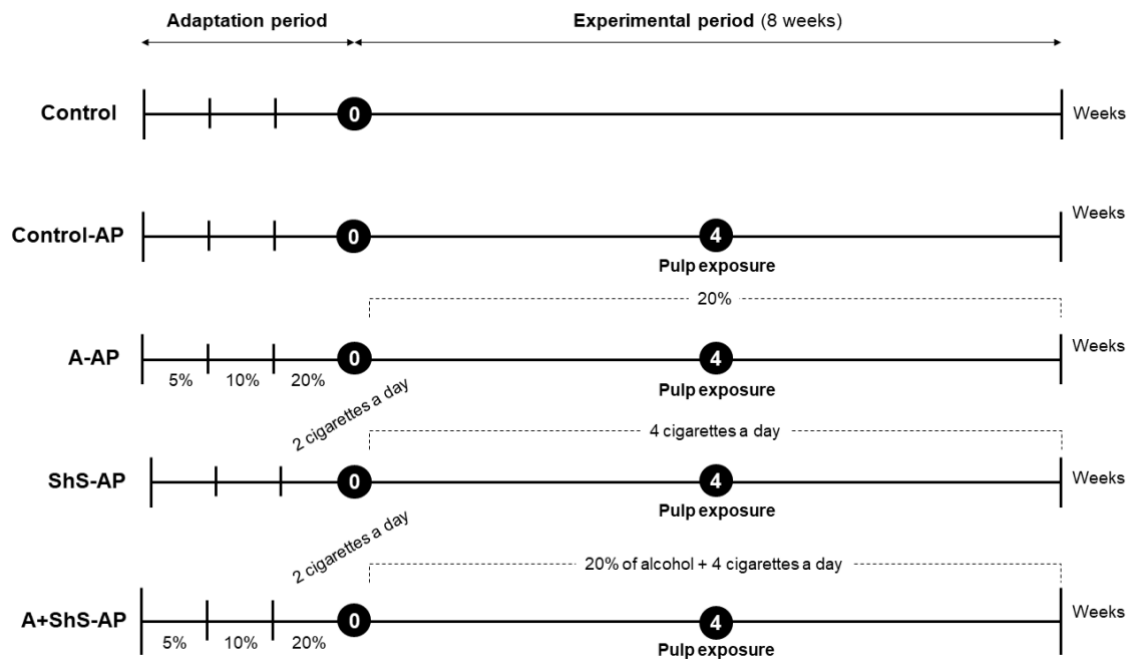


Figure 1 illustrates the experimental setup. The adaptation phase spanned three weeks, followed by an eight-week experimental phase. Apical periodontitis was induced by exposing the pulp in the fourth week of the experimental period. Euthanasia occurred four weeks after pulp exposure.

Apical periodontitis induction

In the eighth week, apical periodontitis (AP) was induced by exposing the pulps of the lower right first molars occlusally. The pulpal exposure was conducted under anesthesia (intramuscular ketamine/xylazine at doses of 70 and 6 mg/kg, respectively) using a high-speed round bur with a diameter of 0.1 mm (KGSorensen® Ind. Com. Ltda, Brazil), with continuous irrigation. Following pulp exposure, the pulp chambers were left open to the oral environment for 4 weeks to facilitate AP development. At the conclusion of the experiment, euthanasia was performed via intraperitoneal administration of a lethal dose of Thiopental.

Sample collection and histological processing

The left lobes of the livers were harvested and submerged in a buffered solution containing 4% paraformaldehyde with a neutral pH for 48 hours to facilitate histological analysis. Meanwhile, the right lobes were frozen at -70 °C for subsequent biochemical analysis. The fixed specimens underwent conventional histological processing for embedding in paraffin. Two consecutive sections, each with a thickness of 5 µm, were obtained for hematoxylin and eosin (H&E) staining, while an additional 2 sections were set aside for Picrosirius Red (PS) staining. A 50 µm interval was maintained before obtaining the subsequent sections, resulting in a semi-serial approach. Ten sections of each staining type were obtained per animal. The left lobes of the livers were harvested and submerged in a buffered solution containing 4% paraformaldehyde with a neutral pH for 48 hours to facilitate histological analysis. Meanwhile, the right lobes were frozen at -70 °C for subsequent biochemical analysis. The fixed specimens underwent conventional histological processing for embedding in paraffin. Two consecutive sections, each with a thickness of 5 µm, were obtained for hematoxylin and eosin (H&E) staining, while an additional 2 sections were set aside for Picrosirius Red (PS) staining. A 50 µm interval was maintained before obtaining the subsequent sections, resulting in a semi-serial approach. Ten sections of each staining type were obtained per animal.

Histomorphometric analyses

The Picrosirius Red (PS)-stained sections were captured using a polarized light microscope (Nikon Eclipse Ti-S) at a 20× magnification. The obtained images were examined to determine the total collagen content in four distinct fields per section, located in equidistant regions. Evaluation was conducted on five sections per animal, resulting in 140 analyses per group. ImageJ software was utilized to quantify the total collagen fibers. Calibration of ImageJ software was performed using the Split Channel and Threshold tools to determine the percentage of type I and type III collagen (reddish-yellow and whitish-green birefringence, respectively). This ensured that all images were adjusted to capture the same color range. Analysis excluded the hepatic capsule and major portal tracts, as they did not represent collagen deposition associated with hepatic damage (Huang et al., 2013).

The hematoxylin and eosin (H&E)-stained sections were captured using a digital scanner coupled with a microscopic camera (ScanScopeAT; Leica Biosystems Imaging, USA) with

a $\times 20$ objective lens. The number of fields, sections per animal, and selection of analyzed areas followed the same pattern as described above for the PS-stained sections. Stained areas within the sinusoids were eliminated using Photoshop v. 24.x (Adobe Systems, San Jose, CA), and then converted into binary images using ImageJ (ImageJ 1.51k, Wayne Rasband, National Institute of Health, USA) to calculate sinusoidal dilation. All analyses were manually performed using ImageJ software (ImageJ 1.51k; Wayne Rasband, National Institute of Health, USA). In both histomorphometric analyses, the percentage of each collagen type and the percentage of area occupied by the sinusoids obtained from each image were tabulated, generating an average per section and per animal. These averages were subjected to analysis using SigmaPlot software (v. 12, Systat Software).

Results

Liver fibrosis analysis

No significant fibrosis was induced by AP or deleterious habits (Figure 2). All groups exhibited no significant differences in collagen type I and III deposition ($p > 0.005$).

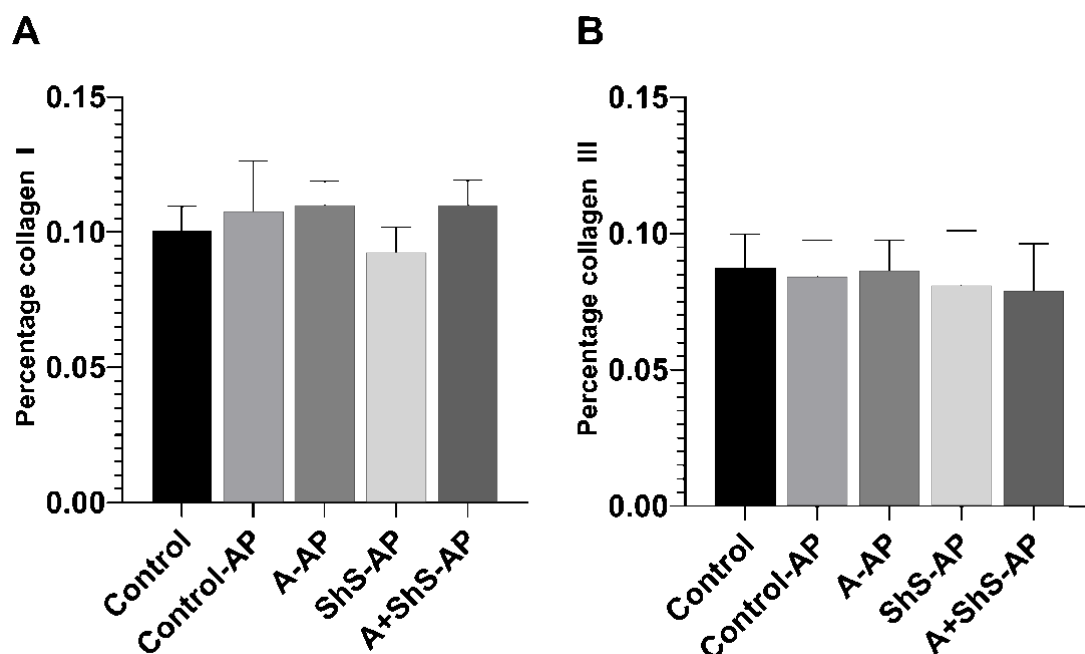


Figure 2. Percentage of collagen type I (a) and III (b) in the liver of rats

AP induces hepatic sinusoidal dilation

The results of the liver sinusoidal analysis are summarized in Figure 3. The group without AP (control) showed significantly smaller sinusoidal spaces than all other groups ($p < 0.001$). All AP groups exhibited a similar sinusoidal dilation percentage among themselves ($p > 0.001$).

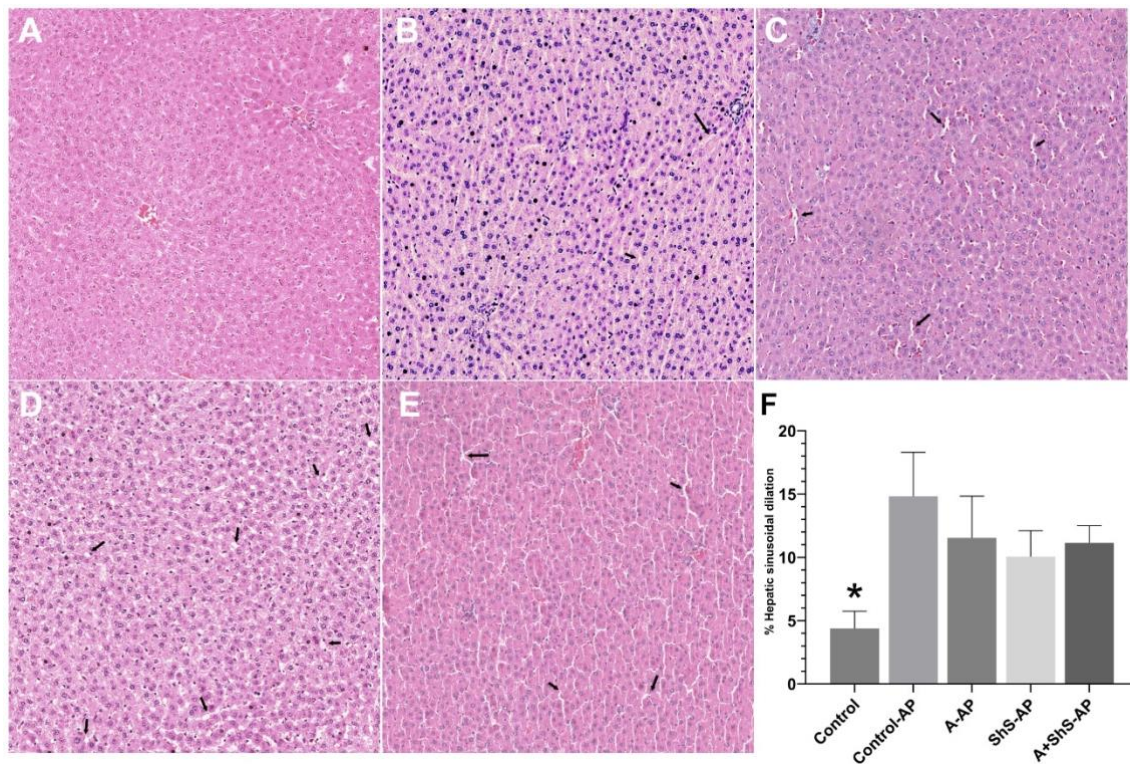


Figure 3. Photomicrographs of liver sections evaluating hepatic sinusoidal dilatation from the control group (a), control-AP (b), A-AP (c), ShS-AP (d), A+ShS-AP ($\times 20$). The hepatic sinusoidal dilation values were expressed in percentage (e). Note the sinusoidal dilation by black arrows. * $p < 0.05$ vs. control group.

Discussion

The analysis of hepatic fibrosis did not reveal any alteration in the volume of fibrotic tissue, either in rats with AP or in rats without AP. However, in the analysis of sinusoidal volume, there were significant differences in the AP-induced group, with an increase in sinusoidal spaces in these animals. Therefore, the null hypothesis was partially rejected. Some studies have already been conducted to analyze the association between sinusoidal spaces, fibrosis in rats, and unhealthy habits (Pinto et al., 2024). However, there is still a lack of research on the effects of AP in the current literature. Our study

used a passive inhalation method, which is an innovative approach to studying the effects of tobacco on the body.

The openings in liver sinusoidal endothelial cells are dynamic structures. Various factors such as ethanol, chemicals, toxins, growth factors, hormones, viruses, and diet have been identified as influencers of the dynamics of these endothelial openings and impact capillary formation in sinusoids (Ki et al., 2021). Chronic liver diseases such as alcoholic liver disease, non-alcoholic fatty liver disease, and chronic viral hepatitis are known to induce capillary formation in sinusoids. Gaps are large pores with a diameter exceeding 300 nm - the largest of fenestrations - and exceed 1 μ m. Gap formation reflects destabilization of fenestrations, resulting in the coalescence of 2 or more small -sized fenestrations (Wisse et al., 1985). Gaps can occur as a result of cell injury, high-pressure perfusion both physiologically and experimentally, hypoxia, tissue fixation procedures, or pathological states (Cogger et al., 2020). Some factors as excessive alcohol consumption (Mark et al., 2021), advanced age (Couteur et al., 2020), pathologies such as sinusoidal ectasia (Oligny et al., 1992) and a fatty diet (Ijaz et al., 2003) could lead alterations on liver morphology.

Our results are consistent with the studies of McConnell et al. (2023) and McCuskey (2006) where sinusoidal spaces were increased in animals with a diet rich in ethanol and tobacco. The injury to hepatocytes caused by chronic consumption of such substances results in the release of free radicals that stimulate Kupffer cells to produce pro-inflammatory and anti-inflammatory cytokines, which play a crucial role in controlling the development and progression of hepatic inflammation and other injurious conditions (Starkel & Leclercq, 2011). In situations of prolonged injury, chronic inflammation and accumulation of extracellular matrix lead to a progressive replacement of healthy liver tissue by scar tissue (Gressner & Weiskirchen, 2006). Although liver fibrosis was not observed in the present study, the increase in these cytokines and inflammatory infiltrate leads to dilation and even loss of sinusoidal fenestrations and consequent greater number of sinusoidal spaces (Wang et al., 2005). Since apical periodontitis is also a factor in increasing inflammatory cytokines in a systemic context (Pinto et al., 2024; Cintra et al., 2014; Samuel et al., 2019), our findings of increased sinusoidal spaces in animals affected by AP are consistent with the

literature. In the study by Antwi et al. (2023), these gaps were larger in areas with visible hepatocyte damage and accumulation of inflammatory cells.

Regarding hepatic fibrosis, our findings were potentially attenuated, possibly due to passive cigarette inhalation, as its effect is milder on hepatic tissue than the nicotine injection used as a smoking induction method in other studies (McCuskey, 2006). In our study, apical periodontitis alone was unable to generate fibrotic tissue in the liver on its own, since the effect of tobacco was minimal. Furthermore, other studies use analysis of liver fibrosis in rats, associated (Cantiga-Silva et al. 2021) or not with AP models, induced liver damage through drugs such as monocrotaline or carbon tetrachloride (CCl₄) (Courtoy et al. 2020, Gewehr et al. 2021), and therefore their findings cannot be compared to the present study.

The use of animals in research on oral pathologies is justified for several fundamental reasons. Firstly, animal models provide a controlled and reproducible platform for studying the progression of oral diseases, such as apical periodontitis (AP), in a complex and relevant biological context, allowing understanding the effects of deleterious habits associated with systemic health damage. In addition, animal studies enable investigation into pathophysiological aspects of oral diseases that may not be feasible to address in humans. However, despite their valuable contributions, it is important to recognize the limitations of animal models, including differences in pathophysiological response and histological analyses, requiring caution in interpreting and integrating clinical evidence from human studies for a comprehensive understanding of conditions such as apical periodontitis.

Conclusions

Apical periodontitis affects liver synergically with chronic alcohol consumption and secondhand smoke exposure in sinusoidal spaces. However, in hepatic tissue of rats didn't show significant collagen type I and II deposition.

References

Aminoshariae, A., Kulild, J., & Gutmann, J. (2020). The association between smoking and periapical periodontitis: A systematic review. *Clinical Oral Investigations*, 24, 533–545. DOI: 10.1007/s00784-019-03117-4

- Antwi, M. B., Dumitriu, G., Simón-Santamaria, J., Sánchez Romano, J., Li, R., Smedsrød, B., Vik, A., Eskild, W., & Sørensen, K. K. (2021). Liver sinusoidal endothelial cells show reduced scavenger function and downregulation of Fc gamma receptor IIb, yet maintain a preserved fenestration in the Glmpgt/gt mouse model of slowly progressing liver fibrosis. *PLoS One*, 16(11), e0293526. DOI: 10.1371/journal.pone.0293526
- Araujo, G. H. S., Lima, J. B. C., Silva, L. C. M., et al. (2021). Análise da imagem histológica assistida por software para identificação e quantificação de dilatação sinusoidal e fibrose hepática centrolubular. *ABCD Arquivos Brasileiros de Cirurgia Digestiva (São Paulo)*, 34(2), e1608. DOI: 10.1590/0102-672020210001e1608
- Bandiera, S., Pulcinelli, R. R., Huf, F., Almeida, F. B., Halmenschlager, G., Bitencourt, P. E. R., Dallegrave, E., Fernandes, M. C., Gomez, R., & Nin, M. S. (2020). Hepatic and renal damage by alcohol and cigarette smoking in rats. *Toxicology Research*, 37(2), 209–219. DOI: 10.1007/s43188-020-00057-y
- Cantiga-Silva, C., Estrela, C., Segura-Egea, J. J., Azevedo, J. P., de Oliveira, P. H. C., Cardoso, C. B. M., Pinheiro, T. N., Ervolino, E., Sivieri-Araújo, G., & Cintra, L. T. A. (2021). Inflammatory profile of apical periodontitis associated with liver fibrosis in rats: Histological and immunohistochemical analysis. *International Endodontic Journal*, 54(8), 1353–1361. DOI: 10.1111/iej.13519
- Cogger, V. C., Hunt, N. J., & Le Couteur, D. G. (2020). Fenestrations in the liver sinusoidal endothelial cells. In I. M. Arias, H. J. Atler, J. L. Boyer, D. E. Cohen, D. A. Shafritz, S. S. Thorgeirsson, & A. W. Wolko (Eds.), *The Liver: Biology and Pathobiology* (6th ed., Vol. 35, pp. 435–443). Hoboken, NJ: John Wiley & Sons, Ltd. DOI: 10.1002/9781119436812.ch35
- Courtoy, G. E., Leclercq, I., Froidure, A., Schiano, G., Morelle, J., Devuyst, O., Huaux, F., & Bouzin, C. (2020). Digital Image Analysis of Picrosirius Red Staining: A Robust Method for Multi-Organ Fibrosis Quantification and Characterization. *Biomolecules*, 10 (1585). DOI: 10.3390/biom10111585
- Dal-Fabbro, R., Marques-de-Almeida, M., Cosme-Silva, L., Ervolino, E., Cintra, L. T. A., & Gomes-Filho, J. E. (2019). Chronic alcohol consumption increases inflammation and osteoclastogenesis in apical periodontitis. *International Endodontic Journal*, 52 (3), 329–336. DOI: 10.1111/iej.13014
- Gracia-Sancho, J., Caparros, E., Fernandez-Iglesias, A., & Frances, R. (2021). Role of liver sinusoidal endothelial cells in liver diseases. *Nature Reviews Gastroenterology & Hepatology*, 18(6), 411–431. DOI: 10.1038/s41575-020-00411-3
- Haapasalo, M., & Shen, Y. (2016). Current therapeutic strategies for endodontic infections and pathogenesis of apical periodontitis. *Endodontic Topics*, 33 (1), 269–297.
- Huang, X., Schulte, B. A., & Zhao, H. (2016). Stem cell-mediated tooth regeneration as a promising therapy for tooth repair: A review. *Stem Cell Research & Therapy*, 7 (1), 1–7.

- Ijaz, S., Yang, W., Winslet, M. C., & Seifalian, A. M. (2004). Impairment of hepatic microcirculation in fatty liver. *Microcirculation*, 11 (1), 49–56.
- Johnson, G. K., & Hill, M. (2004). The effects of cigarette smoking on periodontal health. *Periodontology 2000*, 37 (1), 195–207. DOI: 10.1111/j.1600-0757.2004.00008.x
- Justino, A. B., Pereira, M. N., Peixoto, L. G., et al. (2017). Hepatoprotective Properties of a Polyphenol-Enriched Fraction from *Annona crassiflora* Mart. Fruit Peel against Diabetes-Induced Oxidative and Nitrosative Stress. *Journal of Agricultural and Food Chemistry*, 65 (22), 4428–4438. DOI: acs.jafc.7b01355
- Khalighinejad, N., Aminoshariae, M. R., Aminoshariae, A., Kulild, J. C., Micke, A. L., & Fouad, A. F. (2016). Association between systemic diseases and apical periodontitis. *Journal of Endodontics*, 42, 1427–1434. DOI: 10.1016/j.joen.2016.07.007
- Ki, M. M., Kee, D., & Shin, D. W. (2021). Alcohol-associated capillarization of sinusoids: A critique sin discovery by Schaner and Popper in 1963. *Anatomical Record*. Advance online publication. DOI: 10.1002/ar.24829
- Kojima, A., & Nakano, K. (2017). Periodontitis and systemic diseases: A literature review. *Journal of Oral Biosciences*, 59 (1), 33–38.
- Le Couteur, D. G., Warren, A., Cogger, V. C., Smedsrød, B., Sørensen, K. K., De Cabo, R., Fraser, R., & McCuskey, R. S. (2008). Old age and the hepatic sinusoid. *Anatomical Record (Hoboken)*, 291 (6), 672–683. DOI: 10.1002/ar.20661
- Leite, F. R. M., Nascimento, G., Scheutz, F., & Lopez, R. (2018). Effect of smoking on periodontitis: A systematic review and meta-regression. *American Journal of Preventive Medicine*, 54, 831–841. DOI: j.amepre.2018.02.014
- McConnell, M. J., Kostallari, E., Ibrahim, S. H., & Iwakiri, Y. (2023). The evolving role of liver sinusoidal endothelial cells in liver health and disease. *Hepatology*, 78 (2), 649–669. DOI: 10.1097/HEP.0000000000000207
- McCuskey, R. S. (2006). Sinusoidal endothelial cells as an early target for hepatic toxicants. *Clinical Hemorheology and Microcirculation*, 34 (1-2), 5–10.
- Nagendrababu, V., Kishen, A., Murray, P. E., et al. (2021). PRIASE 2021 guidelines for reporting animal studies in Endodontology: a consensus-based development. *International Endodontic Journal*, 54 (6), 848–857. DOI: 10.1111/iej.13481
- Nair, P. N. R. (2011). Pathogenesis of apical periodontitis and the causes of endodontic failures. *Journal of Endodontics*, 37 (6), 973–990.
- Oligny, L. L., & Lough, J. (1992). Hepatic sinusoidal ectasia. *Human Pathology*, 23 (4), 428–432. DOI: 10.1016/0046-8177(92)90411-u

Pereira Vasconcelos, D. F., Dias Da Silva, M. A., Rocha Marques, M., Gibilini, C., Guimarães Vasconcelos, A. C., & Pereira Barros, S. (2013). Effects of simultaneous nicotine and alcohol use in periodontitis progression in rats: a histomorphometric study. *Journal of Clinical and Experimental Dentistry*, 5, 95–99. DOI: 10.4317/jced.51047

Piano, M. R. (2017). Alcohol and the Cardiovascular System: A Double-Edged Sword. *Nutrition, Metabolism and Cardiovascular Diseases*, 27(2), 957–964.

Pinto, K. P., Ferreira, C. M. A., Guimarães, A. F. C., Lima, C. O., Pires, F. R., Sassone, L. M., & Silva, E. J. N. L. (2020). Effects of alcohol and nicotine consumption on the development of apical periodontitis in rats: a correlative micro-computed tomographic, histological and immunohistochemical study. *International Endodontic Journal*, 53 (9), 1238–1252. DOI: 10.1111/iej.13344

Poisson, J., Lemoine, S., Boulanger, C., Durand, F., Moreau, R., Valla, D., et al. (2017). Liver sinusoidal endothelial cells: Physiology and role in liver diseases. *Journal of Hepatology*, 66 (1), 212–227. DOI: 10.1016/j.jhep.2016.07.009

Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y., & Patra, J. (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *The Lancet*, 373 (9682), 2223–2233. DOI: 10.1016/S0140-6736(09)60746-7

Ríos-Osorio, N., Muñoz-Alvear, H. D., Jiménez-Castellanos, F. A., Quijano-Guauque, S., Jiménez-Peña, O., García-Perdomo, H. A., & Caviedes-Bucheli, J. (2022). Association between cigarette smoking and the prevalence of post-endodontic periapical pathology: a systematic review and meta-analysis. *Restorative Dentistry & Endodontics*, 47 (3), e27. DOI: 10.5395/rde.2022.47.e27

Roux, D., Bioulac-Sage, P., Merlio, J. P., Lamouliatte, H., Quinton, A., & Balabaud, C. (1987). Liver sinusoids and sinusoidal cells in patients with agnogenic myeloid metaplasia. *Ultrastructural Pathology*, 11 (4), 463–469. DOI: 10.1097/00004836-198708000-00027

Sanz, M., Marco Del Castillo, A., Jepsen, S., et al. (2020). Periodontal disease and systemic health: Current status and future directions. *Journal of Clinical Periodontology*, 47 (Suppl 22), S338–S348. DOI: 10.1111/jcpe.13279

Santiago, H. A., Zamarioli, A., Sousa Neto, M. D., & Volpon, J. B. (2017). Exposure to Secondhand Smoke Impairs Fracture Healing in Rats. *Clinical Orthopaedics and Related Research*, 475 (3), 894–902. DOI: 10.1007/s11999-016-5184-6

Segura-Egea, J. J., Martín-Gonzalez, J., & Castellanos-Cosano, L. (2015). Endodontic medicine: connections between apical periodontitis and systemic diseases. *International Endodontic Journal*, 48, 933–951. DOI: 10.1111/iej.12507

Siqueira Jr, J. F., & Rôças, I. N. (2013). Clinical implications and microbiology of bacterial persistence after treatment procedures. *Journal of Endodontics*, 39 (2), 129–138.

Starkel, P., & Leclercq, I. A. (2011). Animal models for the study of hepatic fibrosis. *Best Practice & Research. Clinical Gastroenterology*, 25 (4–5), 319–333. DOI: 10.1016/j.bpg.2011.02.006

Vasques, A. M. V., da Silva, A. C. R., Bueno, C. R. E., Cury, M. T. S., Ervolino, E., Cintra, L. T. A., & Dezan Junior, E. (2023). Inflammatory profile of apical periodontitis exacerbated by cigarette smoke inhalation: Histological and immunohistochemical analysis in rats. *International Endodontic Journal*, 56 (4), 465–474. DOI: 10.1111/iej.13883

Wisse, E., De Zanger, R. B., Charels, K., Van Der Smissen, P., & McCuskey, R. S. (1985). The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. *Hepatology*, 5 (4), 683–692. DOI: 10.1002/hep.1840050427

2. Anexos

2.1. Guidelines para publicação na revista Bioscience Journal

MANUSCRIPT FORMAT

All documents should be created using Microsoft Word. The text should be justified, typed in Calibri font, font size 12, with a 1.0 line spacing and a margin of 2.54 cm throughout the entire document.

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Title Page: must list the article title (up to 35 words and should mention the study design), the author's full name, ORCID identification number (as obtained from orcid.org), institutional affiliation, and the corresponding author's e-mail address. Institutional affiliation must contain the department (if professor or undergraduate student) or name of the postgraduate program (if postgraduate student) or private clinic for example (if you have no institutional affiliation), followed by full name of the institution (do not include the acronym or abbreviation of the institution's name), city, state, and country. Do not include the degree/title of the authors.

Acknowledgments: grants and any other financial support for the study should be acknowledged, always with the funding agency name, and with the protocol number whenever possible. Donation of materials used in the research can and should be acknowledged too. This section should also be used to acknowledge any other contributions from individuals or professionals who have helped in

producing or reviewing the study, and whose contributions to the publication do not constitute authorship.

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Ethics Approval: manuscripts involving research on human beings, animals and systematic reviews should state the public registration database and/or the institutional review board (IRB) or ethics committee and the registry number in this section (see the "ETHICAL PRINCIPLES" section). In addition, a Certificate of Ethical Approval for research with human beings or animals should be sent as a supplementary file in the submission process, if applicable.

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Main Document

Title: must contain the article title with up to 35 words and should mention the study design.

Abstract: must contain an unstructured text with up to 250 words including objectives, methods, results and conclusions. References and credits to suppliers and manufacturers of products or equipment must NOT be cited in this section.

Keywords: must have three to six MeSH or DeCS terms that the authors think express the main article themes, in alphabetical order, separated by a period and starting with a capital letter. These keywords should be different from the words

already used in the title and abstract, so that to improve the discoverability of the article by readers doing a search in PubMed or other databases. Please, check your keywords at <https://www.ncbi.nlm.nih.gov/mesh> and/or <https://decs.bvsalud.org>

Main Text: must contain Introduction, Material and Methods, Results, Discussion and Conclusions. Please, do NOT combine the topics of Results and Discussion, they must always be presented separately. Abbreviations and acronyms should be avoided and when necessary they should be defined the first time they are used in the text. Credit suppliers and manufacturers of equipment, drugs, and other brand-name material mentioned in the manuscript within parentheses, giving the company name and primary location (city and country). The authors can and should use short subheadings, especially those concerning the reporting guideline items. As needed, use italic, superscript and subscript, but do NOT use boldface. Do NOT insert page or section breaks.

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Example: The notion of an invisible college has been explored in the sciences (Crane 1972). Its absence among historians is noted by Stieg (1981b, p. 556). It may be, as Burchard (1965) points out, that they have no assistants, or are reluctant to delegate (Smith 1980; Chapman 1981).

CITATION IN LIST OF REFERENCES

All references must be ordered alphabetically and the authors must be listed if there are up to three authors; if there are four or more, the first author must be cited, followed by the expression "et al." At the end of each reference, please insert the "DOI" number if available. For this, authors must search the metadata for references on the Crossref website (<https://doi.crossref.org/simpleTextQuery>) and copy the DOI whenever available.

2.2 Protocolo Comissão de Ética na Utilização de Animais (CEUA)

**Uniube**

Comitê de Ética em Experimentação Animal

Ofício CEEA-001/2021

Uberaba, 08 de abril de 2021.

CERTIFICADO

Certificamos que o protocolo nº 001/2021 relativos ao projeto intitulado **“Ingestão crônica de álcool associada à inalação da fumaça de tabaco e a repercussão sobre a qualidade óssea do fêmur de rato”** que tem como responsável a **Profª. Sanívia Aparecida de Lima Pereira**, está de acordo com os Princípios Éticos da Experimentação Animal, adotados pelo Comitê de Ética em Experimentação Animal (CEEA/UNIUBE) regido pela lei nº 11.794/08.

CERTIFICATE

We hereby certify that the protocol nº 001/2021 related to the project entitled **“Chronic alcohol intake associated with tobacco smoke inhalation and repercussions on the bone quality of rat femurs”** under the supervision of **Profª. Sanívia Aparecida de Lima Pereira**, is in agreement with the Ethical Principles in Animal Experimentation, adopted by the Ethics Committee in Animal Experimentation (CEEA/UNIUBE) according to the law nº 11.794/08.

Atenciosamente,

Profa. Joely Ferreira Figueiredo Bittar
Coordenadora do CEEA-UNIUBE