UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Romulo de Oliveira Sales Junior

Efeito tardio do consumo de vinho tinto sem álcool no desenvolvimento da periodontite apical induzida em ratos

Araçatuba

2024

Romulo de Oliveira Sales Junior

Efeito tardio do consumo de vinho tinto sem álcool no desenvolvimento da periodontite apical induzida em ratos

Dissertação apresentada ao programa de Pós-graduação da Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Mestre em Ciência, Área de concentração em Endodontia. **Orientador:** Prof. Titular João Eduardo Gomes Filho.

Araçatuba

2024

Catalogação na Publicação (CIP)
Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

Sales Junior, Romulo de Oliveira.
S163e Efeito tardio do consumo de vinho tinto sem álcool no desenvolvimento da periodontite apical induzida em ratos / Romulo de Oliveira Sales Junior. - Araçatuba, 2024
108 f. : il. ; tab.

Dissertação (Mestrado) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba
Orientador: Prof. João Eduardo Gomes Filho

1. Periodontite periapical 2. Vinho 3. Polifenóis 4. Remodelação óssea I. T.

Black D24
CDD 617.67

Claudio Hideo Matsumoto CRB-8/5550

Dedicatória

Aos meus pais,

Romulo Sales e Regina Célia

Por toda luta que tivemos ao longo da vida até aqui, para que eu pudesse concluir este sonho. Afirmo que essa vitória é nossa! Vocês me proporcionaram o melhor presente para um ser humano, a educação. Tudo que passamos na vida, até o momento, foram molas propulsoras que levam eu e meus irmãos a buscar melhoria de vida para todos nós. Saibam que sem vocês esses sonhos não eram possíveis. Eu amo muito vocês e tenho orgulho de ser filho de pessoas tão batalhadoras!

Aos meus irmãos,

Cahio Luccas e Jonatham Ruan

Por todo apoio e cumplicidade que temos ao longo dessa vida. Vocês me inspiram todos os dias em como ser um profissional incrível. Apesar da distância física, o nosso amor nos une e proporciona muito orgulho para os nossos pais. Vocês são seres iluminados e tenho certeza de que vamos voar cada vez mais alto. Eu amo vocês!

A minha segunda mãe,

Marinalda Silva

Por todo amor que você me deu ao longo da vida. Eu sei que você torce diariamente por mim e que sempre zelou para que eu crescesse bem e com muito carinho. Eu amo você! Estou sempre aqui.

Agradecimentos

À Deus,

Por me proporcionar o dom da vida. Você tem sido um Pai fiel por estar comigo em todas as horas, incluindo os desafios, pois é nessas horas que observamos a vulnerabilidade do ser humano. Todos os dias você me proporciona força e coragem que tem sido fundamental para viver aqui. Obrigado por tudo!

Aos familiares e amigos,

Por estarem no meu coração todos os dias e me proporcionarem forças para estar aqui vivenciando um sonho. A cumplicidade que desenvolvendo ao longo da vida se faz cada dia mais imbatível e tenho certeza de que sempre estaremos juntos. Eu amo vocês!

À Instituição,

Universidade Estadual Paulista Júlio de Mesquita Filho

Na pessoa do diretor da Faculdade de Odontologia de Araçatuba **Prof. Titular Alberto Carlos Botazzo Delbem** e vice-diretor **Prof. Adj. Luciano Tavares Ângelo Cintra.**

Ao Programa,

Pós-Graduação em Ciência da FOA/UNESP

Representado pelo seu coordenador **Prof. Dr. Juliano Pelim Pessan**, pela competência e qualidade na condução do Programa de Pós-Graduação. Agradeço a oportunidade de ser representante discente no Conselho de Pós-Graduação em Ciência da FOA/UNESP.

As Agências de Fomento,

Agradeço a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) por me permitir realizar esse sonho, concedendo minha bolsa (88887.668341/2022-00). Agradeço ao auxílio fornecido ao **Professor Titular João Eduardo Gomes Filho** pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (302124/2022-5) e pela Fundação de Amparo a Pesquisa de São Paulo - FAPESP (2022/05023-8) que foram fundamentais para execução do projeto.

Ao meu orientador,

Professor Tit. João Eduardo Gomes Filho

Por me escolher para orientar durante a pós-graduação. Todos os seus ensinamentos são únicos e sei que fazem diferença na minha vida pessoal e profissional. Tenho que agradecer por toda a confiança, incentivo, acolhimento e paciência em todos os nossos momentos. O senhor faz parte das minhas inspirações por ser um professor tão humano, ético e competente no que se propõe a exercer. É uma honra fazer parte do seu time e carregar seu legado ao longo da minha vida acadêmica. Muito obrigado por tudo!

Aos mestres,

Professor Adj. Luciano Tavares Ângelo Cintra

Por todos os ensinamentos e disponibilidade durante esta etapa da pós-graduação. Obrigado pela ajuda em todos os nossos estudos, pois o senhor nos ajudou no momento que mais precisamos para subsidiar nossos experimentos. Agradeço também por fazer parte da minha banca de qualificação. Você é uma inspiração como professor e pesquisador para minha vida profissional. Cruzar com profissionais como o senhor é uma experiência única!

Professor Adj. Rogério de Castilho Jacinto

Agradeço por todos os momentos de ensinamento e oportunidades nessa etapa da vida acadêmica. O senhor me inspira com toda a sua calmaria e dedicação. Agradeço por fazer parte da minha banca de defesa da dissertação e contribuir para meu crescimento pessoal/profissional.

Professor Assist. Edilson Ervolino

Por todo o empenho e acolhimento em seu laboratório para que eu pudesse concluir os experimentos de imuno-histoquímica. Agradeço pelos momentos de ensinamentos e troca de experiência nas longas imunos. Professor, foi uma honra ter sua presença no meu exame de qualificação para contribuir com nosso estudo. Obrigado pela sua dedicação. Espero que possamos ter mais parcerias futuras.

Demais Professores de Endodontia da FOA/UNESP

Os **Prof. Adj. Dr. Eloi Dezan Junior** e **Prof. Assist. Dr. Gustavo Sivieri de Araújo**, deixo aqui meu sentimento de gratidão por me proporcionarem momentos de ensinamentos que irei levar para vida profissional.

Professores Marciane Magnani e Marcos Lima

Agradeço a parceria estabelecida para ajudar na metodologia do estudo com a avaliação dos componentes fenólicos do vinho por meio da cromatografia líquida. Deixo aqui meu total sentimento de gratidão!

Professora Adj. Maria Ângela Arêa Leão Ferraz

Por todo companheirismo ao longo da vida acadêmica. Me sinto honrado por ter sido seu orientando e hoje seguir seus passos na vida acadêmica. Você me inspira com todo seu amor e dedicação pela docência e endodontia. Agradeço por fazer parte da minha banca de defesa da dissertação e espero que possamos continuar com nossas parcerias.

Aos funcionários,

Do Departamento de Odontologia Preventiva e Restauradora da Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista – FOA/UNESP, **Autran Santiago, Carlos Suetake e Jorge Luis Trevelim**, por todos os momentos de amizade e auxílios laboratoriais durante essa etapa. Eu não poderia esquecer de agradecer aos funcionários da Seção Técnica de Pós-Graduação da FOA/UNESP,

Valéria Queiroz e Cristiane Lui, por todo zelo e atenção as demandas solicitadas.

Ao meu grupo de pesquisa,

Nathalia Machado,

Obrigado por todos os ensinamentos que você nos proporciona diariamente. Você é um exemplo de dedicação e zelo em tudo que estamos dispostos a fazer. Eu tenho que te agradecer também por todo acolhimento que você me proporciona aqui nesta jornada. Saiba que você é iluminada e Deus tem muitas dadivas para sua vida.

Bharbara Moura,

Você é luz! Obrigado por ser essa amiga que traz calmaria no meio das minhas ansiedades. A gente compartilha de muito momentos em comum e eu sei que temos ligação incrível. Te agradeço por cada momento de incentivo, carinho e dedicação. Você é um exemplo de muita determinação nesta jornada. Ahhh, obrigado por saber cada meme do *twitter*!

Rafaela Ricci,

Obrigado por todo acolhimento! Tenho que te agradecer por todas as orientações iniciais que recebi de você dentro daquele avião vindo para Araçatuba na minha primeira viagem. Eu aprendi muito sobre estabelecer prioridade e concentração com você nesses últimos anos! Desejo que seu intercâmbio seja repleto de novidade e que você nos conte tudo. Torço muito por você.

Julissa Arguello,

Sua chegada foi especial, eu jamais imaginaria que encontraríamos alguém de uma conexão incrível. Obrigado por ser esse ombro amigo! Você é uma companheira para todos os momentos. Eu sou muito orgulhoso da amizade que construímos e sei que torcemos muito um pelo outro. Você vai longe, acredite! Sei que antes de você retornar ainda vamos colecionar vários momentos.

E os alunos de Iniciação Científica

Eu agradeço ao **Felipe Haddad, Doany Cevada, Emili Minowa**, pela oportunidade de estar com vocês podendo contribuir para formação e pelas longas conversas e convívios no biotério/laboratório. Agradeço em especial a **Ana Beatriz Carreto** por estar em orientação com Prof. João sob minha condução, essa foi uma oportunidade incrível. Vocês se dedicaram e me ajudaram a estar concluindo essa etapa.

Aos demais amigos da Pós-Graduação,

Eu agradeço por tornaram essa jornada mais leve: **Ana Maria Vasques, Ana Claudia Rodrigues, Ana Paula Ribeiro, Cristiane Cantiga, Flávio Duarte, Gladiston Lobo, Henrique Banci, Juliana Goto, Lara Bravo, Larissa Morais, Laura Cesário, Lucas Estrela, Mariana Justo, Pedro Henrique Chaves, Thais Moraes e Thalya Maltarollo.** Obrigado por todo o convívio e sei que cada um de vocês terá um caminho digno quando finalizar cada etapa na Pós-Graduação.

Epígrafe

"O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis".

José de Alencar

Resumo

Sales-Junior RO. **Efeito tardio do consumo de vinho tinto sem álcool no desenvolvimento da periodontite apical induzida em ratos.** [Dissertação] (Mestrado em Endodontia). Araçatuba: UNESP – Univ. Estadual Paulista; 2024.

Objetivo: Analisar e comparar o efeito da suplementação com vinho tinto sem álcool na periodontite apical instalada e no perfil hematológico sistêmico. Metodologia: Foram utilizados trinta e dois ratos Wistar. A periodontite apical (PA) foi induzida nos primeiros e segundos molares direitos superiores e inferiores de todos os animais, totalizando 4 focos. Os animais foram organizados em quatro grupos: controle (C) – ratos sob suplementação placebo; vinho tinto sem álcool (VTSA) - ratos sob suplementação de VTSA; vinho tinto (VT) - ratos sob suplementação de VT; e álcool (AL) - ratos sob suplementação com solução alcoólica. Os animais foram pesados diariamente para análise de ganho de peso e estabelecer a quantidade de suplemento. A suplementação foi feita pelo método de gavagem trinta dias após a indução e instalação da PA que continuou por mais 30 dias. No dia 60, os animais foram anestesiados para coleta de 5 ml de sangue por punção cardíaca e em seguida eutanasiados para retirada das maxilas para análise de microtomografia e as mandíbulas para análise histológica e imunohistoquímica. Testes estatísticos foram aplicados com 5% de significância. Resultados: Os grupos VT e AL tiveram menor ganho percentual de peso ($p<0,05$). Houve aumento significativo da quantidade de hemoglobina, eritrócitos, hematócrito, volume corpuscular médio e plaquetas no grupo AL em comparação aos demais grupos ($p<0,05$). A quantidade de linfócitos aumentou no grupo AL em comparação aos demais grupos ($p<0,05$) e aumentou no grupo C em comparação aos grupos VTSA e VT ($p<0,05$); os neutrófilos apresentaram-se em menor número no grupo AL em comparação aos demais grupos ($p<0,05$) e os monócitos não apresentaram alterações significativas em sua quantidade entre os grupos. O grupo VTSA apresentou menor volume médio de reabsorção óssea, seguido pelos grupos VT, C e AL ($p<0,05$). Os grupos VTSA e VT apresentaram inflamação leve, seguidos do grupo C com inflamação moderada e do grupo AL com inflamação grave ($p<0,05$). A imunorreação foi menor no grupo VTSA do que nos grupos C e AL para as citocinas inflamatórias TNF-α e IL-1β e para células TRAP ($p<0,05$). Além disso, OPG teve maior presença no grupo VTSA do que nos grupos C e AL ($p<0,05$). Conclusões: O vinho tinto sem álcool influencia na inflamação e reabsorção óssea da periodontite apical instalada, e no perfil hematológico sistêmico.

Palavras-chave: Periodontite Periapical; Vinho; Polifenóis; Remodelação Óssea.

Abstract

Sales-Junior RO. **Late effect of non-alcoholic red wine consumption on the development of induced apical periodontitis in rats** [Dissertação] (Mestrado em Endodontia). Araçatuba: UNESP – Univ. Estadual Paulista; 2023.

Objective: To analyse and compare the effect of dealcoholized red wine supplementation on settled apical periodontitis and systemic haematological profile. Methodology: Thirty-two Wistar rats were used. Apical periodontitis (AP) was induced in the upper and lower right first and second molars of all animals, a total of 4 foci. The animals were divided into four groups: control (C) - rats receiving placebo supplementation; dealcoholized red wine (DRW) - rats receiving DRW supplementation; red wine (RW) - rats receiving RW supplementation; and alcohol (AL) - rats receiving alcoholic solution supplementation. Animals were weighed daily to analyse weight gain and to determine the amount of supplementation. Supplementation was performed by gavage thirty days after induction and establishment of AP, which continued for a further 30 days. On day 60, the animals were anaesthetised to obtain 5 ml of blood by cardiac puncture and then euthanised to remove the maxillas for microtomography analysis and the jaws for histological and immunohistochemical analysis. Statistical tests were performed at 5% significance. Results: The RW and AL groups had lower percentage weight gain ($p<0.05$). There was a significant increase in the amount of haemoglobin, erythrocytes, hematocrit, mean corpuscular volume and platelets in the AL group compared to the other groups ($p<0.05$). The number of lymphocytes increased in the AL group compared to the other groups ($p<0.05$) and increased in group C compared to groups DRW and VT ($p<0.05$); neutrophils were fewer in the AL group compared to the other groups ($p<0.05$) and monocytes did not show significant changes in their number between groups. The DRW group had a lower mean volume of bone resorption, followed by the RW, C and AL groups ($p<0.05$). Immunoreactivity for the inflammatory cytokines TNF-α and IL-1β and for TRAP cells was lower in the DRW group than in the C and AL groups ($p<0.05$). Furthermore, OPG was more present in the DRW group than in the C and AL groups ($p<0.05$). Conclusions: Dealcoholized red wine influences inflammation and bone resorption in settled apical periodontitis and the systemic haematological profile.

Keywords: Periapical Periodontitis; Wine; Polyphenols; Bone Remodeling.

Lista de Figuras

Figure 1 – Flowchart of study design: Preferred Reporting Items for Animal Studies in Endodontology (PRIASE). 34

Figura 2 – Structure and chronology of the study 35

Figure 3 – The bar graphs show the mean and standard deviation of the percentage increase in weight obtained 40

Figure 4 – The photomicrographs show the histological characteristics of the regions close to the foraminal opening of the distal root (R) of the lower first molar 42

Figure 5 – Stages and divisions of the study 61

Figure 6 – Microtomography, histology and immunohistochemistry data plots 66

Figure 7 – Representative microtomographic sections 68

Figure 8 – Representative sections from histology and immunohistochemistry (IL-10, TNF-α and IL-1β) 69

Figure 9 – Representative sections from immunohistochemistry (OPG, RANKL and TRAP) 70

Lista de Tabelas

Table 1 - Mean and standard deviation of quantification of phenolic compound..26
Table 2 - Mean and standard deviation of blood cell parameters 29
Table 3 - Scores and Median attributed to inflammatory infiltrate 30

Lista de Abreviaturas

AL	Alcohol
AP	Apical periodontitis
ARRIVE	Animal Research: Reporting of In Vivo Experiments
C	Control
COX	Cyclooxygenase
DRW	Desalcoholzed red wine
EDTA	Ethylenediaminetetraacetic acid
IL-10	Interleukin 10
IL-16	Interleukin 16
IL-1β	Interleukin 1β
MCV	Mean Corpuscular Volume
OPG	Osteoprotegerin
PRIASE	Preferred Reporting Items for Animal Studies in Endodontology
RANK	Receptor activator of nuclear factor kappa B
RANKL	Receptor activator of nuclear factor kappa-B ligand
ROS	Reactive oxygen species
RP-HPLC	Reversed-phase high-performance liquid chromatography
RW	Red Wine
TNF-α	Tumor necrosis factor alpha
TRAP	Tartrate-resistant acid phosphatase

Sumário

Introdução Geral 22
Objetivos 27
Artigo 1 29
Artigo 2 58
Referências Gerais 87
ANEXO A - Comitê de Ética no Uso de Animais (CEUA) 91
ANEXO B - Comprovante de submissão no *International Endodontic Journal* em 09/11/2023........ 92
ANEXO C - Guidelines for Authors on Submission to the *International Endodontic Journal*........ 94
ANEXO D - Guidelines for Authors on Submission to the *Archives of Oral Biology* 99

Introdução Geral

A periodontite apical (PA) é uma doença inflamatória desenvolvida nos tecidos periapicais em resposta a uma infecção microbiana persistente que acomete a polpa dentária. A partir dessa condição, as bactérias e seus subprodutos causam uma resposta inflamatória local que, se não for tratada, podem evoluir para os tecidos periapicais, induzindo o organismo a conter o avanço desta infecção através da reabsorção óssea (Goldman et al., 2019; Kakehashi & Stanley,1965; Yamasaki et al., 1994). Essa resposta imunológica do organismo ocorre de forma adaptativa por meio da sinalização de células de defesa e mediadores inflamatórios pela corrente sanguínea (Nair, 2004). A teoria da infecção focal conclui que a PA pode levar compostos microbianos solúveis, mediadores inflamatórios ativos e fatores hemostáticos do canal radicular para o ambiente sistêmico que podem se instalar em órgãos e tecidos causando uma metástase infeciosa que compromete a saúde do indivíduo (Li et al., 2000; Scannapieco, 1998).

Essa disseminação pode ocorrer devido à conexão com o sistema circulatório, uma vez que não existe uma barreira epitelial entre o canal radicular infectado e o tecido granulomatoso altamente vascular nas infecções periapicais. Esse tecido é um local propenso à concentração dos principais biomarcadores inflamatórios, fator de necrose tumoral e interleucinas (Stashenko, Teles & D'Souza, 1998). Por outro lado, também existem indícios que sustentam a hipótese que a PA pode ter influência na alteração das células de defesas da corrente sanguínea e como também ativar mediadores inflamatórios sistêmicos (Bian et al., 2012; Samuel et al., 2018). Destaca-se a proteína C reativa, interleucinas, TNF-α, e metaloproteinases como esses principais marcadores inflamatórios sistêmicos que sofrem alteração (Georgiou et al., 2019).

O tecido ósseo contém três principais tipos de células responsáveis pela sua composição, sendo elas: osteoblastos, osteócitos e osteoclastos. Sob condições fisiológicas, existe um equilíbrio entre síntese e reabsorção óssea, que define a remodelação óssea. Esse processo inclui como função dos osteoclastos a liberação de enzimas proteolíticas que atuam removendo a superfície óssea enquanto os osteoblastos precipitam nova matriz extracelular (Petean et al.,2022). Na PA com a ativação de citocinas pró-inflamatória acontece um desequilíbrio entre a síntese e reabsorção pelo processo de osteoclastogênese que leva à uma perda óssea mais acentuada por meio de osteoclastos maduros promovendo a degradação dos componentes orgânicos e inorgânicos (Yu et al., 2016). Esse processo de reabsorção conta com a presença de mediadores da osteoclastogênese, sendo eles: o receptor de ativação do fator nuclear NF-kB (RANK), o ligante de RANK (RANKL) e a osteoprotegerina (OPG) (Petean et al.,2022).

RANK é uma proteína receptora encontrada na superfície de células clásticas que, quando ligada ao RANKL induz a expressão de genes que especificam a linhagem osteoclástica com uma enzima de fosfatase ácida resistente ao tartarato (TRAP) causando a ativação de osteoclastos para estimular a reabsorção de tecidos mineralizados. A OPG, por sua vez, atua ligando-se ao RANKL impedindo-o de se ligar ao RANK e sequenciar a formação osteoclástica. O desequilíbrio na expressão de RANKL e OPG em condições inflamatórias, nas quais há um aumento de RANKL e diminuição da atividade de OPG, resulta na exacerbação da osteoclastogênese e reabsorção óssea (Kajiya et al., 2010; Luo et al., 2022; Petean et al.,2022).

A remodelação óssea e inflamação local da PA pode ser regulada com substâncias moduladoras como o vinho tinto (Dal-Fabbro et al., 2021). O vinho tinto (VT) é uma bebida consumida mundialmente e que vem ganhando destaque na sociedade moderna pelas suas ações benéficas: influência na ação protetora dos vasos sanguíneos, capacidade anti-inflamatória, antialérgica e anticancerígena, inclusive nas infecções orais (Castanaldo et al., 2019; Mühlbauer et al., 2003; Muñoz-González et al., 2014; Singh et al., 2018). As principais substâncias que constituem o VT são: açúcares, etanol, ácidos orgânicos, sais de ácidos minerais/orgânicos, vitaminas, anidrido sulfuroso e, principalmente, compostos bioativos de natureza fenólica (Ali et al., 2010; Kutlesa & Mršić, 2016). Esses benefícios foram descobertos a partir da busca por alimentos bioativos que começou na França e Itália quando se percebeu que o consumo leve a moderado de vinho poderia levar a uma menor incidência de doenças coronárias (Pignatelli et al., 2006; Vinson, Teufel & Wu, 2001).

De forma geral, os efeitos do VT dependem da composição, quantidade e frequência de consumo, sendo está em torno de 150ml/dia para mulheres e 300ml/dia para homens (Dal-Fabbro et al., 2021; U.S. Department of Agriculture & U.S. Department of Health & Human Services, 2020). Apesar das bebidas alcoólicas estarem sendo relacionadas com benefícios gerais para a saúde humana pela sua ingestão leve ou moderada, este consumo não pode ser generalizado por conta das diferenças genéticas, estilo de vida dos indivíduos e sua associação com diversas doenças crônicas (Ferrières, 2004; Fernandez-Sola et al., 2015; Santos, Gonzalez-Manzano & Gonzalez-Paramás, 2021).

Nosso grupo de pesquisa observou em um estudo prévio, que embora a administração profilática do VT em ratos com PA induzida possa diminuir a intensidade inflamatória e a marcação de TRAP, somente o grupo que recebeu a administração de Resveratrol + Quercetina proporcionou resultados estatisticamente melhores que o ao grupo controle (com suplementação placebo), sendo eles: menor intensidade do processo inflamatório, menor tamanho da lesão observado pela microtomografia e maior expressão de OPG. Entretanto, quando o álcool foi administrado na mesma dose e concentração observadas no VT, observou-se um aumento da reabsorção óssea e aumento da severidade do perfil inflamatório (Dal-Fabbro et al., 2021).

O álcool apresenta efeitos nocivos para saúde sistêmica, uma vez que altera a fisiologia dos neutrófilos, facilitando a proliferação e penetração bacteriana; e reduz a produção de citocinas inflamatórias pelos monócitos, o que pode ser possivelmente benéficas para a proliferação microbiana (Malherbe & Messaoudi, 2022) podendo, assim, afetar diretamente a PA. Em outros estudos com ratos Wistar sobre ação local, o consumo de álcool nas concentrações acima de 10% provocou uma maior perda óssea alveolar e processo inflamatório local pela alteração da proporção de RANKL/OPG, explicado através da capacidade do álcool em potencializar a liberação de RANKL e não alterar a liberação de OPG, o que contribui para um desequilíbrio e estimulação da osteoclastogênese (Dal-Fabbro et al., 2019; Wagner et al., 2019).

Com esses resultados fortalece-se, portanto, a hipótese de que o VT possui efeitos benéficos em função dos polifenóis e que o álcool repercute de forma antagônica em relação à prevenção da reabsorção óssea e da inflamação na PA. Uma alternativa para estudar apenas as propriedades favoráveis dos compostos fenólicos sem a interferência do álcool, que viriam a otimizar os resultados do grupo VT, é a utilização do vinho tinto sem álcool (VTSA).

Nos últimos anos, enólogos e pesquisadores desenvolveram várias técnicas para a redução do teor alcoólico dos vinhos e bebidas, mantendo as boas qualidades sensoriais, a fim de satisfazer a procura dos consumidores mediante a grande demanda do mercado (Sam et al., 2021). Essas técnicas podem ser classificadas de acordo com a fase de produção do vinho em: fase de préfermentação, fermentação e pós-fermentação. A redução alcoólica nesses estágios inclui a redução do álcool e açúcares fermentáveis através da separação por uma membrana ou aparelhos sem membrana que causam mínimas alterações nos componentes fenólicos do vinho e podem inclusive aumentar suas concentrações no produto (Belisário-Sánchez et al., 2009).

O VTSA pode possuir em sua composição até 24% dos teores de compostos fenólicos, fenólicos totais, flavonol, éster tartárico e antocianinas (Belisário-Sánchez et al., 2009). Os principais parâmetros de qualidade do VTSA em diferentes níveis de concentração alcoólica variam de 9,8% a 0,3% no processo de destilação osmótica. A partir do tratamento de osmose reversa evaporativa, houve um aumento de fenólicos (particularmente as antocianinas), intensidade de cor e ácidos orgânicos, devido à redução da precipitação de sais de tartarato de vinho, uma vez que esses podem absorver polifenóis (Liguori et al., 2019).

Até o momento, não há estudos que relacionem a suplementação alimentar com VTSA, como uma terapia sistêmica coadjuvante ao tratamento endodôntico convencional, cujo potencial com a presença dos polifenóis e ausência do componente álcoolico poderá auxiliar na modulação da atividade inflamatória sistêmica e local frente a ocorrência de PA, tornando possível o seu uso para controle de progressão dessa lesão no organismo.

Objetivos

OBJETIVO GERAL

Analisar e comparar a lesão periapical instalada em ratos Wistar com suplementação de vinho tinto sem álcool, via gavagem, por meio da análise histológica do perfil inflamatório local, análise de imuno histoquímica local, análise microtomográfica dos tecidos periapicais e o perfil hematológico para perfil inflamatório sistêmico.

OBJETIVOS ESPECÍFICOS

- Analisar e compara o ganho de peso dos animais ao longo do protocolo experimental por meio do peso final em relação ao inicial;
- Analisar o perfil hematológico (plaquetas, eritrócitos, hemoglobina (HGB), hematócrito, volume corpuscular médio, proteínas plasmáticas, neutrófilos, linfócitos e monócitos), por meio da análise sanguínea em analisador automático;
- Analisar o perfil inflamatório da lesão periapical por meio da análise histológica em H&E e imunohistoquímica para as citocinas pró-inflamatórias IL-1β, IL-10 e TNF-α;
- Analisar e quantificar as áreas da lesão periapical por meio da análise microtomográfica;
- Analisar o processo de remodelação óssea da lesão periapical em ratos por meio da análise imuno-histoquímica utilizando a expressão OPG e RANKL somado com a presença de células TRAP.

Artigo 1

Dealcoholized red wine influences the systemic hematological profile and apical periodontitis in rats

Artigo formatado e submetido ao periódico *International Endodontic Journal*
(Qualis A1 e Fator de impacto 5 em 2023)

ABSTRACT

Objective: to analyze the effect of consumption of dealcoholized red wine on the settled apical periodontitis and its effect on the systemic hematological profile. Methodology: Thirty-two Wistar rats were used. Apical periodontitis (AP) was induced in the upper and lower right first and second molars of all animals, a total of 4 AP foci. The animals were divided into four groups: control (C) - rats receiving placebo supplementation; dealcoholized red wine (DRW) - rats receiving DRW supplementation; red wine (RW) - rats receiving RW supplementation; and alcohol (AL) - rats receiving alcoholic solution supplementation. Animals were weighed daily to analyse weight gain and to determine the amount of supplementation. Supplementation was administered by gavage 30 days after the induction of AP and continued for a further 30 days. On day 60, the animals were anaesthetised to obtain 5 ml of blood by cardiac puncture and then euthanised to remove the jaws for histological analysis. Statistical tests were performed. Results: The RW and AL groups had lower percentage weight gain ($p<0.05$). There was a significant increase in the amount of haemoglobin, erythrocytes, hematocrit, mean corpuscular volume and platelets in the AL group compared to the other groups ($p<0.05$). The number of lymphocytes increased in the AL group compared to the other groups ($p<0.05$); and increased in group C compared to the DRW and RW groups ($p<0.05$); neutrophils were fewer in the AL group compared to the other groups ($p<0.05$) and monocytes did not show significant changes in their quantity between the groups. The mean inflammatory process score in the AP was significantly lower in the RW and DRW (2) groups than in the C (3) and AL (4) groups, ($p<0.05$). Conclusions: Supplementation with dealcoholized red wine influences the reduction of local inflammatory infiltrate and systemic hematological profile in rats with settled AP.

Keywords: Periapical Periodontitis; Wine; Polyphenols; Bone Remodeling.

INTRODUCTION

Apical periodontitis (AP) is an inflammatory disease of the periapical tissues in response to a microbial infection of the dental pulp causing that, if left untreated, stimulates the organism to limit its advance through increasing of inflammation and bone resorption (Goldman et al., 2019; Kakehashi & Stanley,1965; Yamasaki et al., 1994). This immune response of the body occurs adaptively through the signalling of defense cells and inflammatory mediators through the bloodstream (Nair, 2004). The bloodstream can also transport soluble microbial compounds, active inflammatory mediators and haemostatic factors from the root canal to be deposited in organs and tissues causing infectious metastasis and compromising the health of the individual (Li et al., 2000; Scannapieco, 1998).

Beverages containing bioactive compounds can help protecting against certain diseases, including the use of alcoholic beverages such as red wine (RW) (Vejarano & Luján-Corro, 2022). The interest regarding active foods began in France and Italy where it was found that light to moderate wine consumption could lead to a lower incidence of coronary heart disease (Pignatelli et al., 2006; Vinson, Teufel & Wu, 2001). The beneficial effects of red wine may vary between health organizations but are known to depend on the composition, quantity and frequency of consumption, generally around 150 ml/day for women and 300 ml/day for men (Dal-Fabbro et al., 2021; U.S. Department of Agriculture & U.S. Department of Health & Human Services, 2020;). The consumption can also be based on the number of cups as up to one cup for women and up to two cups for men (NHLBI, 2021).

Red wine has been the subject of studies that reveal its phenolic composition with flavonoid and non-flavonoid components whose most beneficial effects are related to: vascular protection, anti-inflammatory, anticancer, antibacterial capacity, and and bone remodelling (Castanaldo et al., 2019; Mühlbauer et al., 2003; Muñoz-González et al., 2014; Singh et al., 2018). There are studies in periodontal medicine that show the possibility of the red wine modulator action that improves periodontal health (Kongstad et al., 2004; Wagner et al., 2019). Similarly, it was showed lower inflammation and reduced apical periodontitis area with red wine or its phenolic compounds (resveratrol associated with quercetin) but not when alcohol was administered at the same dose and concentration as in red wine that increased bone resorption and the severity of the inflammatory profile (Dal-Fabbro et al., 2021a).

Although alcoholic beverages are associated with general benefits for human health, their consumption can not be widespread due to genetic differences, individual lifestyles and their association with various chronic diseases (Fernandez-Sola, 2015; Ferrières, 2004; Hu et al., 2022; Santos, Gonzalez-Manzano & Gonzalez-Paramás, 2021). Alcohol has harmful effects as it alters the physiology of neutrophils, facilitating bacterial proliferation and penetration; and reduces the production of inflammatory cytokines by monocytes, which may be possibly beneficial for microbial proliferation (Malherbe & Messaoudi, 2022) and may therefore directly affect the apical periodontitis.

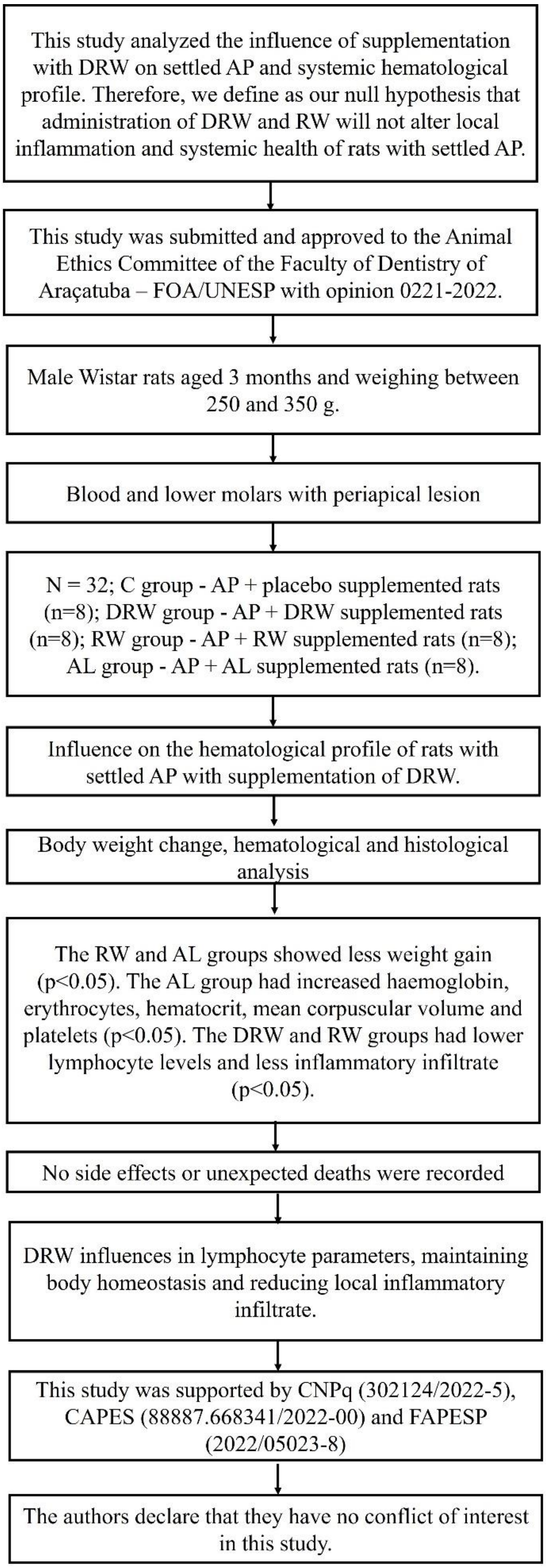
An alternative to study only the desired properties of the red wine based on the phenolic compounds without the interference of alcohol is the use of dealcoholized red wine (DRW) (Bai et al., 2019). In recent years, winemakers and researchers have developed several techniques to reduce the alcohol content of wines and other beverages to meet consumer demand (Sam et al., 2021). Dealcoholized red wine can contain up to 24% of phenolic compounds, total phenolics, flavonol, tartaric acid esters and anthocyanins (Belisário-Sánchez et al., 2009). DRW has been listed in the literature as a beneficial bioactive for inhibiting atherosclerosis, reducing insulin resistance, lowering blood pressure and its high antioxidant capacity (Chiva et al., 2013; Chiva-Blanch et al., 2013; Vinson, Teufel & Wu, 2001; Serafini, Maiani & Ferro-Luzzi, 1998).

To date, there is no scientific evidence linking dietary supplementation with dealcoholized red wine as an adjuvant systemic therapy of the apical periodontitis, whose potential properties could help modulating the inflammatory activity and consequently influencing on the systemic health. Thus, this study aimed to analyze the effect of consumption of dealcoholized red wine on the settled apical periodontitis and its effect on the systemic hematological profile. Therefore, our null hypothesis is that administration of dealcoholized red wine will not alter local inflammation and systemic hematological profile in rats with settled apical periodontitis.

MATERIAL AND METHODS

The present in vivo study and its experimental protocols were approved by the Institutional Ethics Committee on Animal Use (0221-2022) of the Universidade Estadual Paulista, São Paulo, Brazil. For the design of this study, the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) guideline (Nagendrababu et al., 2021) was used with the experimental report in Figure 1.

Figure 1 - Flowchart of study design: Preferred Reporting Items for Animal Studies in Endodontology (PRIASE).



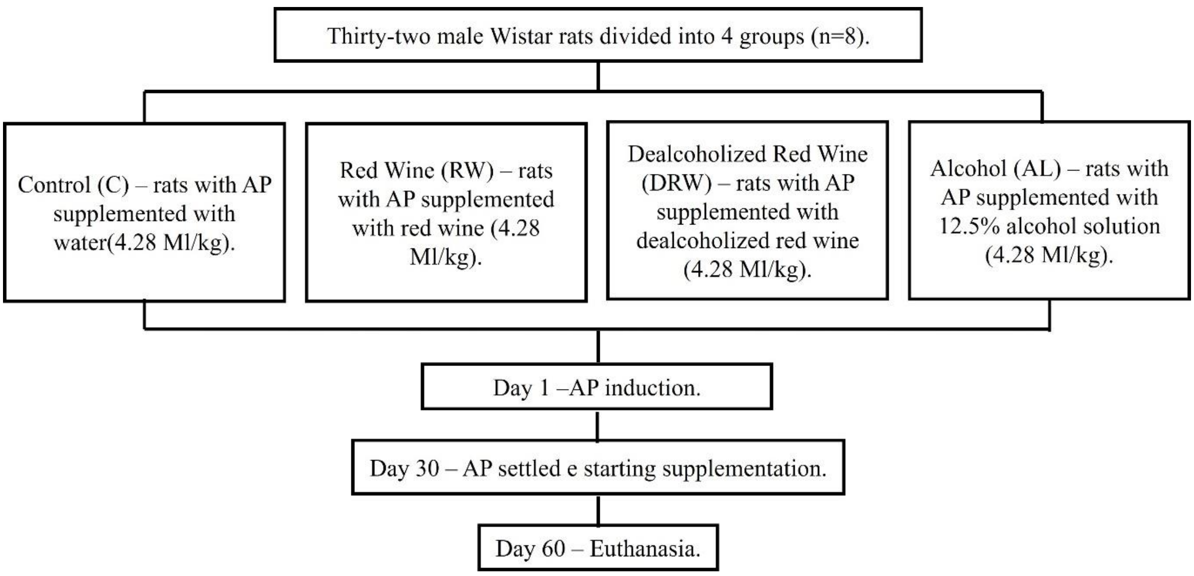
Sample calculation

The sample was calculated on the scientific basis of previous studies with similar methodology, based on the parameters used, with an alpha error of 5% and a power of 95%, satisfactory values to present a difference, which was obtained as required with a minimum of seven animals per group (Cintra et al., 2014; Cosme-Silva et al., 2021; Pinto et al., 2020). However, considering the long duration of the experiment and being aware of the susceptibility to complications, one animal was added to each group in a total of eight animals per group.

Animals

Thirty-two male Wistar rats (Rattus norvegicus albinus, three months old, weighing between 250 and 350g, were used, kept in mini-isolators at 22°, 70% humidity and light control (12 h light and 12 h dark), with solid food and water ad libitum. These animals were arranged into 4 groups, with a sample size of 8 animals, as follows Control (C): rats with AP; dealcoholized red wine (DRW): rats with AP and diet supplemented with DRW; red wine (RW): rats with AP and diet supplemented with RW; alcohol (AL): rats with AP and diet supplemented with alcoholic solution. The experiment lasted 60 days, with supplementation by gavage starting 30 days after the AP onset. The design and chronology of the study are shown in Figura 2.

Figura 2 - Structure and chronology of the study.



Detection and quantification of phenolic compounds in wine samples

Before starting the experimental protocol, samples of red wine and dealcoholized red wine (Vinoh brand, Bento Gonçalves, RS, Brazil) were obtained for quantification of phenolic components using reversed-phase high-performance liquid chromatography (RP-HPLC). The regular red wine tested was a sample of Merlot wine harvest used for the dealcoholization. Briefly, a Zorbax pre-column (USA, C18; 12.6 × 4.6 mm, 5 μm) and a Zorbax column (USA, Eclipse Plus RP-C18; 100 × 4.6 mm, 3.5 μm) were used, divided into two phases with an injection volume of 20 μL of sample. In phase A, acidified water (pH 2 with 0.1 mM/L phosphoric acid) was used for dilution and filtered through a 0.45 μm membrane. In phase B, methanol acidified with 0.5% phosphoric acid was used with the gradient used for the separation: 0 to 5 min - 5%; 5 to 14 min - 23%; 14 to 30 min - 50%; 30 to 33 min: 80%. The column temperature was kept at 35°C. Flow rate was maintained at 0.8 mL/min. Data acquisition was performed using OpenLAB CDS ChemStation EditionTM software (Agilent Technologies, Santa Clara, California, USA). Peaks of phenolic compounds were identified by comparison of their retention times with external standards (purity ≥ 98%) from Cayman Chemical Company (Ann Arbor, MI, USA). Calibration curves showed R2 ≥ 0.998 for all compounds. The spectral purity of the peaks was checked using the Threshold tool to ensure the accuracy of the identification against the external standard (Padilha et al., 2017).

Induction and development of apical periodontitis

On the first day of the experimental protocol, all groups underwent general intramuscular anesthesia with a combination of 10 mg/kg of 2% xylazine (Ceva Saúde Animal Ltda, Paulinia, SP, Brazil) and 80 mg/kg of 10% ketamine hydrochloride (Ceva Saúde Animal Ltda, Paulinia, SP, Brazil) for the induction of apical periodontitis. This procedure consisted of exposing the pulp with a coronal opening of the first and second maxillary and mandibular right molars using a long neck maillefer drill (Dentsply Sirona, Charlotte, North Carolina, USA) with a diameter of 0.5 mm, standardizing the pulp exposures (Goldman et al., 2019; Yamasaki et al., 1994). The teeth were kept open to the oral environment during 30 days without any further intervention for the development the apical periodontitis (Tani-Ishii et al., 1994; Yoneda et al., 2017).

Dietary supplementation for the treatment groups

Supplements started to be administered by gavage in the morning of the 31th day after the pulp exposure day. A daily dose was based on a previous study with a beneficial dose for a volume equal to 4.28 mL of red wine/kg of animal body weight (Dal-Fabbro et al., 2021a). Rats from C group received 4.28 mL sterilized water as sham intervention; for the RW and DRW groups, red wine and de-alcoholized red wine were used (Vinoh, Bento Gonçalves, RS, Brazil); The rats from AL group received a balanced solution of 12.5%.

Blood analysis and euthanasia

After 60 days from the pulp exposure day, the rats were anesthetized intramuscularly and 5 mL of blood was collected through a cardiac puncture. These samples were mixed with Ethylenediaminetetraacetic acid (EDTA) (Labsynth Produtos, São Paulo, Brazil) and transferred to a veterinarian for processing and then analyzed using an automatic hematology analyzer (BC 2800 Vet; Shenzhen Mindray Animal Medical Technology Co., China) for quantification of: hemoglobin, red blood cells, corpuscular volume, hematocrit, neutrophils, lymphocytes and monocytes (Azuma et al., 2021; Cosme-Silva et al., 2019). The animals were then euthanized with an overdose of sodium thiopental (Thiopentax, Cristalia Produtos Químicos Farmacêuticos Ltda., São Paulo, Brazil) (Cantiga-Silva et al., 2021; Justo et al., 2022).

Collection and histological analysis

After euthanasia, the right hemimandibles were removed, fixed in 10% formalin and demineralized in 10% EDTA (Labsynth Produtos, São Paulo, Brazil). They were then washed in running water, dehydrated in alcohol, clarified in xylol and embedded in paraffin for semi-serial sections in the mesiodistal plane of 5 µm thickness. They were then stained with hematoxylin-eosin to mount slides for analysis of the inflammatory infiltrate based on its intensity and extension under an optical microscope (DM 4000 B; Leica, Wetzlar, Germany). For the intensity assessment, the area of the apical foramen of the distal root of the lower first molar was used with a series of 10 images at 400x magnification to count inflammatory cells and assign the following scores: 1 - absent (absent or few inflammatory cells); 2 - mild (up to 25 cells and mild reaction); 3 - moderate (25-125 inflammatory cells and moderate reaction); and 4 - severe (more than 125 cells and severe reaction) (Conti et al., 2020).

Statistical analysis

Data were analyzed using SigmaPlot 14.0™ (Chicago, IL, USA) with the Shapiro-Wilk test to verify normal distribution. The T-student test was then used to compare the two wine samples to quantify phenolic compounds by liquid chromatography. For blood and histological analyses, one-way analysis of variance (ANOVA) followed by the Tukey test was used for parametric data, and the Kruskal-Wallis test followed by Dunn's test was used for non-parametric data. A 5% significance level ($p<0.05$) was used for all statistical analyses.

RESULTS

Quantification of phenolic compounds in wine samples

Several phenolic compounds were detected and quantified in the RW and DRW samples, as shown in Table 1. It was found that, in general, both wines contained the same amounts of phenolic acids, flavanols, proanthocyanidins, stilbenes, and flavanones, with no statistical difference between the samples. As for the flavonol components, myricetin and quercetin-3-glucoside, their amounts were significantly higher in DRW ($p<0.05$); already one flavanol component, rutin, and three anthocyanins, delphinidin-3-glucoside, pelargonidin-3-glucoside and malvidin-3-glucoside, were significantly higher in RW ($p<0.05$).

Table 1 - Mean and standard deviation of quantification of phenolic compounds

Phenolic Components (mg/L)	**Samples**	
	DRW	**RW**
Phenolic acids		
Gallic acid	56.92 ± 1.38[a]	56.40 ± 1.17[a]
Caftaric acid	105.80 ± 2.40[a]	104.92 ± 2.24[a]
Caffeic acid	3.10 ± 0.05[a]	2.91 ± 0.01[a]
p_Coumaric acid	0.77 ± 0.01[a]	0.78 ± 0.04[a]
Flavanols		
Catechin	6.73 ± 0.15[a]	6.77 ± 0.12[a]
Epigallocatechin gallate	0.05 ± 0.00[a]	0.05 ± 0.00[a]

Epicatechin	11.60 ± 0.18[a]	11.93 ± 0.27[a]
Epicatechin gallate	0.96 ± 0.01[a]	0.98 ± 0.02[a]
Proanthocyanidins		
Procyanidin B1	14.65 ± 0.40[a]	14.10 ± 0.19[a]
Procyanidin B2	29.25 ± 0.71[a]	29.60 ± 0.73[a]
Stilbenes		
Cis-Resveratrol	0.91 ± 0.02[a]	1.48 ± 0.20[a]
Trans-Resveratrol	0.43 ± 0.00[a]	0.42 ± 0.01[a]
Flavanones		
Naringenin	4.56 ± 0.37[a]	5.47 ± 0.46[a]
Flavonols		
Myricetin	19.82 ± 0.39[a]	17.15 ± 0.51[b]
Quercetin 3-Glucoside	3.69 ± 0.02[a]	3.57 ± 0.02[b]
Rutin	0.68 ± 0.01[b]	0.84 ± 0.01[a]
Kaempferol 3-glucoside	1.42 ± 0.29[a]	1.48 ± 0.29[a]
Anthocyanins		
Delphinidin 3-glucoside	4.54 ± 0.14[b]	5.13 ± 0.13[a]
Cyanidin 3-glucoside	2.93 ± 0.03[a]	3.11 ± 0.07[a]
Pelargonidin 3-glucoside	17.80 ± 0.30[b]	20.03 ± 0.28[a]
Peonidin 3-glucoside	3.19 ± 0.05[a]	3.42 ± 0.05[a]
Malvidin 3-glucoside	76.94 ± 0.85[b]	85.74 ± 0.92[a]

a-b Mean ± standard deviation with different lowercase letters on the same line differed by Student's t test ($p < 0.05$) between samples.

Change in body weight

The percentages of change in body weight of the animals were calculated, considering the final in relation to the initial weight. It was observed that all animals had weight gain during the experimental period, with the mean and standard deviation being 64.00%±12.45 for the C group, 52.12±8.75 for the DRW group, 48.62%±7.03 for the RW group and 37.75%±4.21 for the AL group (see Figure 3). Means of body weight gain showed that the C group gained significantly more weight than the RW and AL groups ($p < 0.05$) and that the DRW group gained significantly more weight than the AL group ($p < 0.05$).

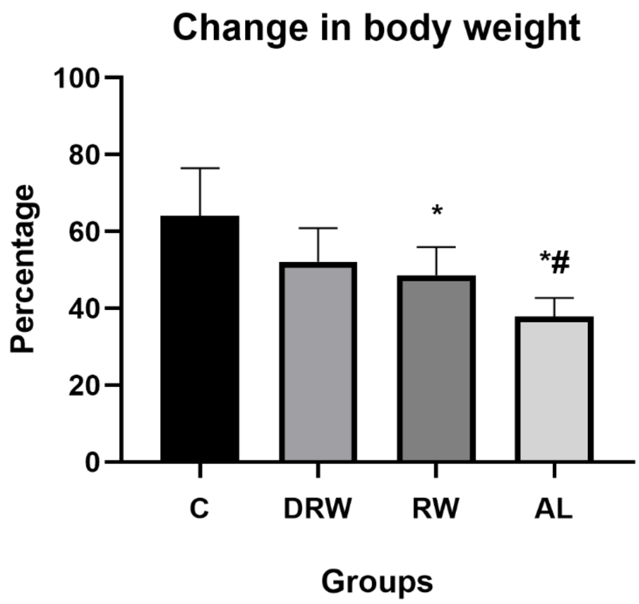


Figure 3 - The bar graphs show the mean and standard deviation of the percentage increase in weight obtained in the ANOVA test and the different symbols show the statistical differences found in the Tukey test ($p < 0.05$). Symbols: *$p < 0.05$ versus C; # $p < 0.05$ versus DRW.

Blood profile

The following blood parameters were quantified: hemoglobin, erythrocytes, mean corpuscular volume, platelets, hematocrit, neutrophils, lymphocytes and monocytes, which the results are shown in Table 2.

Regarding the quantity of the main red blood cells, hemoglobin, erythrocytes, platelets, hematocrit and mean corpuscular volume had significantly higher quantitative values in the AL group than in the C, RW and DRW groups ($p < 0.05$). For the percentage of the main inflammatory cells in the white series, it was found that lymphocytes had a significantly higher concentration in the AL group ($p < 0.05$) in relation to the other groups and C group had a significantly higher concentration than DRW and RW groups ($p < 0.05$); neutrophil count was lower in the AL group ($p < 0.05$) compared to the other groups; and monocytes count was similar among the groups ($p>0.05$).

Table 2 - Mean and standard deviation of blood cell parameters.

Parameters Hematological	Groups (Mean ±SD)				
	Reference Values	C	DRW	RW	AL
Hemoglobin (g/dL)	13–15	14,18±0,50[a]	13,67±0,41[a]	14,50±0,39[a]	16,17±1,00[b]
Erythrocytes (x10^6/μL)	6–8	7,99±0,35[a]	7,93±0,35[a]	7,83±0,24[a]	9,23±0,54[b]
Mean Corpuscular Volume (fL)	48-54	49,91±2,13 [a]	49,81±1,70 [a]	51,61±1,04[a]	62,24±2,02[b]
Plaquetas (x10^3/μL)	923–1580	640,00±58,55[a]	597,50±93,92[a]	497,50±124,41[a]	1137,50±130,24[b]
Hematocrit (%)	42–49	40,37±1,84[a]	40,00±1,77[a]	40,75±1,66[a]	57,00±2,20[b]
Neutrophils (%)	6–22	20,75±2,43[b]	21,87±0,99[b]	20,37±2,32[b]	14,25±2,31[a]
Lymphocyte (%)	69–86	76,00±2,56[b]	71,00±1,30[a]	71,25±1,48[a]	83,37±2,20[c]
Monocytes (%)	3–10	3,62±0,74[a]	3,12±0,35[a]	3,25±0,46[a]	3,75±0,46[a]

* Mean ± standard deviation obtained in the ANOVA test and the different lowercase letters in the same line show the statistical differences found in the Tukey test ($p < 0.05$) between the groups. The reference values come from the article by He et al. (2017).

Histological analysis

All groups showed total pulp necrosis with inflammatory infiltrate in the periapical region, with disruption of the periodontal ligament and areas of bone resorption, demonstrating the effectiveness of the method of inducing the periapical lesion its maintenance along 60 days. The inflammation scores assigned to each animal in each group are shown in Table 3 and representative hematoxylin-eosin stained sections are shown in Figure 4. The animals from the Control group had a moderate level of inflammation with a median score of 3, significantly higher than the DRW and RW groups, which had a median score of 2 with mild inflammation ($p<0.05$). There was no statistical difference when comparing the DRW and RW groups with a median score of 2 ($p>0.05$). However, ALC group had a higher inflammation score with a median of 4 when compared to the DRW and RW groups ($p<0.05$).

Table 3 - Scores and Median attributed to inflammatory infiltrate.

Analysis Parameters		**Groups**				**P value**
		C	**DRW**	**RW**	**AL**	
Inflammatory Infiltrate	**1 - Absent**	0/8	1/8	2/8	0/8	*0,001*
	2 - Mild	0/8	7/8	6/8	0/8	
	3 - Moderate	6/8	0/8	0/8	0/8	
	4 - Severus	2/8	0/8	0/8	8/8	
	Median*	3^{a}	2^{b}	2^{b}	4^{a}	

*Median obtained in the Kruskal Wallis test and the different lowercase letters in the same line show the statistical differences found in the Dunn's test ($p < 0.05$) among the groups.

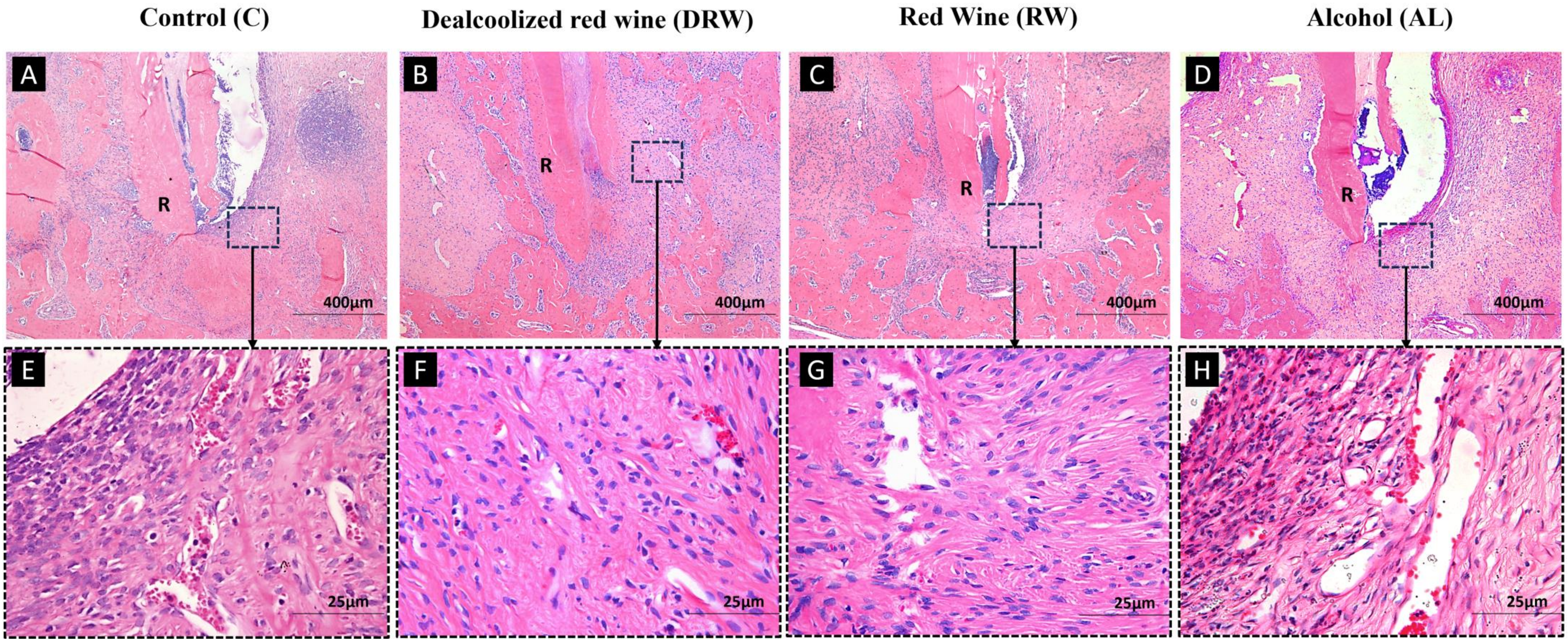

Control (C)
Dealcoolized red wine (DRW)
Red Wine (RW)
Alcohol (AL)
A
B
C
D
R
R
R
R
400µm
400µm
400µm
400µm
E
F
G
H
25µm
25µm
25µm
25µm

Figure 4 - The photomicrographs show the histological characteristics of the regions close to the foraminal opening of the distal root (R) of the lower first molar, stained with hematoxylin-eosin. In the C group (A and E, magnifications 50× and 400×, respectively), an inflammatory infiltrate of moderate intensity can be observed. In the DRW group (B and F, magnifications ×50 and ×400) and in the RW group (C and G, magnifications ×50 and ×400) a mild inflammatory infiltrate can be seen. The AL group (D and H, magnifications 50x and 400x) shows a severe inflammatory infiltrate. The rectangles in figures A, B, C, and D indicate the magnified area at the next magnification. (A, B, C, and D) Scale bars: 400 μm and (E, F, G, and H) scale bars: 25 μm.

DISCUSSION

The present study was the first one to use dealcoholized (DRW) and regular red wine (RW) as a modulator of the local inflammatory process and blood profile of rats with settled AP. The null hypothesis was rejected in the present study once the supplementation with DRW or RW influenced on the reduction of the local inflammatory infiltrate and the systemic hematological profile in rats with settled AP. The use of wine was already shown as a beneficial modulator in the development of the inflammatory response caused by AP (Dal-Fabbro et al., 2021a).

The polyphenol quantification method used has already been reported in the literature for the determination of phenolic compounds in red wine (Burin et al., 2011; Padilha et al., 2017; Stecher et al., 2001;) due to its efficiency in separation and rapid and accurate quantification of phenolic components (Stecher et al., 2001). The animal model was chosen due to its wide acceptance in alcoholic beverage research, the similarity of physiological and neurobiological aspects of rats to humans, experimental convenience due to ease of handling, low cost and availability of scientific information (Malherbe & Messaoudi, 2022; McBride et al., 2014; Kehinde, 2013; Ponnappa & Rubin, 2000). The method used to induce apical periodontitis includes the pulp exposition to contamination by oral microorganisms, resulting in pulp necrosis and effects on the periapical tissues (Goldman et al., 2019; Yamasaki et al., 1994).

The supplementation was initiated after a 30-day waiting period, which is appropriate to assess the progression of the inflammatory process and bone loss associated with apical periodontitis. During this period, no signs of acute inflammation are observed to avoid confounding the analyses, and the model used reflects the pathogenesis observed in humans (Justo et al., 2022; Tani-Ishii et al., 1994; Yamasaki et al., 1994; Yoneda et al., 2017). Supplementation was performed by oral gavage, a method used in other systemic evaluation studies (Cosme-Silva et al., 2019; Wagner et al., 2019; Wolle et al., 2013), which consists in the forced administration of substances through an orogastric catheter so that each animal receives the dose according to its weight in a standardized and personalized manner (Justo et al., 2022). In this study, gavage was performed in the morning after adapting to the animals natural circadian cycle (Tosini et al., 2008).

The supplementation was administered daily in a single dose to each animal, using 4.28 ml/kg body weight (Dal-Fabbro et al., 2021a). This dose was calculated based on studies recommending a consumption of 300 ml/day of red wine for a 70 kg human with health benefits (Kikura et al., 2004; Pavlidou et al., 2018). The AL group received a prepared concentration of 12.5% to simulate the effect of alcohol, as alcohol concentrations in wine vary between 9 and 15%. This concentration has also been used in studies comparing the presence of alcohol in wine (Araya et al., 2003; Dal-Fabbro et al., 2021a; Wagner et al., 2019; Wolly et al., 1999) and in studies evaluating the relationship between alcohol and AP (Dal-Fabbro et al., 2019; Pinto et al., 2020).

The individual dose was determined based on the daily weight rats, which allowed to analyze the body weight percentage increase (Dal-Fabro et al., 2021b). It was calculated using the difference between the final weight and the initial weight. The group receiving alcohol supplementation showed the lowest average weight gain, with a statistically significant difference compared to the C and DRW groups. This can be attributed to the alcohol high caloric value (7 kcal/g) that reduces appetite (Nelson et al., 2016; Traversy & Chaput, 2015). Therefore, it may influence the reduction of food intake to compensate for the calories from alcohol, as observed in other studies comparing weight gain with alcohol (Nelson et al., 2016; Pinto et al., 2020). The RW group showed similar weight gain to the AL group possibly due to the presence of alcohol among its compounds and its ability to prevent weight gain and maintain body homeostasis through the influence of its polyphenols on fat absorption and oxidation (Vadilho et al., 2006). The rats in the

DRW group showed more weight gain compared to the AL group, so its consumption could be an alternative to the use of polyphenol compounds in weight control without alcohol interference, since this substance can affect the body's internal balance through the action of fatty malnutrition (Li et al., 2023).

The quantification of the DRW and RW components showed a strong similarity, but the absence of alcohol in the DRW. Some variations in concentration were observed, especially in phenolic acids, flavonols, proanthocyanidins, stilbenes, and flavanones. It is known that the phenolic composition of wine is directly related to the original profile of the grape, as well as to the extraction and vinification techniques used (Lorrain et al., 2006). The wines selected for this present study were produced from grapes of the Merlot variety which is known to be a rich source of bioactive phenolic compounds (Đorđević et al., 2017)

To the best of our knowledge, this is the first study to monitor the hematological profile of rats to evaluate the influence of alcoholic beverages, such as wine (with or without alcohol) and ethanol, on the body homeostasis in the face of apical periodontitis suggesting an adaptive response of the immune system. The comparative analysis of blood cell homeostasis was based on a study that established specific reference ranges for hematological and biochemical parameters (He et al., 2017).

In the present study, it was not observed significant changes in hemoglobin, a protein transported by erythrocytes, once all groups except alcohol group remained within healthy reference values. An increase in hemoglobin was observed in the group that received alcohol, possibly due to its influence on the level of ferritin, an iron storage protein that can increase the absorption of dietary iron (Charlton et al., 1964; Milman & Pedersen, 2009). This may be a systemic effect of the AP inflammatory process that can affect iron availability through bacterial action, thereby impairing erythropogenesis (Ross, 2017). In this study, an increase in red blood cell count and haematocrit was observed in the AL group compared with reference values. This contrasts with the literature, which generally associates alcohol with a higher risk of anaemia depending on the concentration and duration (Akhtar et al., 2020; Lee et al., 2015; Reddy et al., 2013). Alcohol may directly affect the bone marrow, but further research is needed to clarify such relationship (Ballart, 1997). The other groups had less red blood cells compared to the normal parameters probably due to the progression of the apical periodontitis and the activation of inflammatory cytokines such as IL-16, IL-10 and TNF-alpha, which can cause inhibition of erythropoietin (Patel et al., 2014).

There was also a significant increase in the number of platelets in the AL group compared to the other groups, possible due to the influence of ethanol on tissue plasminogen activating factor, which allows partial degranulation of platelets, allowing them to continue circulating with altered function (Bau et al., 2017). The relationship between periodontal infection and platelet function is not fully understood but it is thought that the decrease in platelet numbers in severe periodontal disease may be related to the increased consumption of large, activated platelets at sites of inflammation (Zhang et al., 2016). Polyphenols in the RW and DRW groups may also inhibit the binding between platelets and fibrinogen, altering their normal role in coagulation (Rabai et al., 2010).

There was also an increase in Mean Corpuscular Volume (MCV) in the AL group. It is known that MCV can be a biomarker for alcohol abuse by the activation of alcohol dehydrogenase enzymes that help in the metabolization of this compound into acetaldehyde that triggers reactive oxygen species (ROS) that damage, through phenazine methosulfate, the morphology of erythrocytes (Pavanello et al., 2012; Rabai et al., 2010). Since phenolic agents can react with ROS, they can prevent the environment to become an oxidative one (Chiva-Blanch et al., 2012; Rabai et al., 2010; Romeo et al., 2007), justifying the results found in the maintenance of this parameter for the RW and DRW groups.

The white blood cells in this study maintained normal standards but the AL group showed a significant concentration of lymphocytes in the blood, such cells play an important role in fighting apical periodontitis especially in the chronic phase (Braz-Silva et al., 2019; Loos et al., 2004). Due to the diversity of lymphocytes with specific functions in adaptive defense, there is a prevalence of T lymphocytes (Rothenberg et al., 2016). This result can be attributed to the stimulus of alcohol, which favors the maturation of naive lymphocytes into memory T lymphocytes, rendering their receptors more sensitive, even at low stimulation, and capable of producing a pro-inflammatory cytokine, gamma interferon (Cho et al., 2000; Song et al., 2002; Zhang & Meadows, 2005). These findings are in line with previous studies that observed an increase in lymphocyte count in rats infected with lipopolysaccharides, while a decrease was observed in rats supplemented with wine (Percival & Sims, 2000). On the other hand, the DRW and RW groups had lower count of lymphocytes, possibly due to polyphenols, which have antioxidant properties and protect immune cells from damage caused by reactive oxygen species (Romeo et al., 2007; Chiva et al., 2012). These results were also found in a study showing no adverse effects on human immune cell function in healthy men exposed to RW and DRW (Watzl et al., 2004).

Neutrophil concentrations decreased in the AL group, although there is a possibility that alcohol may increase the recruitment of these cells in innate immune response, in the adaptive immune response it acts as suppressive effect, which can be a risk factor for bacterial infections in organism (Molina et al., 2010). Meanwhile, the DRW and RW groups achieved higher counting probably due to the protective effect of polyphenols on the immune cells once they react with ROS (Watzl et al., 2004). The monocytes counting in the present study was not different among the groups and maintained values within the normal range probably due to the a chronic state of AP. It is suggested that monocytes are more present in the acute phase of infections, acting as the body's first line of defense, while their presence in chronic infections is generally lower (Molina et al., 2010).

In the AP, there is a significant local increase in inflammatory cells, mainly lymphocytes, which can trigger and intensify specific systemic health effects through the activation of pro-inflammatory cytokines that increase oxidative stress (Bian et al., 2018; Samuel et al., 2018). The histological analysis of the present study confirmed the AP induction method with the presence of inflammatory infiltrate in all groups. However, it was shown that DRW and RW wines had a mild score with reduced inflammatory cells, which may be correlated with reduced blood cells. This fact may be due to the polyphenols, which act to reduce lymphocytes and oxidative stress, inhibiting phospholipase A, cyclooxygenase (COX)-2,5-lipoxygenases, reducing reactive oxygen and the production of nitrous oxide (López et al., 2007). These results are consistent with the previous study that highlighted the anti-inflammatory benefits of RW and its polyphenols in AP (Dal-Fabbro et al., 2021a). The isolated administration of 12.5% alcohol exacerbated the inflammatory infiltrate in apical periodontitis, confirming previous findings related to the capacity of alcohol to recruit pro-inflammatory cytokines, highlighting its inflammatory effect in the present study (Dal-Fabbro et al., 2019; Dal-Fabbro et al., 2021a; Pinto et al. 2019).

There are conflicting views in the literature regarding the effects of alcohol on the wine polyphenols. Some suggest that polyphenols may partially offset the harmful effects of alcohol while others claim that alcohol does not affect the absorption of phenolic compounds. (Dal-Fabbro et al., 2021a; Fiore et al., 2020; Manach et al., 2004; Rabai et al., 2010; Rothes-Ribalta et al., 2012). There is also evidence that it can facilitate the absorption of these compounds by destabilizing the intestinal barrier with the ability to extract and dissolve lipids from the mucus layer, reducing hydrophobicity and increasing intestinal permeability (Bai et al., 2019; Qin & Deitch, 2015). However, these studies are known to have limitations, as most participants are young and the data are generally epidemiological associations that require more in-depth discussion and support (Chiva & Badimon, 2019).

The present study provides prospective insight into the potential of DRW to modulate apical periodontitis (AP) and general health. Furthermore, the consumption of DRW may benefit those who avoid alcoholic beverages by providing antioxidants to combat oxidative stress (Noguer et al., 2012). However, due to some limitations, such as the use of animal models, the discouragement of alcohol consumption to promote health, and the frequency and duration of treatment of periapical lesions, the present results cannot be directly extrapolated to humans. Therefore, it is important to encourage new research that takes these relevant aspects into account.

CONCLUSION

Supplementation with dealcoholized red wine influences the systemic hematological profile of rats with settled AP, resulting in an improvement in lymphocytes parameters, maintaining body homeostasis and reducing local inflammatory infiltrate.

REFERENCES

Akhtar, M., Mehmood, R., Ullah, H. & Irfan, M. (2020) Quality, quantity and hematological disorders in blood under Ethanol analyte an in vitro study. *Pakistan Journal of Pharmaceutical Sciences*, 33(2).

Araya, J., Rodrigo, R., Orellana, M. & García, V. (2003) Effects of red wine consumption on kidney FA composition. *Lipids*, 38(3), 275-279.

Azuma, M.M., Cardoso, C.B.M., Samuel, R.O., Pipa, C.B., Bomfim, S.R.M., Narciso, L.G., Gomes-Filho, J.E. & Cintra, L.T.A. (2021) Omega-3 Fatty Acids Alter Systemic Inflammatory M ediators Caused by Apical Periodontitis. *Jounal of Endodontics*, 47(2), 272-277.

Ballard, H.S. (1997) The hematological complications of alcoholism. *Alcohol Health and Research World,* 21(1), 42-52.

Bai, S., Li, P., Liu, J., Cui, C., Li, Q. & Bi, K. (2019) A UFLC-MS/MS method for the simultaneous determination of eight bioactive constituents from red wine and dealcoholized red wine in rat plasma: Application to a comparative pharmacokinetic study. *Biomedical Chromatography*, 33(3), 4437.

Bau, P.F., Bau, C.H., Rosito, G.A., Manfroi, W.C. & Fuchs, F.D. (2007) Alcohol consumption, cardiovascular health, and endothelial function markers. *Alcohol*, 41(7), 479-488.

Belisario-Sanchez, Y.Y., Taboada-Rodriguez, A., Marin-Iniesta, F., & Lopez-Gomez, A. (2009) Dealcoholized wines by spinning cone column distillation: phenolic compounds and antioxidant activity measured by the 1, 1-diphenyl-2-picrylhydrazyl method. *Journal of Agricultural and Food Chemistry*, 57(15), 6770-6778.

Bian, Z., Guo, Y., Ha, B., Zen, K. & Liu Y. (2012) Regulation of the inflammatory response: braenhancing neutrophil infiltration under chronic inflammatory conditions. *The Journal Immunology*, 188(2), 844-853.

Braz-Silva, P.H., Bergamini, M.L., Mardegan, A.P., Rosa, C.S., Hasseus, B. & Jonasson, P. (2019) Inflammatory profile of chronic apical periodontitis: a literature review. *Acta Odontologica Scandinavica*, 77(3),173-180.

Burin, V.M., Arcari, S.G., Costa, L.L. & Bordignon-Luiz, M.T. (2011) Determination of some phenolic compounds in red wine by RP-HPLC: method development and validation. *Journal of Chromatographic Science*, 49(8), 647-651.

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 Endodontics Journal*, 54(8), 1353-1361.

Castaldo, L., Narváez, A., Izzo, L., Graziani, G., Gaspari, A., Di, M.G. & Ritieni, A. (2019) Red wine consumption and cardiovascular health. *Molecules,* 24(19), 3626.

Cintra, L.A.T., da Silva, F.A.C., Prieto, A.K., Sumida, D.H., Narciso, L.G., Mogami, B.S.R., Oliveira, S. C., Dezan-Júnior, E. & Gomes-Filho, J.E. (2014) Blood profile and histology in oral infections associated with diabetes. *Jounal of Endodontics*, 40(8), 1139-44.

Charlton, R.W., Jacobs, P., Sefte, H. & Bothwell, T. H. (1964) Effect of alcohol on iron absorption. *British Medical Journal,* 2(5422), 1427.

Chiva, B.G., Urpi, S. M, Ros, E., Arranz, S., Valderas, M.P., Casas, R., Sacanella, E., Llorach, R., Lamuela, R.R.M., Andres, L.C. & Estruch, R. (2012) Dealcoholized red wine decreases systolic and diastolic blood pressure and increases plasma nitric oxide: short communication. *Circulation Research*, 111(8), 1065-8.

Chiva-Blanch, G., Urpi-Sarda, M., Ros, E., Valderas-Martinez, P., Casas, R., Arranz, S., Guillén, M., Lamuela-Raventós, R.M., Llorach, R., Andrés-Lacueva C.& Estruch, R. (2013) Effects of red wine polyphenols and alcohol on glucose metabolism and the lipid profile: a randomized clinical trial. *Clinical nutrition*, 32(2), 200-206.

Chiva, B.G. & Badimon, L. (2019) Benefits and Risks of Moderate Alcohol Consumption on Cardiovascular Disease: Current Findings and Controversies. *Nutrients*, 12(1), 108.

Cho, B.K., Rao, V.P., Ge, Q., Eisen, H.N. & Chen, J. (2000) Homeostasis-stimulated proliferation drives naive T cells to differentiate directly into memory T cells. *Journal of Experimental Medicine,* 192(4), 549-56.

Conti, L.C., Segura-Egea, J.J., Cardoso, C.B.M., Benetti, F., Azuma, M.M., Oliveira, P.H.C., Bomfim, S.R.M & Cintra, L.T.A. (2020) Relationship between apical periodontitis and atherosclerosis in rats: lipid profile and histological study. *International Endodontics Journal*, 53(10), 1387-1397.

Cosme-Silva, L., Dal-Fabbro, R., Cintra, L.T.A., Dos Santos, V.R., Duque, C., Ervolino, E., Mogami, B.S. & Gomes-Filho, J.E. (2019) Systemic administration of probiotics reduces the severity of apical periodontitis. *International Endodontics Journal*, 52(12), 1738-1749.

Cosme-Silva, L., Dal-Fabbro, R., Cintra, L.T.A., Ervolino, E., Prado, A.S.D., Oliveira, D.P., Marcelos, P.G.C.L. & Gomes-Filho, J.E. (2021) Dietary supplementation with multi-strain formula of probiotics modulates inflammatory and immunological markers in apical periodontitis. *Journal of Applied Oral Science*, 29, 20210483.

Dal-Fabbro, R., Cosme-Silva, L., Rezende, S.M.O.F., Capaldo, L.C., Plazza, F.A., Ervolino, E., Cintra, L.T.A. & Gomes-Filho, J.E. (2021a) Effect of red wine or its polyphenols on induced apical periodontitis in rats. *International Endodontics Journal*, 54(12), 2276-2289.

Dal-Fabbro, R., Cosme-Silva, L., Capalbo, L.C., Chaves-Neto, A.H., Ervolino, E., Cintra, L.T.A & Gomes-Filho, J.E. (2021b) Excessive caffeine intake increases bone resorption associated with periapical periodontitis in rats. *International Endodontics Journal*, 54(10), 1861-1870.

Dal-Fabbro, R., Marques, A. 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 Endodontics Journal*, 52(3), 329- 336.

Đorđević, N.O., Pejin, B., Novaković, M.M., Stanković, D.M., Mutić, J.J., Pajović, S.B. & Tešević, V.V. (2017) Some chemical characteristics and antioxidant capacity of novel Merlot wine clones developed in Montenegro. *Scientia Horticulturae*, 225, 505-511.

Fernandez-Solà, J. (2015) Cardiovascular risks and benefits of moderate and heavy alcohol consumption. *Nature Reviews Cardiology*, 12(10), 576-587.

Ferrières, J. (2004) The French paradox: lessons for other countries. *Heart*, 90(1), 107-111.

Goldman, E., Reich, E., Abramovitz, I. & Klutstein, M. (2019*)* Inducing Apical Periodontitis in Mice. *Journal of Visualized Experiments, (*150).

He, Q., Su, G., Liu, K., Zhang, F., Jiang, Y., Gao, J., Liu, L., Jiang, Z., Jin, M. & Xie, H. (2017) Sex-specific reference intervals of hematologic and biochemical analytes in Sprague-Dawley rats using the nonparametric rank percentile method. *PLOS ONE*, 12(12), 0189837.

Hu, C., Huang, C., Li, J., Liu, F., Huang, K., Liu, Z., Yang, X., Liu, X., Cao, J., Chen, S., Li, H., Shen, C., Yu, L., Wu, X., Li, Y., Hu, D., Huang, J., Lu, X. & Gu, D. (2022) Causal associations of alcohol consumption with cardiovascular diseases and all-cause mortality among Chinese males. *The American Journal of Clinical Nutrition,* 116(3), 771-779.

Justo, M.P., Cardoso, C.B.M., Cantiga-Silva, C., de Oliveira, P.H.C., Sivieri-Araújo, G., Azuma, M.M., Ervolino, E. & Cintra, L.T.A. (2022) Curcumin reduces inflammation in rat apical periodontitis. *International Endodontics Journal*, 55(11), 1241-1251.

Kakehashi, S., Stanley, H.R. & Fitzgerald, R.J. (1965) The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral surgery, oral medicine, oral pathology,* 20(3), 340-349.

Kehinde, E.O. (2013) They see a rat, we seek a cure for diseases: the current status of animal experimentation in medical practice. *Medical Principles and Practice*, 22 (1), 52-61.

Kikura, M., Levy, J.H., Safon, R.A., Lee, M.K. & Szlam, F. (2004) The influence of red wine or white wine intake on platelet function and viscoelastic property of blood in volunteers. *Platelets,* 15(1), 37-41.

Kongstad, J., Hvidtfeldt, U.A., Grønbaek, M., Jontell, M., Stoltze, K.& Holmstrup, P. (2004) Amount and type of alcohol and periodontitis in the Copenhagen City Heart Study. *Journal of Clinical Periodontology*, 15(1), 37-41.

Lee, S. Y., Park, H. J., Best-Popescu, C., Jang, S. & Park, Y. K. (2015) The effects of ethanol on the morphological and biochemical properties of individual human red blood cells. *PloS one*, 10(12), e0145327.

Li, Q., Wang, O., Ji, B., Zhao, L. & Zhao, L. (2023) Alcohol, White Adipose Tissue, and Brown Adipose Tissue: Mechanistic Links to Lipogenesis and Lipolysis. *Nutrients*, 15(13), 2953.

Li, X., Kolltveit, K. M., Tronstad, L., & Olsen, I. (2000). Systemic diseases caused by oral infection. *Clinical microbiology reviews*, 13(4), 547-558.

Loos, B.G., Roos, M.T., Schellekens, P.T., Velden, U.D.V. & Miedema, F. (2004) Lymphocyte numbers and function in relation to periodontitis and smoking. *Journal of Periodontology*, 75(4), 557-564.

López, D., Pavelkov, M., Gallova, L., Simonetti, P., Gardana, C., Lojek, A., Loaiza, R. & Mitjavila, M.T. (2007) Dealcoholized red and white wines decrease oxidative stress associated with inflammation in rats. *British Journal of Nutrition,* 98(3), 611-619.

Lorrain, B., Chira, K. & Teissedre, P.L. (2006) Phenolic composition of Merlot and Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage: Comparison to 2006, 2007 and 2008 vintages. *Food Chemistry,* 126(4), 1991-1999.

Manach, C., Scalbert, A., Morand, C., Rémésy, C. & Jiménez, L. (2004) Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79(5), 727-747.

Malherbe, D.C. & Messaoudi, I. (2022) Transcriptional and Epigenetic Regulation of Monocyte and Macrophage Dysfunction by Chronic Alcohol Consumption. *Frontiers in Immunology*, 13, 911951.

McBride, W.J., Rodd, Z.A., Bell, R.L., Lumeng, L. & Li, T.K. (2014) The alcohol-preferring (P) and high-alcohol-drinking (HAD) rats--animal models of alcoholism. *Alcohol,* 48(3), 209-215.

Milman,,N. & Pedersen, A.N. (2009) Blood haemoglobin concentrations are higher in smokers and heavy alcohol consumers than in non-smokers and abstainers—should we adjust the reference range?. *Annals of hematology,* 88, 687-694.

Molina, P.E., Happel, K.I., Zhang, P., Kolls, J.K. & Nelson, S. (2010) Focus on: Alcohol and the immune system. *Alcohol Research & Health*, 33(1-2), 97-108.

Mühlbauer, R.C., Lozano, A., Reinli, A. & Wetli, H. (2003) Various selected vegetables, fruits, mushrooms and red wine residue inhibit bone resorption in rats. *Journal of Nutrition*, 133(11), 3592-7.

Muñoz-González, I., Thurnheer, T., Bartolomé, B., & Moreno-Arribas, M. V. (2014) Red wine and oenological extracts display antimicrobial effects in an oral bacteria biofilm model. *Journal of agricultural and food chemistry*, 62(20), 4731-4737.

Nagendrababu, V., Kishen, A., Murray, P.E., Nekoofar, M.H., de Figueiredo, J.A.P., Priya, E., Jayaraman, J., Pulikkotil, S.J., Camilleri, J., Silva, R.M. & Dummer, P.M.H. (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: a consensus-based development. *International Endodontics Journal*, 54(6),848-857.

Nair, P. R. (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical Reviews in Oral Biology & Medicine*, 15(6), 348-381.

Nelson, N.G., Suhaidi, F.A., DeAngelis, R.S. & Liang, N.C. (2016) Appetite and weight gain suppression effects of alcohol depend on the route and pattern of administration in Long Evans rats. *Pharmacology Biochemistry and Behavior*, 150-151, 124-133.

NHLBI. (2021) Your Guide to Lowering Your Blood Pressure with DASH.

Noguer, M.A., Cerezo, A.B., Donoso, N.E. & Garcia-Parrilla, M.C. (2012) Intake of alcohol-free red wine modulates antioxidant enzyme activities in a human intervention study. *Pharmacological Research*, 65(6), 609-614.

Pavanello, S., Snenghi, R., Nalesso, A., Sartore, D., Ferrara, S. D., & Montisci, M. (2012) Alcohol drinking, mean corpuscular volume of erythrocytes, and alcohol metabolic genotypes in drunk drivers. *Alcohol*, 46(1), 61-68.

Pavlidou, E., Mantzorou, M., Fasoulas, A., Tryfonos, C., Petridis, D. & Giaginis, C. (2018) Wine: An Aspiring Agent in Promoting Longevity and Preventing Chronic Diseases. *Diseases*, 6(3), 73.

Padilha, C.V., Miskinis, G.A., de Souza, M.E., Pereira, G.E., de Oliveira, D., Bordignon-Luiz, T. & Lima, M.D. (2017) Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian varieties of grape. *Food Chemistry,* 1(228), 106-115.

Patel, M.D., Shakir, Q.J., & Shetty, A. (2014) Interrelationship between chronic periodontitis and anemia: a 6-month follow-up study. *Journal of Indian Society of Periodontology*, 18(1), 19.

Percival, S.S. & Sims, C.A. (2000) Wine modifies the effects of alcohol on immune cells of mice. *Journal of Nutrition*, 30(5), 1091-1094.

Pignatelli, P., Ghiselli, A., Buchetti, B., Carnevale, R., Natella, F., Germanò, G., Fimognari, F., Di Santo, S., Lenti, L. & Violi, F. (2006) Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. *Atherosclerosis,* 188(1), 77-83.

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 Endodontics Journal*, 53(9), ponna1238-1252.

Ponnappa, B.C. & Rubin, E. (2000) Modeling alcohol's effects on organs in animal models. *Alcohol Research & Health*, 24(2), 93-104.

Qin, X. & Deitch, E.A. (2015) Dissolution of lipids from mucus: a possible mechanism for prompt disruption of gut barrier function by alcohol. *Toxicology Letters*, 232(2), 356-362.

Rabai, M., Toth, A., Kenyeres, P., Mark, L., Marton, Z., Juricskay, I., Toth, K. & Czopf, L. (2010) In vitro hemorheological effects of red wine and alcohol-free red wine extract. *Clinical Hemorheology and Microcirculation,* 44(3), 227-236.

Reddy, K. R., Reddy, V. D., Padmavathi, P., Kavitha, G., Saradamma, B. & Varadacharyulu, N. C. (2013) Gender differences in alcohol-induced oxidative stress and altered membrane properties in erythrocytes of rats. *Indian Journal of Biochemistry and Biophysics*, 50(1), 32-39.

Romeo, J., Wärnberg, J., Nova, E., Díaz, L.E., Gómez-Martinez, S. & Marcos, A. (2007) Moderate alcohol consumption and the immune system: a review. *British Journal of Nutrition*, 98(1), 111-115.

Ross, A.C. (2017) Impact of chronic and acute inflammation on extra- and intracellular iron homeostasis. *The American Journal of Clinical Nutrition*, 106(6), 1581S-1587S.

Rothenberg, E.V., Kueh, H.Y., Yui, M.A. & Zhang, J.A. (2016) Hematopoiesis and T-cell specification as a model developmental system. *Immunological Reviews,* 271(1), 72-97.

Sam, F. E., Ma, T. Z., Salifu, R., Wang, J., Jiang, Y. M., Zhang, B., & Han, S. Y. (2021) Techniques for dealcoholization of wines: Their impact on wine phenolic composition, volatile composition, and sensory characteristics. *Foods*, 10(10), 2498.

Samuel, R.O., Gomes-Filho, J.E., Azuma, M.M., Sumida, DH., de Oliveira, S.H.P., Chiba, F.Y., Bomfim, S.R.M., Ciarlini, P.C., Narciso, L.G. & Cintra, LTA. (2018) Endodontic infections increase leukocyte and lymphocyte levels in the blood. *Clinical Oral Investigations*, 22(3), 1395-1401.

Santos, B.C., González-Manzano, S. & González-Paramás, A.M. (2021) Wine, Polyphenols, and Mediterranean Diets. What Else Is There to Say? *Molecules,* 26(18), 5537.

Scannapieco, F. A., Papandonatos, G. D., & Dunford, R. G. (1998). Associations between oral conditions and respiratory disease in a national sample survey population. *Annals of periodontology*, 3(1), 251-256.

Serafini, M., Maiani, G., & Ferro-Luzzi, A. (1998). Alcohol-free red wine enhances plasma antioxidant capacity in humans. *The Journal of Nutrition*, 128(6), 1003-1007

Singh, A. K., Bishayee, A., & Pandey, A. K. (2018) Targeting histone deacetylases with natural and synthetic agents: an emerging anticancer strategy. *Nutrients,* 10(6), 731.

Song, K., Coleman, R.A., Zhu, X., Alber, C., Ballas, Z.K., Waldschmidt, T.J. & Cook, RT. (2002) Chronic ethanol consumption by mice results in activated splenic T cells. *Journal of Leukocyte Biology* ,72(6), 1109-1116.

Stecher, G., Huck, C.W., Popp, M. & Bonn, G.K (2001) Determination of flavonoids and stilbenes in red wine and related biological products by HPLC and HPLC-ESI-MS-MS. *Fresenius' Journal of Analytical Chemistry*, 371(1), 73-80.

Stocker, R. & O'Halloran, R.A. (2004) Dealcoholized red wine decreases atherosclerosis in apolipoprotein E gene-deficient mice independently of inhibition of lipid peroxidation in the artery wall. *The American Journal of Clinical Nutrition*, 79(1), 123-130.

Tani-Ishii, N., Wang, C.Y., Tanner, A. & Stashenko, P. (1994) Changes in root canal microbiota during the development of rat periapical lesions. *Oral Microbiology and Immunology*, 9(3),129-135.

Traversy, G. & Chaput, J.P. (2015) Alcohol Consumption and Obesity: An Update. *Current Obesity Reports*, 4(1), 122-130.

Tosini, G., Pozdeyev, N., Sakamoto, K. & Iuvone, P.M. (2008) The circadian clock system in the mammalian retina. *Bioessays*, 30(7), 624-633.

U.S. Department of Agriculture and U.S. Department of Health and Human Services. (2020) *Dietary guidelines for Americans, 2020–2025*, 9th edition. Washington, DC.

Vadillo, M., Ardévol, A., Fernández-Larrea, J., Pujadas, G., Bladé, C., Salvadó, M.J., Arola, L. & Blay, M. (2006) Moderate red-wine consumption partially prevents body weight gain in rats fed a hyperlipidic diet. *Journal of Nutritional Biochemistry,* 17(2), 139-142.

Vejarano, R. & Luján-Corro, M. (2022) Red Wine and Health: Approaches to Improve the Phenolic Content During Winemaking. *Frontiers in Nutrition*, 25(9), 890066.

Vinson, J.A., Teufel, K. & Wu, N. (2001) Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis,* 156(1), 67-72.

Wagner M.C., Cavagni J., Gaio E.J., Brum V.S., Jesus L.H., Filho M.S., Carrard V.C., Dorneles G.P., Peres A. & Rösing C.K.(2019) Effect of red wine and its major components on periodontitis and systemic inflammation in rats. *International Academy of Periodontology,* 21(4), 139-147.

Watzl, B., Bub, A., Pretzer, G., Roser, S., Barth, S.W. & Rechkemmer, G. (2004) Daily moderate amounts of red wine or alcohol have no effect on the immune system of healthy men. *European Journal of Clinical Nutrition*, 58(1), 40-45.

Wolle, C.F., Zollmann, L.A., Bairros, P.O., Etges, A., Leite, C.E., Morrone, F.B. & Campos, M.M. (2013) Outcome of periapical lesions in a rat model of type 2 diabetes: refractoriness to systemic antioxidant therapy. *Journal of Endodontics*, 39(5), 643-7.

Yamasaki, M., Kumazawa, M., Kohsaka, T., Nakamura, H. & Kameyama, Y. (1994) Pulpal and periapical tissue reactions after experimental pulpal exposure in rats. *Journal of Endodontics*, 20(1), 13-17.

Yoneda, N., Noiri, Y., Matsui, S., Kuremoto, K., Maezono, H., Ishimoto, T., Nakano, T., Ebisu, S. & Hayashi, M. (2017) Development of a root canal treatment model in the rat. *Scientific Reports*, 7(1), 3315.

Zhang, H. & Meadows, G.G. (2005) Chronic alcohol consumption in mice increases the proportion of peripheral memory T cells by homeostatic proliferation. *Journal of Leukocyte Biology*, 78(5), 1070-1080.

Zhan, Y., Lu, R., Meng, H., Wang, X. E., & Hou, J. (2016) Platelet activation and platelet–leukocyte interaction in generalized aggressive periodontitis. *Journal of Leucocyte Biology,* 100(5), 1155-1166.

Artigo 2

Supplementation with dealcoholized red wine reduces inflammation and bone resorption of settled apical periodontitis

Artigo formatado para submissão ao periódico *Archives of Oral Biology*

(Qualis A1 e Fator de impacto 3 em 2023)

Abstract

Objective: To analyze the effect of supplementation with dealcoholized red wine on the apical periodontitis severity after it had been settled.

Design: Thirty-two male Wistar rats were arranged into four groups: water as control (C), dealcoholized red wine (DRW), red wine (RW) and alcohol (AL). Apical periodontitis was induced by pulp exposure during 30 days. When the AP was settled, supplementation was started and continued daily for extra 30 days. After the supplementation period, the animals were euthanized, the maxillas were removed to evaluate bone resorption through microtomographic analysis, and the jaws were removed to analyze the inflammatory response through histological and immunohistochemical analysis. Statistical tests were applied at 5% of significance .

Results: The DRW group had a lower mean bone resorption volume, followed by the RW, C and AL groups ($p<0.05$). The DRW and RW groups showed mild inflammation, followed by the C group with moderate inflammation and the AL group with severe inflammation ($p<0.05$). The immunoreaction was lower in the DRW group than C and AL groups for the inflammatory cytokines TNF-α and IL-1β, and for TRAP cells ($p<0.05$). Moreover, OPG was more present in the DRW than C and AL groups ($p<0.05$).

Conclusion: Supplementation with dealcoholized red wine in rats reduced inflammation and bone resorption in settled apical periodontitis.

Keyword: Apical Periodontitis; Dealcoholized Red Wine; Inflammation; Bone Resorption.

Highlights

- Reduction of inflammation in settled apical periodontitis supplementation of dealcoholized red wine in rats;
- Decreased osteoclastogenesis with lower TRAP and less periapical bone resorption with dealcoholized red wine.
- In settled apical periodontitis, alcohol consumption increases inflammation and bone resorption.

Clinical relevance

This study provides initial insights into the use of dealcoholized red wine as a potential benefit on the inflammatory activity and bone metabolism of settled apical periodontitis.

1.Introduction

Apical periodontitis is an inflammatory disease that affects the periapical tissues in response to a polymicrobial infection in the root canal system (Möller et al., 1981; Siqueira & Roças, 2007). That response develops a highly vascular granulomatous tissue in the periapical region with a high concentration of cells such as lymphocytes and inflammatory biomarkers such as tumor necrosis factor (TNF-α) and pro-inflammatory interleukins recruited from the bloodstream (Mehrazarin et al., 2017; Siqueira & Roças, 2007; Stashenko & Teles, 1998; Yoshimura et al., 1997).

The pathogenesis of this disease is complex and results in bone resorption of periapical tissues with intense osteoclastic activity that degrades the organic and inorganic components of the bone surface (Petean et al., 2022; Yu et al., 2016). The bone resorption is mediated by the receptor-activating nuclear factor NF-kB (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) balance (Petean et al., 2022; Vernal et al., 2006). An imbalance exacerbates osteoclastogenesis under inflammatory conditions (Luo et al., 2022; Kajiya et al., 2010).

There is evidence that the endodontic infections treatment outcome depends on the general health and nutrition (Holland et al., 2017). Therefore, new substances that can contribute to systemic health with positive effects on apical periodontitis through dietary supplementation with systemic effects, such as omega-3 fatty acids, curcumin, lactobacilli, and red wine with its phenolic compounds have been investigated (Azuma et al., 2017; Cosme-Silva et al., 2019; Dal-Fabbro et al., 2021a; Justo et al., 2022).

The benefits of red wine are related to French paradox once it was noted that the presence of this drink in the Mediterranean diet was associated with the reduction of cardiovascular diseases (Artero et al., 2015; Finicelli et al., 2019; St-leger et al., 1979). Wine contains several components, including water, ethanol, glycerol, polysaccharides, various acids, and mainly bioactive phenolic compounds (Finicelli et al., 2019; Snopek et al., 2018). The nutraceutical science states that the presence of bioactive phenolic compounds is related to health benefits (Souza-Bomfim et al., 2022; Piccolella et al., 2019). On the other hand, the consumption of alcoholic beverages cannot be promoted due to the association of ethanol with adverse health effects (WHO, 2019). When red wine or its polyphenols were used starting its administration before the development of apical periodontitis to evaluate their effect on inflammation and bone resorption, the results were more promising when only polyphenols were used and the presence of ethanol in wine was pointe out as a substance that could reduce the beneficial effects (Dal-Fabbro et al., 2021a). Moreover, alcohol was related to the increase the severity and osteoclastogenesis of apical periodontitis (Dal-Fabbro et al., 2019; Dal-Fabbro et al., 2021a).

Trying to keep the benefits of red wine based on its phenolic compounds without the downside of alcohol, the industry has developed techniques for its dealcoholization (Belisário-Sánchez et al., 2009; Liguori et al., 2019; Sam et al., 2021). Several beneficial effects were already associated with dealcoholization red wine (DRW), such as reduction of oxidative stress (López et al., 2007); increased oxidative defenses (Modun et al., 2008); inhibition of atherosclerosis (Wang et al., 2005); reduced blood pressure and increased plasma nitric oxide (Chiva-Blanch et al., 2012); anti-cancer properties (Lan et al., 20-23); and antimicrobial potential (Muñoz-González et al., 2014).

Once it was demonstrated the benefits of red wine and its phenolic compounds in an administration starting before the apical periodontitis induction, the relationship of alcohol with the increase of apical periodontitis and there is no study relating dealcoholization red wine to apical periodontitis (Dal-Fabbro et al., 2021a), the present study aimed to analyze the effect of dealcoholization red wine supplementation on the severity of the pathogenesis of settled apical periodontitis. The null hypothesis tested was that dealcoholization red wine supplementation will not alter the pathogenesis of settled apical periodontitis in rats.

2. Material and methods

2.1 Experimental design

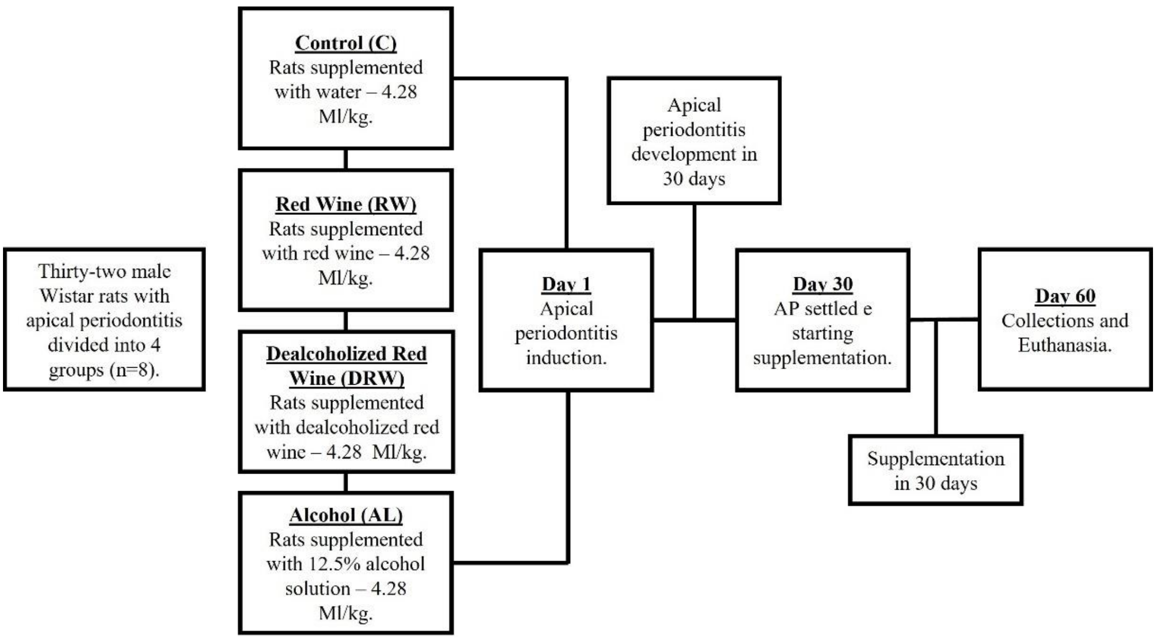
This in vivo study followed all experimental protocols approved by the Institutional Ethics Committee on Animal Use (0221-2022) of the Universidade Estadual Paulista, São Paulo, Brazil. For the design of this study, the ARRIVE Animal Research: Reporting of In Vivo Experiments guidelines were followed according to the ten essential items (Percie et al., 2020).

To determine the number of animals required for the study sample, a calculation was made using data from previous scientific studies with similar methodologies, taking into account specific parameters of alpha error at 5% and power at 95%, sufficient to detect significant differences (Dal-Fabbro et al., 2021a;

Justo et al., 2022). The calculation indicated the need for at least seven animals per group. However, due to the long duration of the experiment and awareness of the susceptibility to complications, one additional animal was included in each group, resulting in a total of eight animals per group (Dal-Fabbro et al., 2021a).

Thirty-two male Wistar rats (Rattus norvegicus albinus), aged 3 months and weighing between 250-350 g, were used. All animals were had induced apical periodontitis and arranged into 4 groups as follows: Control (C) - rats with water supplementation; dealcoholized red wine (DRW): rats with DRW supplementation; red wine (RW): rats with RW supplementation; alcohol (AL): rats with 12% alcoholic solution supplementation. The experimental protocol lasted 60 days, and supplementation began after 30 days from induction, when AP was settled. For this purpose, the animals were housed in mini-isolators where environmental conditions, including temperature (22º), humidity (70%), and light cycles (12 hours light, 12 hours dark), were carefully controlled with a solid diet and water at will. The general health of the animals was monitored throughout the experimental period. The stages and divisions of the study are shown in Figure 5.

Figure 5 – Stages and divisions of the study.



2.2. Induction of Apical Periodontitis

For the apical periodontitis induction, the rats were anesthetized intramuscularly with a combination of xylazine (10 mg/kg, Ceva Saúde Animal Ltda, Paulinia, SP, Brazil) and ketamine hydrochloride (80 mg/kg, Ceva Saúde Animal Ltda, Paulinia, SP, Brazil). Then, in all groups, a coronal opening was made in the first and second maxillary and mandibular right molars using a ¼ LN long-neck maillefer drill (Dentsply Sirona, Charlotte, North Carolina, USA) to expose the pulp to the oral environment (compatible with the size of the drill), causing to pulp necrosis and subsequent development and installation of the periapical lesion on 30 days (Dal-Fabbro et al., 2019; Goldman et al., 2019; Justo et al., 2022).

2.3. Supplementation

Thirty days after induction of the periapical lesion, it was considered settled and the supplementation by gavage was started in all groups with a favorable daily dose adapted to the animal model of 4.28 ml of red wine per kg of animal body weight (Dal-Fabbro et al., 2021a). Control group received sterilized drinking water as a placebo solution; the DRW and RW groups received dealcoholized red wine and red wine (Vinoh, Bento Gonçalves, RS, Brazil) and the AL group received a 12.5% alcoholic solution.

2.4. Euthanasia

After 30 days from the apical periodontitis has been settled (60 days from the induction start), the animals were sedated with a combination of anesthetics, described previously, and euthanized under an overdose of anesthetic with Sodium Thiopental (Thiopentax, Cristalia Produtos Químicos Farmacêuticos Ltda., São Paulo, Brazil) (Cintra et al., 2016). Then, the right maxillas were removed and frozen in liquid nitrogen and sent for microtomographic analysis; and the right jaws were fixed in 4% formaldehyde for 24h for processing and histological/immunohistochemical analysis (Dal-Fabbro et al., 2021b).

2.5. Microtomographic processing and analysis

This analysis was based on the method previously used (Dal-Fabbro et al.,2021b), where the maxilla was scanned by microtomography (SkyScan 1272, Kontich, Belgium) with the following parameters: 100kV, 250μA, 11 Mp (4032x2688 pixels) 14-bit, with a nominal resolution of 0.45 mm to 27 mm and a space scanning tool with a diameter of 75 mm and a length of 70 mm. The images obtained were reconstructed in three dimensions (3D) using the Data Viewer software (SkyScan, version 1.4.4 64-bit) and analyzed in the sagittal plane using the CTAnalyser - CTAn software (SkyScan, version 1.12.4. 0, Belgium) to measure the tissue volume (TV) and alveolar bone volume (BV) in grey scales and the expression in cubic millimeters (mm3) of the periradicular bone resorption space involving the periapical region of the distal root of the first molar, accurately traced from its beginning to its disappearance (Justo et al., 2022; Kalatzis-Sousa et al., 2017).

2.6. Histological/immunohistochemical processing and analysis

After the fixation period, the right jaws were demineralized in 10% EDTA (Labsynth Produtos, São Paulo, Brazil) for two months. They were then washed in running water, dehydrated in alcohol (Labsynth Produtos, São Paulo, Brazil), clarified in xylene (Labsynth Produtos, São Paulo, Brazil), and embedded in paraffin (Merck KGaA, Darmstadt, Germany) for semi-thin sectioning of sections serially in a 5 µm thick sagittal plane (Cantiga-Silva et al., 2021; Cosme-Silva et al., 2019). These sections were organized into slides containing 3 species per animal for histological analysis and each immunohistochemical analysis.

For histological analysis, the slides were stained with hematoxylin-eosin to analyze the inflammatory infiltrate in the area of the apical foramen of the distal root of the lower first molar by its intensity under a light microscope (DM 4000 B; Leica, Wetzlar, Germany). Ten images were used at 400× magnification to count inflammatory cells and assign scores as follows: 0 - absent (absent or few inflammatory cells), 1 - mild (up to 25 cells and mild reaction), 2 - moderate (25-125 inflammatory cells and moderate reaction) and 3 - severe (more than 125 cells and severe reaction) (Cosme-Silva et al., 2019).

Immunohistochemical analysis was performed using the immunoperoxidase technique as previously described (Dal-Fabbro et al., 2019). The remaining six slides from each animal were incubated with the following primary antibodies diluted 1:100: Interleukin-10 (IL-10) (orv221323 antibody, Biorbyt Ltd, Cambridge, UK); Receptor activator of nuclear factor kappa-B ligand (RANKL) (antibody sc-377079, Santa Cruz Biotechnology, Texas, USA); Osteoprotegerin (OPG) (antibody sc-390518, Santa Cruz Biotechnology, Texas, USA); tumor necrosis factor alpha (TNF-alpha) (orb11495 antibody, Biorbyt Ltd, Cambridge, UK); interleukin 1-beta (IL-1beta) (orb382131 antibody, Biorbyt Ltd, Cambridge, UK); and tartrate-resistant acid phosphatase (TRAP) (orb2250 antibody, Biorbyt Ltd, Cambridge, UK).

Immunohistochemistry was performed using the study samples and the negative reaction control (where the use of the primary antibody was suppressed and no immunostaining was obtained). Positive immunoreactivity was indicated by brownish staining in the cytoplasm and extracellular matrix. For the semiquantitative analysis of IL-10, RANKL, OPG, TNF-α and IL-1β, scores were used according to the immunostaining pattern assigned by Cantiga-Silva et al. (2021), where 0: no immunostaining (complete absence of immunoreactivity); 1: <20% - immunoreactive cells and little staining in the extracellular matrix; 2: >20% and <40% - immunoreactive cells and little staining in the extracellular matrix; 3: >40% and <60% - immunoreactive cells and moderate staining in the extracellular matrix; 4: >60% and <80% - immunoreactive cells and high staining in the extracellular matrix; 5: >80% - immunoreactive cells and high staining in the extracellular matrix. TRAP analysis was performed using the ratio between the amount of positive multinucleated TRAP and the perimeter obtained in the delineation of bone resorption in apical periodontitis, with this analysis expressed in cells per millimeter (cells/mm) (Gomes-Filho et al., 2015).

The analysis described above was performed by a histologist blinded to the groups using a light microscope (DM 4000 B; Leica) and a color camera (DFC 500; Leica).

2.7. Statistical analysis

Data analysis was performed using GraphPad Prism software (version 8.0.2) (La Jolla, CA, USA). After testing for normality using the Shapiro-Wilk test, the Kruskal-Wallis test was used for non-parametric data, followed by Dunn's multiple comparisons. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used for parametric data. A 5% significance level was used.

3.Results

3.1. Microtomographic analysis

All groups presented increased radiolucent areas in the periapical region caused by the apical periodontitis. The graph of the data resulting from the microtomographic analysis is explained in Figure 6 and the representative images of each group are shown in Figure 7. It was observed that the dealcoholized red wine group presented a lower mean volume of bone resorption of 0.38mm3±0.058mm3, followed by the red wine group with 0.49mm3±0.038mm3, the control group with 0.621mm3±0.009mm3 and the alcohol group with 0.91mm3±0.058 mm3. All groups present significant differences when compared to each other ($p<0.05$).

3.2. Histological analysis

It was observed that all groups had completely necrotic pulp tissue and the periodontal ligament was disorganized and inflamed with areas of resorption, demonstrating the effectiveness of the induction method using pulp exposition. The graph of the inflammation scores assigned to each group with the median is explained in Figure 6 and the representative sections of hematoxylin-eosin staining are explained in Figure 8. After assigning the scores, it was evident that the dealcoholized red wine and red wine groups presented the lowest score (1) with mild inflammation, with no statistical difference between them ($p>0.05$). They were followed by the control group with a score of (2) corresponding to moderate inflammation and the alcohol group with a higher score of (3) corresponding to severe inflammation, with no statistical difference between them ($p>0.05$). The control and alcohol groups were statistically higher than the dealcoholized red wine and red wine groups ($p<0.05$).

3.3 Immunohistochemical analysis

The pattern of immunoreactivity for IL-10, TNF-α, IL-1β, OPG, RANKL, and TRAP is described in the graphs in Figure 6 and the representative sections for each group in Figures 8 and 9:

IL-10: All groups had the same pattern of immunoreactivity (median 1) ($p>0.05$).

TNF-α: The immunoreaction pattern was lower in the dealcoholized red wine group (median 1), different from the control group (median 2.5) and the alcohol group (median 3) ($p<0.05$); followed by the red wine group (median 2), different from the alcohol group (median 3) ($p<0.05$).

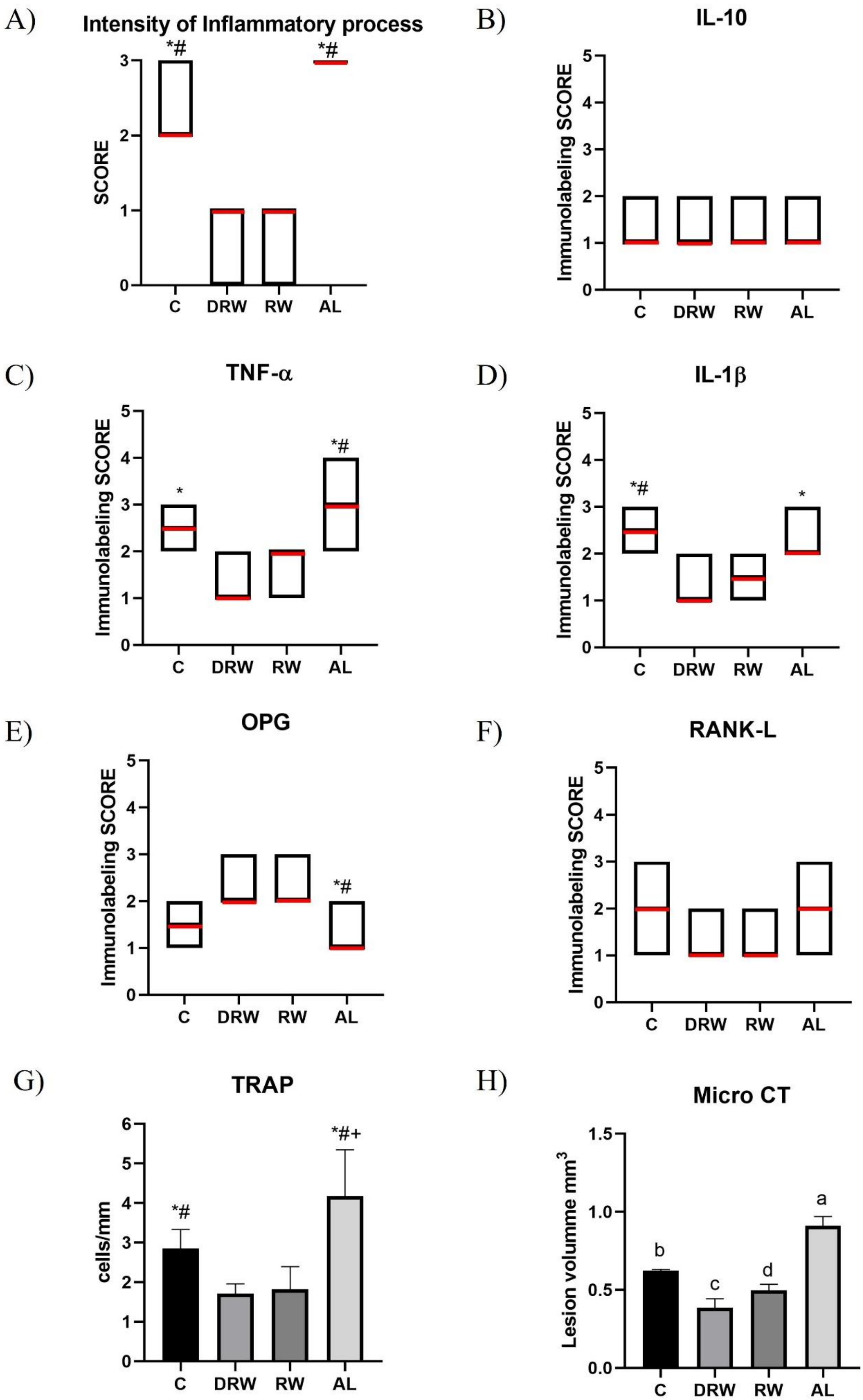
IL-1β: The immunoreaction pattern was lower in the dealcoholized red wine group (median 1), than the control group (median 2.5) and the alcohol group (median 2) ($p<0.05$), followed by the red wine group (median 1.5) different from the control group ($p<0.05$).

OPG: The dealcoholized red wine and red wine groups had a more immunoreaction (median 2), when compared to the alcohol group (median 1) ($p<0.05$).and similar to group control ($p<0.05$).

RANKL: all groups were similar ($p > 0.05$).

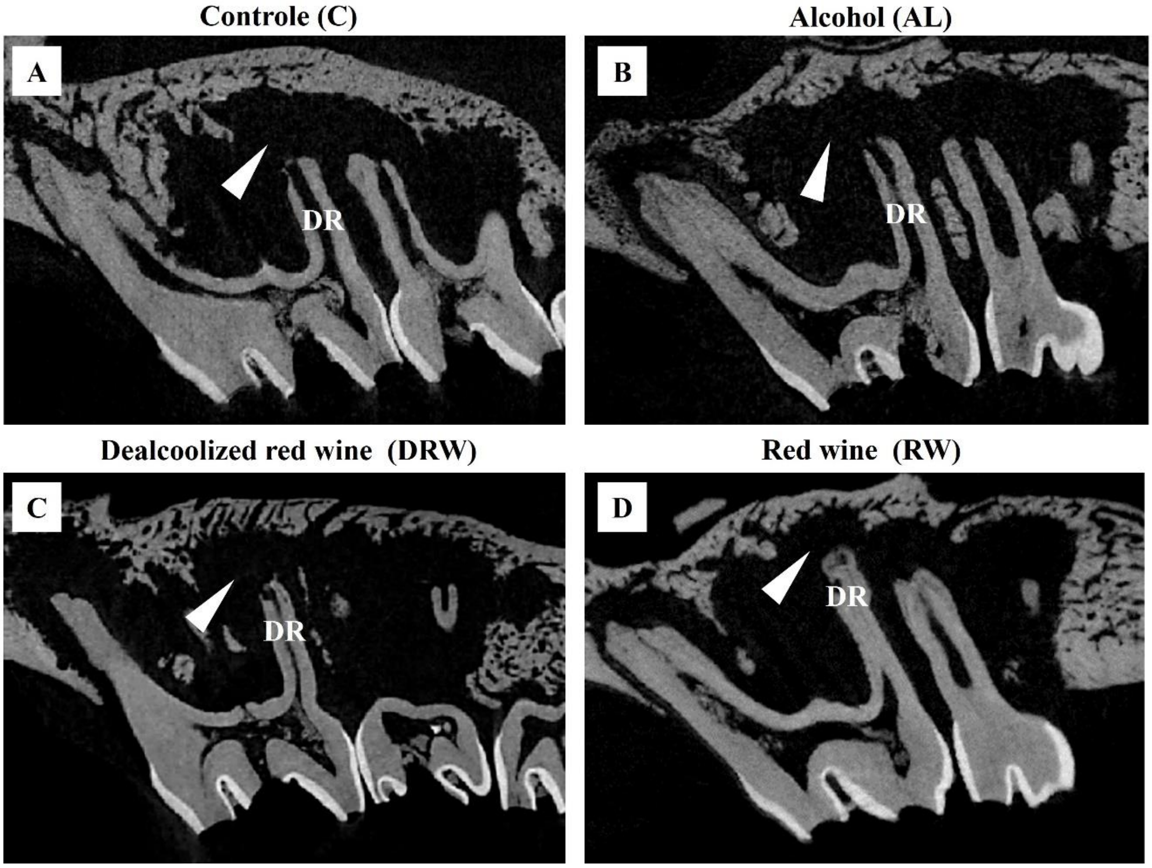
TRAP: The pattern of immunoreactivity for TRAP was specific for osteoclastic cells. It was found that the dealcoholized red wine and red wine groups had a similar number of cells per mm, 1.71±0.24 and 1.81±0.57 ($p > 0.05$). Both groups showed less immunoreactivity compared to control group (2.85±0.48) and alcohol group (4.17±1.17) ($p<0.05$). The alcohol group was found to be the most osteoclastic cells inducer differently from control group ($p<0.05$).

Figure 6 - Microtomography, histology and immunohistochemistry data plots.


A)
Intensity of Inflammatory process
SCORE
*#
*#
C DRW RW AL
B)
IL-10
Immunolabeling SCORE
C DRW RW AL
C)
TNF-α
Immunolabeling SCORE
*
*#
C DRW RW AL
D)
IL-1β
Immunolabeling SCORE
*#
*
C DRW RW AL
E)
OPG
Immunolabeling SCORE
*#
C DRW RW AL
F)
RANK-L
Immunolabeling SCORE
C DRW RW AL
G)
TRAP
cells/mm
*#
*#+
C DRW RW AL
H)
Micro CT
Lesion volumme mm3
b
c
d
a
C DRW RW AL

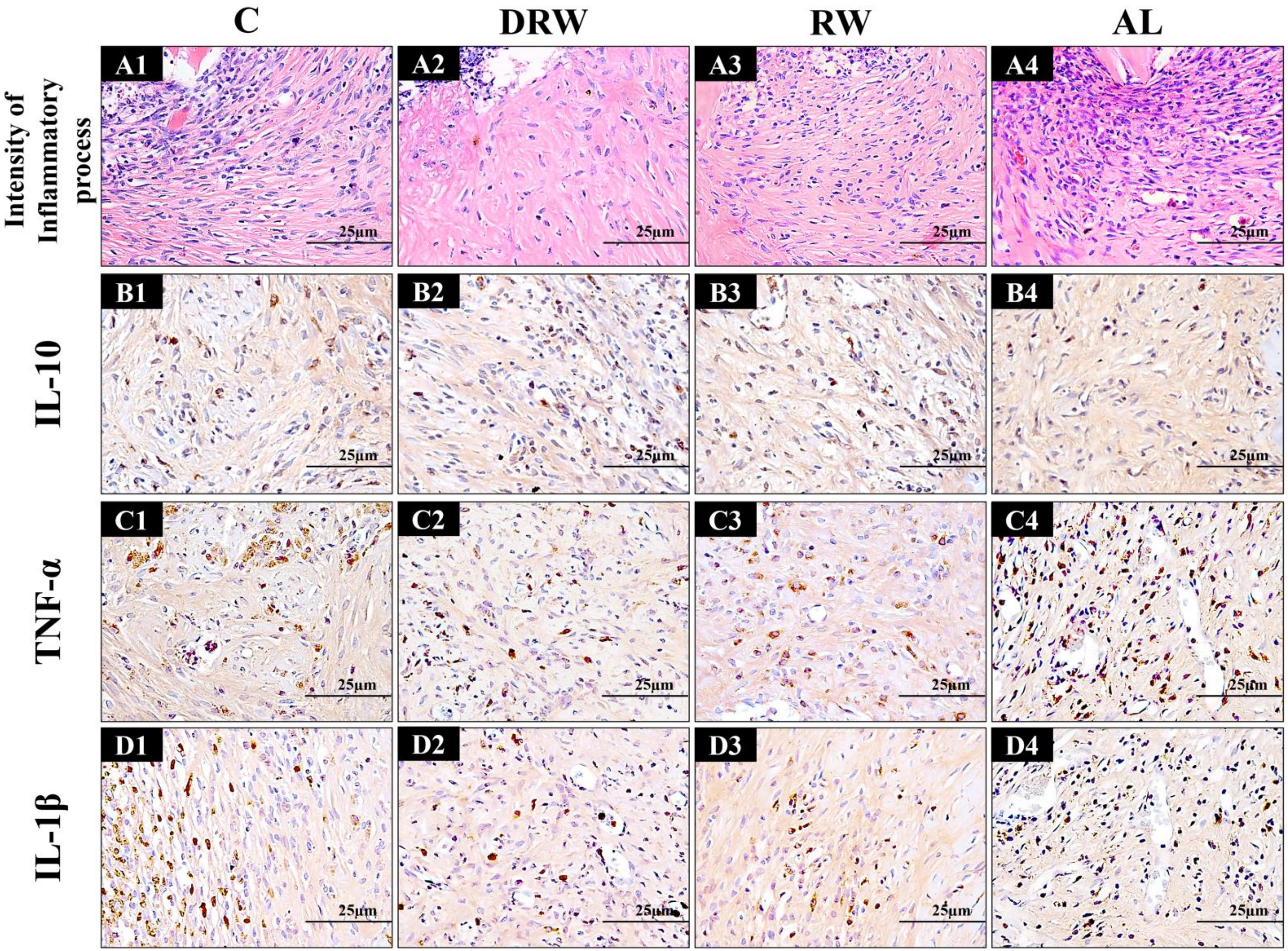
(a) The graph of the intensity of the inflammatory process with the medians for each group shown in red. (b, c, d, e, f) The graphs show the scores of the immune response with the medians shown in red for each group for IL-10, TNF-α, IL-1β, OPG, RANKL, respectively. (g) Bar graph of TRAP immunoreactive cells per millimeter perimeter of bone resorption in apical periodontitis with mean and standard deviation for each group and statistical differences indicated by symbols. (h) Bar graph of lesion volume with mean and standard deviation for each group. Statistical differences indicated by symbols or letters. Symbols: *$p < 0.05$ versus Dealcoholized Rede Wine (DRW); #$p < 0.05$ versus Red Wine (RW); +$p < 0.05$ versus Control (C)

Figure 7 - Representative microtomographic sections



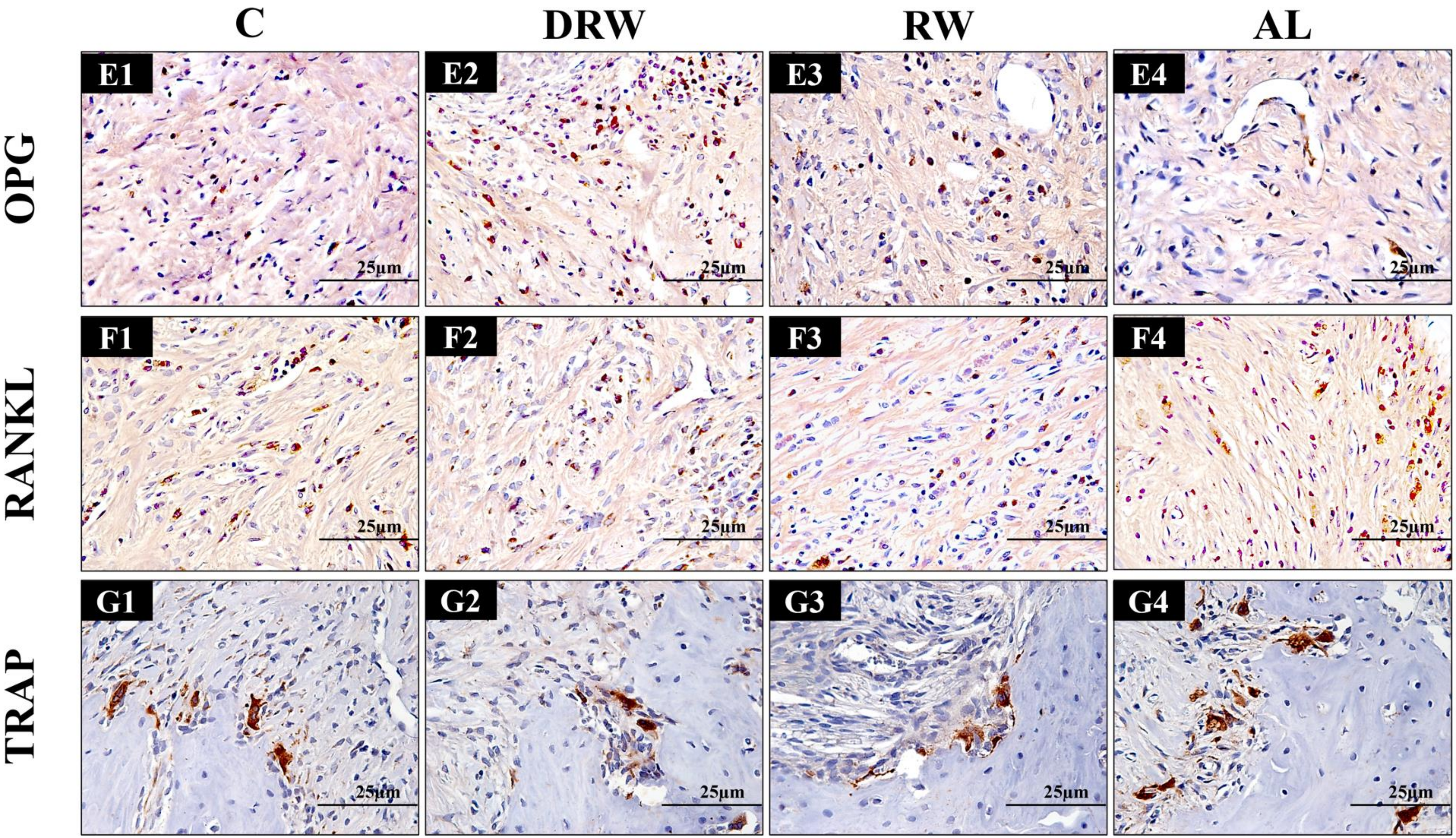
Micro-computed tomography (μCT) images of apical periodontitis aspects in sagittal sections and the maxillary first molars of each group. All groups showed bone loss along the apex of the distal root (DR) of the maxillary first molars, indicated by the white arrow. Reduced bone resorption on microtomography is observed in the DRW group (C), followed by the RW group (D) and the C group (A). The AL group (B) shows more bone resorptio

Figure 8 - Representative sections from histology and immunohistochemistry (IL-10, TNF-α and IL-1β).


C
DRW
RW
AL
Intensity of Inflammatory process
IL-10
TNF-α
IL-1β
A1
A2
A3
A4
B1
B2
B3
B4
C1
C2
C3
C4
D1
D2
D3
D4
25µm

Images show representative sections of the histological and immunohistochemical aspects of the periapical region around the foraminal opening of the distal root of the lower first molar 30 days after injury induction. Hematoxylin and eosin staining in groups Control (C) (A1), Dealcoholized Red Wine (DRW) (A2), Red Wine (RW) (A3) and Alcohol (AL) (A4) shows the area of bone resorption and inflammatory infiltrate around the apex (400x magnification). Immunohistochemistry images show the expression of IL-10, TNF-α and IL-1β in groups C (B1, C1 and D1), DRW (B2, C2 and D2), RW (B3, C3 and D3) and AL (B4, C4 and D4).

Figure 9 - Representative sections from immunohistochemistry (OPG, RANKL and TRAP).


C
DRW
RW
AL
OPG
RANKL
TRAP
E1
E2
E3
E4
F1
F2
F3
F4
G1
G2
G3
G4
25μm

Images show representative sections of the immunohistochemical aspects of the periapical region around the foraminal opening of the distal root of the lower first molar 30 days after injury induction. Images show the expression of OPG, RANKL and TRAP in groups Control (C) (E1, F1 and G1), Dealcoholized Red Wine (DRW) (E2, F2 and G2), Red Wine (RW) (E3, F3 and G3) and Alcohol (AL) (E4, F4 and G4), respectively.

4. Discussion

The present study is the first to analyze the influence of dealcoholized red wine (DRW) on the severity of induced apical periodontitis in rats. The results obtained contradicted the null hypothesis as there was a beneficial effect of Dealcoholized Red Wine (DRW) supplementation with reduction in the extent of the inflammatory process and osteoclastogenesis in settled apical periodontitis corroborating the highlights from. Nutraceutical science regarding the red wine polyphenols benefits for human health (Piccolella et al., 2019).

The rat model was chosen due to its biological responses are similar to humans in studies involving alcoholic beverages (Dal-Fabbro et al., 2019; Malherbe & Messaoudi, 2022; McBride et al., 2014). In addition, animal research is essential to support human clinical trials in endodontics to minimize health risks (Nagendrababu et al., 2021). Female rats have the highest circulation of pro-inflammatory cytokines due to hormonal influences while the use of male rats has been better defined (Bain et al., 2019).

Exposure of the dental pulp to the oral environment was the method used to induce apical periodontitis as it is consolidated in rodents causing pulp necrosis through infection with oral pathogens and repercussions on the apical tissues (Dal-Fabbro et al., 2021b; Goldman et al., 2019; Justo et al., 2022). The pulp remained exposed for 30 days without any further intervention to allow the pathology to be settled and to start the supplementation. That period of time was considered to reproduce the pathogenesis of apical periodontitis in rodents similarly to humans (Justo et al., 2022; Tani-Ishii et al., 1994). The apical lesion was histologically confirmed by pulp necrosis and the presence of inflammatory infiltrate in the periapical lesions (Conti et al., 2020; Cantiga-Silva et al., 2021).

The solutions were administered daily in the morning by the gavage method using a gastric cannula respecting a dose based on the weight of the animals (Azuma et al., 2018; Cosme-Silva et al., 2019; Dal-Fabbro et al., 2021a; Dal-Fabbro et al., 2021b; Dal-Fabbro et al., 2019; Justo et al., 2022). A dose of 4.28 ml/kg was considered beneficial, equivalent to the daily consumption of 300 ml of wine for a man weighing 70 kg (Pavlidou et al., 2018). The dealcoholized red wine and red wine groups received Merlot wine, which is recognized for its high concentration of bioactive phenolic compounds (Đorđević et al., 2017). The control group received water to simulate the stress of gavage and to allow substance free comparisons

(Dal-Fabbro et al., 2021b; Justo et al., 2022). The AL group received 12.5% alcohol to mimic the amount of ethanol in wine (9-15%) (Dal-Fabbro et al., 2019; Wagner et al., 2019).

In the present study, histological analysis showed that the inflammatory infiltrate was mild in the dealcoholized red wine and red wine groups compared to control and alcohol groups. Wine appears to attenuate osteoimmune inflammatory responses during the apical periodontitis development (Dal-Fabbro et al., 2021a). This effect may be explained by the presence of phenolic compounds (Dal-Fabbro et al., 2021; Wagner et al., 2019). These compounds can inhibit inflammatory stimuli from nuclear factor kappa-B (NF-kB), cyclooxygenase, and lipoxygenase, which can help neutralize pro-inflammatory cytokines (Laughton et al., 1991; Lopez et al., 2007; Santalego et al., 2007). As a result, the activation of infection-fighting leukocytes is reduced (Petean et al., 2022). On the other hand, alcohol is reported to induce an increase in inflammatory cells and cytokines in periodontitis, which justifies the result of the alcohol group in this study with a severe inflammatory infiltrate with a concentration of defense cells (Dal-Fabbro et al., 2019; Pinto et al., 2020).

In the present study, it was also found the reduction of inflammatory cytokines in the settled apical periodontitis of animals treated with DRW compared to animals treated with water and alcoholic solution. Different form previous study, the present one that found significant reduction in IL-1β and TNF-α (Dal-Fabbro et al., (2021a). That result was obtained in supplementation schedule that started in the day of the apical periodontitis induction and the present one after the apical periodontitis has been settled. More over, the brand of wine was changed, which can affect the amount of polyphenols (Đorđević et al., 2017). In oral infections including apical periodontitis, these mediators can be released by the interaction of host cells with bacteria and their by-products leading to tissue inflammation (Groeger & Meyle, 2019; Petean et al., 2022), being IL1-β and TNF-α prevalent in apical periodontitis and related to bone resorption (Artese et al., 1991; Nair, 2004). Modulating the activation of inflammatory mediators is a promising pathway for the application of the red wine polyphenols (Majkić et al., 2019). It is believed that in the present study, the combination of several polyphenols may have produced a beneficial effect, as these components regulate gene expression in the reduction of inflammatory mediators through precursor inflammasomes (Tangney & Rasmussen et al., 2013).

Regarding IL-10, the present study was similar to previous one showing no difference among the groups (Dal-Fabbro et al., 2021a). Interleukine 1 combats and prevents inflammatory pathologies at different stages of the immune response by macrophages and dendritic cells in response to microbial products (Saraiva & Garra, 2010). Polyphenols have been reported as possible substances that induce the release of anti-inflammatory cytokines to protect the body and apical periodontitis (Magrone et al., 2008; Dal-Fabbro et al., 2021a). It is possible that a longer time for the development of AP has relation to the amount of anti-inflammatory cytokines (Kawashima & Stashenko, 1999; Sasaki et al., 2000).

In this study, the OPG-RANKL pathway was analyzed. The results showed a higher expression of OPG in the dealcoholized red wine and red wine groups compared to the alcohol group. On the other hand, there were no significant differences between the groups for RANKL. OPG, a protein secreted by the body that binds to RANKL and prevents the osteoclastic sequence, resulted in less bone resorption (Petean et al., 2022). Mediators such as TNF-α cause greater osteoclast maturation (Yu et al., 2016) through the activation of RANK-L (Sabeti et al., 2005). The increased number of mature osteoclasts affects bone metabolism through increased degradation of organic and inorganic compounds on the bone surface. These actions unbalance the RANK/RANKL/OPG system that is important for maintaining the bone metabolism (Boyce & Xing, 2008; Petean et al., 2022). Polyphenols were found to be effective as antioxidant inhibiting reactive oxygen species (ROS) that promote RANK activation and to reduce cytokines that are precursors of bone resorption, such as TNF-α and interleukin-6, and to decrease RANKL-induced osteoclast differentiation and ROS generation and increased osteoclast apoptosis in vitro analysis (Wattel et al., 2004; Choi & Hwang; He et al., 2010).

Bone resorption could also be observed in the microtomography analysis, where the dealcoholized red wine group had less for bone resorption, which is correlated to less formation of TRAP-positive multinucleated cells, followed by the red wine, control and alcohol groups. The improvement of bone parameters under the influence of red wine and its isolated components in apical periodontitis was also observed in other studies (Dal-Fabbro et al., 2021a; Kongstad et al., 2008; Wagner et al., 2019).

The present study is a preliminary comparison showing that dealcoholized red wine had a superior performance in the parameters of OPG, TRAP, and bone resorption expression. However, alcohol increased the expression of TRAP-positive multinucleated cells and bone resorption similar to other studies linking craniofacial bone metabolism to the alcoholic component (Correa et al., 2016; Dal-Fabbro et al., 2019; Dal-Fabbro et al., 2021a; Souza et al., 2009). Alcohol can activate osteoclasts via RANK with high production of ROS and kinases (Correa et al., 2016), in addition to altering RANKL (Dal-Fabbro et al., 2019; Dal-Fabbro et al., 2021a).

Although this study represents an advance in endodontic science, these results cannot be directly extrapolated to humans due to limitations in the experimental model, the dosage of wine, and the complexity of the treatment of apical periodontitis that aims to improve the healing process. Therefore, future investigations are encouraged to make further advances in understanding the use of dealcoholized red wine to assist the human body in inflammatory and osteoclastic conditions of the apical periodontitis.

5.Conclusion

Supplementation of dealcoholized red wine in rats reduced the inflammation of settled apical periodontitis by reducing the local inflammatory infiltrate and the levels of the pro-inflammatory cytokines TNF-α and IL-1β. In addition, there was a reduction in bone resorption as evidenced by increased expression of OPG, decreased immunoreactivity to TRAP cells and reduced periapical bone resorption.

References

Artese, L., Piattelli, A., Quaranta, M., Colasante, A. & Musani, P. (1991) Immunoreactivity for interleukin 1-beta and tumor necrosis factor-alpha and ultrastructural features of monocytes/macrophages in periapical granulomas. *Journal of Endodontics*, 17(10),483-487.

Artero, A., Artero, A., Tarín, J.J. & Cano, A. (2015) The impact of moderate wine consumption on health. *Maturitas*, 80(1),3-13.

Azuma, M.M., Cardoso, C.B.M., Samuel, R.O., Pipa, C.B., Bomfim, S.R.M., Narciso, L.G., Gomes-Filho, J.E. & Cintra, L.T.A. (2021) Omega-3 Fatty Acids Alter Systemic Inflammatory M ediators Caused by Apical Periodontitis. *Jounal of Endodontics*, 47(2), 272-277.

Bain, J.L., Lester, S.R., Henry, W.D., Bishop, C.M., Turnage, A.A., Naftel J.P. & Johnson RB. (2009) Comparative gender differences in local and systemic concentrations of pro-inflammatory cytokines in rats with experimental periodontitis. *Journal of Periodontal Research*, 44(1), 133-140.

Belisario-Sanchez, Y. Y., Taboada-Rodriguez, A., Marin-Iniesta, F., & Lopez-Gomez, A. (2009). Dealcoholized wines by spinning cone column distillation: phenolic compounds and antioxidant activity measured by the 1, 1-diphenyl-2-picrylhydrazyl method. *Journal of Agricultural and Food Chemistry*, 57(15), 6770-6778.

Biasi, F., Astegiano, M., Maina, M., Leonarduzzi, G. & Poli G. (2011) Polyphenol supplementation as a complementary medicinal approach to treating inflammatory bowel disease. *Current Medicinal Chemistry,* 18(31), 4851-4865.

Boyce, B.F. & Xing, L. (2008) Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Archives of Biochemistry and Biophysics*, 473(2),139-146.

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 Endodontics Journal*, 54(8), 1353-1361.

Chen, S., Lei, H., Luo, Y., Jiang, S., Zhang, M., Lv, H., Cai, Z. & Huang, X. (2019) Micro-CT analysis of chronic apical periodontitis induced by several specific pathogens. *International Endodontics Journal*, 52(7), 1028-1039.

Chiva, B.G., Urpi, S. M, Ros, E., Arranz, S., Valderas, M.P., Casas, R., Sacanella, E., Llorach, R., Lamuela, R.R.M., Andres, L.C. & Estruch, R. (2012) Dealcoholized red wine decreases systolic and diastolic blood pressure and increases plasma nitric oxide: short communication. (2012) *Circulation Research*, 111(8), 1065-1068.

Choi, E.M. & Hwang, J.K. (2003) Effects of (+)-catechin on the function of osteoblastic cells. *Biological and Pharmaceutical Bulletin,* 26(4):523-526.

Cintra, L. T., Samuel, R. O., Azuma, M. M., de Queiroz, A. O., Ervolino, E., Sumida, D. H., Gomes-Filho, J. E. (2016) Multiple Apical Periodontitis Influences Serum Levels of Cytokines and Nitric Oxide. *Journal of Endodontics*, 42(5),747-751.

Corrêa, M.G., Gomes, C.M.L., Marques, M.R., Ambrosano, G.M., Casati, M.Z., Nociti, F.H.J. & Sallum, E.A. (2016) Alcohol intake may impair bone density and new cementum formation after enamel matrix derivative treatment: histometric study in rats. *Journal of Periodontal Research*, 51(1), 60-69.

Conti, L.C., Segura-Egea, J.J., Cardoso, C.B.M., Benetti, F., Azuma, M.M., Oliveira P.H.C., Bomfim, S.R.M. & Cintra, L.T.A. (2020) Relationship between apical periodontitis and atherosclerosis in rats: lipid profile and histological study. *International Endodontics Journal*, 53(10),1387-1397.

Cosme-Silva, L., Dal-Fabbro, R., Cintra, L.T.A., Santos, V.R., Duque, C., Ervolino, E., Bomfim, M.S & Gomes-Filho J.E. (2019) Systemic administration of probiotics reduces the severity of apical periodontitis. *International Endodontics Journal*, 52(12), 1738-1749. doi: 10.1111/iej.13192.

Dal-Fabbro, R., Cosme-Silva, L., Rezende, S.M.O.F., Capaldo, L.C., Plazza, F.A., Ervolino, E., Cintra, L.T.A. & Gomes-Filho, J.E. (2021a) Effect of red wine or its polyphenols on induced apical periodontitis in rats. *International Endodontics Journal*, 54(12), 2276-2289.

Dal-Fabbro, R., Cosme-Silva, L., Capalbo, L.C., Chaves-Neto, A.H., Ervolino, E., Cintra, L.T.A & Gomes-Filho, J.E. (2021b) Excessive caffeine intake increases bone resorption associated with periapical periodontitis in rats. *International Endodontics Journal*, 54(10), 1861-1870.

Dal-Fabbro, R., Marques, A. 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 Endodontics Journal*, 52(3), 329- 336.

Finicelli, M., Squillaro, T., Cristo, F., Salle, A., Melone, M.A.B., Galderisi, U. & Peluso, G. (2019) Metabolic syndrome, Mediterranean diet, and polyphenols: Evidence and perspectives. *Journal of Cellular Physiology*,234(5), 5807-5826.

Goldman, E., Reich, E., Abramovitz, I. & Klutstein, M. (2019) Inducing Apical Periodontitis in Mice. *Journal of Visualized Experiments,* (150).

Gomes-Filho, J.E., Wayama, M.T., Dornelles, R.C., Ervolino, E., Coclete, G.A., Duarte, P.C., Yamanri, G.H., Lodi, C.S., Dezan-Júnior, E. & Cintra, L.T. (2015) Effect of raloxifene on periapical lesions in ovariectomized rats. *Journal of Endodontics*, 41(5):671-675.

Groeger, S. & Meyle, J. (2019) Oral Mucosal Epithelial Cells. *Frontiers in Immunology,* 10, 208.

Habold, C., Momken, I., Ouadi, A., Bekaert, V. & Brasse, D. (2011) Effect of prior treatment with resveratrol on density and structure of rat long bones under tail-suspension. *Journal of Bone and Mineral Metabolism,29*(1),15-22.

He, X., Andersson, G., Lindgren, U. & Li, Y. (2010) Resveratrol prevents RANKL-induced osteoclast differentiation of murine osteoclast progenitor RAW 264.7 cells through inhibition of ROS production. *Biochemical and Biophysical Research Communications,* 401(3), 356-362.

Holland, R., Gomes-Filho, J.E., Cintra, L.T.A, Queiroz, Í.O.A & Estrela, C. (2017) Factors affecting the periapical healing process of endodontically treated teeth. *Journal of Applied Oral Science,* 25(5):465-476.

Jhang, J.J., Lu, C.C., Ho, C.Y., Cheng, Y.T. & Yen, G.C. (2015) Protective Effects of Catechin against Monosodium Urate-Induced Inflammation through the Modulation of NLRP3 Inflammasome Activation. *Journal of Agricultural and Food Chemistry*, 63(33):7343-7352

Justo, M.P., Cardoso, C.B.M., Cantiga-Silva, C., de Oliveira, P.H.C., Sivieri-Araújo, G., Azuma, M.M., Ervolino, E. & Cintra, L.T.A. (2022) Curcumin reduces inflammation in rat apical periodontitis. *International Endodontics Journal*, 55(11), 1241-1251.

Kajiya, M., Giro, G., Taubman, M.A., Han, X., Mayer, M.P. & Kawai, T. (2010) Role of periodontal pathogenic bacteria in RANKL-mediated bone destruction in periodontal disease. *Journal of Oral Microbiology,* 8, 2.

Kalatzis-Sousa, N.G., Spin-Neto, R., Wenzel, A., Tanomaru-Filho, M. & Faria, G. (2017) Use of micro-computed tomography for the assessment of periapical lesions in small rodents: a systematic review. *International Endodontics Journal*, 50(4), 352-366.

Kawashima, N. & Stashenko, P. (1999) Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. *Archives of Oral Biology,* 44(1), 55-66.

Kongstad, J., Hvidtfeldt, U.A., Grønbaek, M., Jontell, M., Stoltze, K. & Holmstrup P. (2008) Amount and type of alcohol and periodontitis in the Copenhagen City Heart Study. *Journal of Clinical Periodontology,* 35(12), 1032-1039.

Kutleša, Z. & Budimir, M. D. (2016) Wine and bone health: a review. *Journal of Bone and Mineral Metabolism,* 34(1), 11-22.

Lan, Y., Wang, K., Lan, Z., Zhou, H. & Sun, J. (2023) Dealcoholized red wine inhibits occurrence and progression of hepatocellular carcinoma possibly by inducing cell cycle arrest and apoptosis. *Nan Fang Yi Ke Da Xue Xue Bao,* 43(8), 1297-1305.

Laughton, M.J., Evans, P.J., Moroney, M.A., Hoult, J.R. & Halliwell, B. (1991) Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochemical Pharmacology,*42(9),1673-1681.

Liguori, L., Albanese, D., Crescitelli, A., Matteo, M. & Russo, P. (2019) Impact of dealcoholization on quality properties in white wine at various alcohol content levels. *Journal of Food Science and Technology,* 56(8):3707-3720.

López, D., Pavelkov, M., Gallova, L., Simonetti, P., Gardana, C., Lojek, A., Loaiza, R. & Mitjavila, M.T. (2007) Dealcoholized red and white wines decrease oxidative stress associated with inflammation in rats. *British Journal of Nutrition*, 98(3), 611-619.

Luo, X., Wan, Q., Cheng, L., & Xu, R. (2022) Mechanisms of bone remodeling and therapeutic strategies in chronic apical periodontitis. *Frontiers in Cellular and Infection Microbiology,* 12, 908859.

Magrone, T., Candore, G., Caruso, C., Jirillo, E. & Covelli, V. (2008) Polyphenols from red wine modulate immune responsiveness: biological and clinical significance. *Current Pharmaceutical Design,*14(26), 2733-2748

Majkić, T.M., Torović, L.D., Lesjak, M.M., Četojević-Simin, D.D. & Beara, I.N. (2019) Activity profiling of Serbian and some other European Merlot wines in inflammation and oxidation processes. *Food Research International,*121,151-160.

Malherbe, D.C. & Messaoudi, I. (2022) Transcriptional and Epigenetic Regulation of Monocyte and Macrophage Dysfunction by Chronic Alcohol Consumption. *Frontiers in Immunology,* 13, 911951.

McBride, W.J., Rodd, Z.A., Bell, R.L., Lumeng, L. & Li, T.K. (2014) The alcohol-preferring (P) and high-alcohol-drinking (HAD) rats--animal models of alcoholism. *Alcohol*, 48(3), 209-215

Mehrazarin, S., Alshaikh, A. & Kang, M.K. (2017) Molecular Mechanisms of Apical Periodontitis: Emerging Role of Epigenetic Regulators. *Dental Clinics of North America,*61(1), 17-35.

Modun, D., Music, I., Vukovic, J., Brizic, I., Katalinic, V., Obad, A., Palada, I., Dujic, Z. & Boban, M. (2008) The increase in human plasma antioxidant capacity after red wine consumption is due to both plasma urate and wine polyphenols. *Atherosclerosis.,* 197(1), 250-256.

Möller, A.J., Fabricius, L., Dahlén, G., Ohman, A.E. & Heyden, G. (1981) Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. *Scandinavian Journal of Dental Research,,* 89(6), 475-84.

Muñoz-González, I., Thurnheer, T., Bartolomé, B., & Moreno-Arribas, M. V. (2014) Red wine and oenological extracts display antimicrobial effects in an oral bacteria biofilm model. *Journal of agricultural and food chemistry*, 62(20), 4731-4737.

Nagendrababu, V., Kishen, A., Murray, P.E., Nekoofar, M.H., de Figueiredo, J.A.P., Priya, E., Jayaraman, J., Pulikkotil, S.J., Camilleri, J., Silva, R.M. & Dummer, P.M.H. (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: a consensus-based development. *International Endodontics Journal,* 54(6),848-857.

Nair, P. R. (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical Reviews in Oral Biology & Medicine,* 15(6), 348-381.

Pavlidou, E., Mantzorou, M., Fasoulas, A., Tryfonos, C., Petridis, D. & Giaginis, C. (2018) Wine: An Aspiring Agent in Promoting Longevity and Preventing Chronic Diseases. *Diseases*, 6(3), 73.

Percie, S.N.,Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Hurst, V., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T. & Würbel, H. (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biology,* 18(7), e3000411.

Petean, I. B. F., Silva-Sousa, A. C., Cronenbold, T. J., Mazzi-Chaves, J. F., Silva, L. A. B. D., Segato, R. A. B., Castro, G. P. A., Kuchler, E.C., Paula-Silva, F.W.G. & SousaNeto, M.

D. (2022) Genetic, cellular and molecular aspects involved in apical periodontitis. *Brazilian Dental Journal,* 33, 1-11.

Piccolella, S., Crescente, G., Candela, L. & Pacifico, S. (2019) Nutraceutical polyphenols: New analytical challenges and opportunities. *Journal of Pharmaceutical and Biomedical Analysis,*175, 112774.

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 Endodontics Journal,* 53(9),1238-1252.

Roe, K. (2021) An inflammation classification system using cytokine parameters. *Scandinavian Journal of Immunology*, 93(2), e12970.

Sabeti, M., Simon, J., Kermani, V., Valles, Y. & Rostein, I. (2005) Detection of receptor activator of NF-kappa beta ligand in apical periodontitis. *Journal of Endodontics*, 31(1), 17-18

Sam, F.E., Ma, T.Z., Salifu, R., Wang, J., Jiang, Y.M., Zhang, B. & Han, S.Y. (2021) Techniques for Dealcoholization of Wines: Their Impact on Wine Phenolic Composition, Volatile Composition, and Sensory Characteristics. *Foods*, 10(10), 2498.

Santangelo, C., Varì, R., Scazzocchio, B., Benedetto, D.R., Filesi, C. & Masella, R. (2007) Polyphenols, intracellular signalling and inflammation. *Annali dell'Istituto Superiore di Sanità*, 43(4), 394-405.

Saraiva, M. & O'Garra, A. (2010) The regulation of IL-10 production by immune cells. *Nature Reviews Immunology,* 10(3), 170-181.

Sasaki, H., Hou, L., Belani, A., Wang, C.Y., Uchiyama, T., Müller, R. & Stashenko, P. (2000) IL-10, but not IL-4, suppresses infection-stimulated bone resorption in vivo. *Journal of Immunology,*165(7), 3626-3630.

Siqueira, J.F.J. & Rôças I.N. (2007) Bacterial pathogenesis and mediators in apical periodontitis. *Brazilian Dental Journal,*18(4):267-80.

Snopek, L., Mlcek, J., Sochorova, L., Baron, M., Hlavacova, I., Jurikova, T., Kizek, R., Sedlackova, E. & Sochor J. (2018) Contribution of Red Wine Consumption to Human Health Protection. *Molecules,*23(7), 1684.

Souza-Bomfim, G.H., Musial, D.C., Rocha, K., Jurkiewicz, A. & Jurkiewicz, N.H. (2022) Red wine but not alcohol consumption improves cardiovascular function and oxidative stress of the hypertensive-SHR and diabetic-STZ rats. *Clinical and Experimental Hypertension*, 44(6), 573-584.

Souza, D.M., Ricardo, L.H., Kantoski, K.Z. & Rocha, R.F. (2009) Influence of alcohol consumption on alveolar bone level associated with ligature-induced periodontitis in rats. *Brazilian Oral Research,* 23(3), 326-332.

St Leger, A.S., Cochrane, A.L. & Moore, F. (1979) Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *The Lancet,* 1(8124), 1017-1020.

Tangney, C.C. & Rasmussen, H.E. (2013) Polyphenols, inflammation, and cardiovascular disease. *Current Atherosclerosis Reports,* 15(5), 324.

Tani-Ishii, N., Wang, C.Y., Tanner, A. & Stashenko, P. (1994) Changes in root canal microbiota during the development of rat periapical lesions. *Oral Microbiology and Immunology,* 9(3),129-135.

Vernal, R., Dezerega, A., Dutzan, N., Chaparro, A., León, R., Chandía, S., Silva, A. & Gamonal J. (2006) RANKL in human periapical granuloma: possible involvement in periapical bone destruction. Oral Diseases, 12(3):283-289.

Wagner M.C., Cavagni J., Gaio E.J., Brum V.S., Jesus L.H., Filho M.S., Carrard V.C., Dorneles G.P., Peres A. & Rösing C.K.(2019) Effect of red wine and its major components on periodontitis and systemic inflammation in rats. *International Academy of Periodontology,* 21(4), 139-147.

Wang, C., Pan, Y., Zhang, Q.Y., Wang, F.M. & Kong, L.D. (2012) Quercetin and allopurinol ameliorate kidney injury in STZ-treated rats with regulation of renal NLRP3 inflammasome activation and lipid accumulation. *PLoS One*, 7(6), e38285.

Wang, Z., Zou, J., Cao, K., Hsieh, T.C., Huang, Y. & Wu, J.M. (2005) Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels. *International Journal of Molecular Medicine,*16(4), 533-540.

Waterhouse, A.L. (2002) Wine phenolics. *Annals of the New York Academy of Sciences*, 957, 21-36.

Wattel, A., Kamel, S., Prouillet, C., Petit, J.P., Lorget, F., Offord, E. & Brazier, M. (2004) Flavonoid quercetin decreases osteoclastic differentiation induced by RANKL via a mechanism involving NF kappa B and AP-1. *Journal of Cellular Biochemistry,*, 92(2), 285-295.

World Health Organization. (2019). Global status report on alcohol and health 2018. *World Health Organization.*

Yang, S.J. & Lim Y. (2014) Resveratrol ameliorates hepatic metaflammation and inhibits NLRP3 inflammasome activation. *Metabolism,* 63(5):693-701.

Yoshimura, A., Hara, Y., Kaneko, T. & Kato, I. (1997) Secretion of IL-1 beta, TNF-alpha, IL-8 and IL-1ra by human polymorphonuclear leukocytes in response to lipopolysaccharides from periodontopathic bacteria. *Journal of Periodontal Research,* 32(3), 279-86.

Yu, Y.Q., Qu, L., Qiu, L.H., Guo, J.J., Ma, N. & Zhu L., (2016) TNF-α mechanism in the bone defect of chronic apical periodontitis. *Xangai Kou Qiang Yi Xue*, 25 (4), 414–419.

Referências Gerais

Ali, K., Maltese, F., Choi, Y. H., & Verpoorte, R. (2010) Metabolic constituents of grapevine and grape-derived products. *Phytochemistry Reviews*, 9, 357-378.

Azuma, M.M., Cardoso, C.B.M., Samuel, R.O., Pipa, C.B., Bomfim, S.R.M., Narciso, L.G., Gomes-Filho, J.E. & Cintra, L.T.A. (2021) Omega-3 Fatty Acids Alter Systemic Inflammatory M ediators Caused by Apical Periodontitis. *Jounal of Endodontics*, 47(2), 272-277.

Belisario-Sanchez, Y. Y., Taboada-Rodriguez, A., Marin-Iniesta, F., & Lopez-Gomez, A. (2009). Dealcoholized wines by spinning cone column distillation: phenolic compounds and antioxidant activity measured by the 1, 1-diphenyl-2-picrylhydrazyl method. *Journal of Agricultural and Food Chemistry*, 57(15), 6770-6778.

Bian, Z., Guo, Y., Ha, B., Zen, K., Liu, Y. (2012) Regulation of the inflammatory response: enhancing neutrophil infiltration under chronic inflammatory conditions. *The Journal of Immunology.* 188(2), 844-53.

Castaldo, L., Narváez, A., Izzo, L., Graziani, G., Gaspari, A., Di, M.G. & Ritieni, A. (2019) Red wine consumption and cardiovascular health. *Molecules*, 24(19), 3626.

Dal-Fabbro, R., Cosme-Silva, L., Rezende, S.M.O.F., Capaldo, L.C., Plazza, F.A., Ervolino, E., Cintra, L.T.A. & Gomes-Filho, J.E. (2021) Effect of red wine or its polyphenols on induced apical periodontitis in rats. *International Endodontics Journal*, 54(12), 2276-2289.

Dal-Fabbro, R., Marques, A. M., Cosme-Silva, L., Ervolino, E., Cintra, L.T.A. & GomesFilho, J.E. (2019) Chronic alcohol consumption increases inflammation and osteoclastogenesis in apical periodontitis. *International Endodontics Journal*, 52(3), 329- 336.

Fernandez-Sola, J. (2015). Cardiovascular risks and benefits of moderate and heavy alcohol consumption. *Nature Reviews Cardiology*, 12(10), 576-587.

Ferrières, J. (2004) The French paradox: lessons for other countries. *Heart,* 90(1), 107-111.

Georgiou, A.C., Crielaard, W., Armenis, I., Vries, R., Van der Waal, S.V. Apical Periodontitis Is Associated with Elevated Concentrations of Inflammatory Mediators in Peripheral Blood: A Systematic Review and Meta-analysis. (2019) *Journal of Endodontics*. 45(11,1279-1295.

Goldman, E., Reich, E., Abramovitz, I. & Klutstein, M. (2019) Inducing Apical Periodontitis in Mice. *Journal of Visualized Experiments*, (150).

Kajiya, M., Giro, G., Taubman, M. A., Han, X., Mayer, M. P., & Kawai, T. (2010) Role of periodontal pathogenic bacteria in RANKL-mediated bone destruction in periodontal disease. *Journal of oral microbiology*, 2(1), 5532.

Kakehashi, S., Stanley, H.R. & Fitzgerald, R.J. (1965) The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral surgery, oral medicine, oral pathology*, 20(3), 340-9.

Kutleša, Z., & Budimir Mršić, D. (2016). Wine and bone health: a review. *Journal of bone and mineral metabolism*, 34, 11-22.

Mühlbauer, R.C., Lozano, A., Reinli, A. & Wetli, H. (2003) Various selected vegetables, fruits, mushrooms and red wine residue inhibit bone resorption in rats. *Journal of Nutrition*, 133(11), 3592-7.

Muñoz-González, I., Thurnheer, T., Bartolomé, B., & Moreno-Arribas, M. V. (2014). Red wine and oenological extracts display antimicrobial effects in an oral bacteria biofilm model. *Journal of agricultural and food chemistry*, 62(20), 4731-4737.

Li, X., Kolltveit, K. M., Tronstad, L., & Olsen, I. (2000) Systemic diseases caused by oral infection. *Clinical microbiology reviews*, 13(4), 547-558.

Liguori, L., Albanese, D., Crescitelli, A., Di Matteo, M., & Russo, P. (2019). Impact of dealcoholization on quality properties in white wine at various alcohol content levels. *Journal of food science and technology*, 56, 3707-3720.

Luo, X., Wan, Q., Cheng, L., & Xu, R. (2022) Mechanisms of bone remodeling and therapeutic strategies in chronic apical periodontitis. F*rontiers in Cellular and Infection Microbiology*, 12, 908859.

Malherbe, D.C. & Messaoudi, I. (2022) Transcriptional and Epigenetic Regulation of Monocyte and Macrophage Dysfunction by Chronic Alcohol Consumption. *Frontiers in Immunology*, 13, 911951.

Nair, P. R. (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical Reviews in Oral Biology & Medicine*, 15(6), 348-381.

Petean, I. B. F., Silva-Sousa, A. C., Cronenbold, T. J., Mazzi-Chaves, J. F., Silva, L. A. B. D., Segato, R. A. B., Castro, G. P. A., Kuchler, E.C., Paula-Silva, F.W.G. & SousaNeto, M. D. (2022) Genetic, cellular and molecular aspects involved in apical periodontitis. *Brazilian Dental Journal*, 33, 1-11.

Pignatelli, P., Ghiselli, A., Buchetti, B., Carnevale, R., Natella, F., Germanò, G., Fimognari, F., Di Santo, S., Lenti, L. & Violi, F. (2006) Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. *Atherosclerosis*, 188(1), 77-83.

Sam, F. E., Ma, T. Z., Salifu, R., Wang, J., Jiang, Y. M., Zhang, B., & Han, S. Y. (2021) Techniques for dealcoholization of wines: Their impact on wine phenolic composition, volatile composition, and sensory characteristics. *Foods*, 10(10), 2498.

Samuel, R.O., Gomes-Filho, J.E., Azuma, M.M., Sumida, DH., de Oliveira, S.H.P., Chiba, F.Y., Bomfim, S.R.M., Ciarlini, P.C., Narciso, L.G. & Cintra, LTA. (2018) Endodontic infections increase leukocyte and lymphocyte levels in the blood. *Clinical Oral Investigations*, 22(3), 1395-1401.

Santos, B.C., González-Manzano, S. & González-Paramás, A.M. (2021) Wine, Polyphenols, and Mediterranean Diets. What Else Is There to Say? *Molecules*, 26(18), 5537.

Scannapieco, F. A., Papandonatos, G. D., & Dunford, R. G. (1998). Associations between oral conditions and respiratory disease in a national sample survey population. *Annals of periodontology*, 3(1), 251-256.

Singh, A. K., Bishayee, A., & Pandey, A. K. (2018) Targeting histone deacetylases with natural and synthetic agents: an emerging anticancer strategy. *Nutrients*, 10(6), 731.

Stashenko, P., Teles, R., D'Souza, R. (1998) Periapical inflammatory responses and their modulation. *Critical Reviews in Oral Biology & Medicine,* 9(4), 498-521.

U.S. Department of Agriculture and U.S. Department of Health and Human Services. (2020) *Dietary guidelines for Americans, 2020–2025*, 9th edition. Washington, DC.

Vinson, J.A., Teufel, K. & Wu, N. (2001) Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis,* 156(1), 67-72.

Wagner M.C., Cavagni J., Gaio E.J., Brum V.S., Jesus L.H., Filho M.S., Carrard V.C., Dorneles G.P., Peres A. & Rösing C.K. (2019) Effect of red wine and its major components on periodontitis and systemic inflammation in rats. *International Academy of Periodontology*, 21(4), 139-147.

Yamasaki, M., Kumazawa, M., Kohsaka, T., Nakamura, H. & Kameyama, Y. (1994) Pulpal and periapical tissue reactions after experimental pulpal exposure in rats. *Journal of Endodontics,* 20(1), 13-17.

Yu Y. U., Liu, Q. U., Li-hong, Q. I. U., Jia-jie, G. U. O., Nan, M. A., & Li, Z. H. U. (2016). Mechanism of TNF-α in bone defect of chronic apical periodontitis. *Shanghai Journal of Stomatology*, 25(4), 414.

ANEXO A - Comitê de Ética no Uso de Animais (CEUA)

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado **"Efeito do consumo de vinho tinto sem álcool no desenvolvimento da periodontite apical induzida em ratos"**, Processo FOA nº 0221-2022, sob responsabilidade de João Eduardo Gomes Filho apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 25 de Abril de 2022.

VALIDADE DESTE CERTIFICADO: 30 de Maio de 2025.
DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 30 de Junho de 2025.

CERTIFICATE

We certify that the study entitled **"Effect of alcohol-free red wine consumption on the developmente of induced apical periodontitis in rats"**, Protocol FOA nº 0221-2022, under the supervision of João Eduardo Gomes Filho presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on April 25, 2022.

VALIDITY OF THIS CERTIFICATE: May 30, 2025.
DATE OF SUBMISSION OF THE FINAL REPORT: June 30, 2025.

Prof. Dr. João Carlos Callera
Coordenador da CEUA
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais
Faculdade de Odontologia de Araçatuba
Faculdade de Medicina Veterinária de Araçatuba
Rua José Bonifácio, 1193 – Vila Mendonça - CEP: 16015-050 – ARAÇATUBA – SP
Fone (18) 3636-3234 Email CEUA: ceua.foa@unesp.br

ANEXO B - Comprovante de submissão no *International Endodontic Journal* em 09/11/2023

WILEY

My Submissions Romulo

INTERNATIONAL ENDODONTIC JOURNAL
The official journal of the British Endodontic Society and the European Society of Endodontology

JOURNAL HOME AUTHOR GUIDELINES EDITORIAL CONTACT

Powered by Atypon ReX

Submission Overview

Initial Submission This manuscript has been submitted to the editorial office for review. Changes cannot be made during editorial review, but you can view the information and files you submitted, below.

Article Type

Original Article

Title

Dealcoholized red wine influences the systemic hematological profile and apical periodontitis in rats

Manuscript Files

Name	Type of File	Size
Artigo 1 - VTD Sistemico IEJ.docx	Anonymized Main Document - MS Word	2.2 MB
Title page IEJ.docx	Title Page	15.1 KB
Checkliste PRIASE - IEJ.docx	Supplementary Material for Review	29.8 KB
Ethics Committee in Portuguese.pdf	Supplementary Material for Review	177.3 KB
Ethics Committee in English.pdf	Supplementary Material for Review	177.3 KB

Abstract

Objective: To analyze the influence of late supplementation with dealcoholized red wine on the systemic hematological profile and apical periodontitis in rats
Methodology: Forty Wistar rats were used. Apical periodontitis (AP) was induced in the animals' right first and second molars. The animals were arranged into four groups: control (C) - rats with AP; dealcoholized red wine (DRW) - rats with AP and administration of DRW; red wine (RW) - rats with AP and administration of RW; and alcohol (AL) - rats with AP and administration with alcoholic solution. The animals received therapeutic supplementation by gavage thirty days after AP induction that continued along extra 30 days. On day 60, the animals were anesthetized to collect 5 ml of blood by cardiac puncture and then euthanized to remove the jaws for histological analysis. The T-student test was used to compare the phenolic composition of the wines. The Shapiro-Wilk test was then used to check normality. It ended with the Kruskal-Wallis test was used for non-parametric data and ANOVA followed by Tukey's test for parametric data ($p < 0.05$).
Results: The RW and AL groups had a lower percentage weight gain ($p<0.05$). There was a significant increase in hemoglobin, erythrocytes, hematocrit, mean corpuscular volume and platelets in the AL group compared to the other groups ($p<0.05$). There was an increase in plasma proteins in the RW group. Leukocytes increased in the AL group compared to the other groups ($p < 0.001$), and it was increased in the C group compared to the DRW and RW groups ($p < 0.001$); neutrophils were lower in the AL group compared to the other groups ($p < 0.001$), and monocytes did not show significant changes among the groups. The mean inflammatory process score in the AP was significantly lower in the RW and DRW groups (2) than in the C (3) and AL (4) groups, $p < 0.001$.
Conclusions: Late supplementation with dealcoholized red wine influences the systemic hematological profile of rats with AP, resulting in an improvement in leukocyte parameters, an increasing in plasma proteins, maintaining body homeostasis and reducing local inflammatory infiltrate.

Authors

Name	Email	Country/Location
Romulo de Oliveira Sales-Junior [1] Submitting Author	romulojr_99@hotmail.com	Brazil
Bharbara de Moura Pereira [1]	bharbara.moura@unesp.br	Brazil
Rafaela Ricci [1]	rafaela.ricci@unesp.br	Brazil
Nathália Evelyn da Silva Machado [1]	nathalia.machado@unesp.br	Brazil
Ana Beatriz Carreto [1]	a.carreto@unesp.br	Brazil
Murilo Catelani Ferraz [2]	murilo.ferraz@unesp.br	Brazil
Marcos dos Santos Lima [3]	marcos.santos@ifsertao-pe.edu.br	Brazil

Marciane Magnani [3]	magnani2@gmail.com	Brazil
Luciano Tavares Ângelo Cintra [1]	luciano.cintra@unesp.br	Brazil
João Eduardo Gomes-Filho [1] Corresponding Author	joao.eduardo@unesp.br	Brazil

Affiliations

We will use the best match from our database to determine if your manuscript is eligible for special benefits. Matched organizations are for internal purposes and will not be published.

1. Department of Preventive and Restorative Dentistry. São Paulo State University (Unesp), School of Dentistry, Araçatuba
   Matched organization
   Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Odontologia
   ARAÇATUBA, Brazil
2. Department of Clinic, Surgery and Animal Reproduction, São Paulo State University (Unesp), Faculty of Veterinary Medicine, Araçatuba
   Matched organization
   Organization is not listed
3. Department of Food Technology, Federal University of Paraiba (UFPB), Petrolina
   Matched organization
   Organization is not listed

Additional Information

Is your data available?

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funders

National Council for Scientific and Technological Development [CNPq grants 302124/2022-5]

CAPES – Financial 88887.668341/2022-00

FAPESP 2022/05023-8

Keywords

Dealcoholized red wine; Animal model; Inflammation; Blood; Apical periodontitis

Manuscript sub-type

Laboratory study - Animal

Animal Subjects and Tissue Samples

Yes, animal subjects or samples were involved

Is this submission for a special issue?

No, this is not for a special issue

Has this manuscript been submitted previously to this journal?

No, it wasn't submitted previously

Informed Consent

No, there were no human subjects

Cover Letter / Comments

No, I don't have additional comments

History

Submitted On 9 November 2023 by Romulo Sales

Show this version history

Submission Started 9 November 2023 by Romulo Sales

ANEXO C - Guidelines for Authors on Submission to the *International Endodontic Journal*

Submission and Peer Review Process

Free Format submission

International Endodontic Journal now offers Free Format submission for a simplified and streamlined submission process. Before you submit, you will need:

Your manuscript: this should be an editable file including text, figures, and tables, or separate files – whichever you prefer. All required sections should be contained in your manuscript, including abstract (which does need to be correctly styled), introduction, methods, results, and conclusions. Figures and tables should have legends. Figures should be uploaded in the highest resolution possible. References may be submitted in any style or format, as long as it is consistent throughout the manuscript. Supporting information should be submitted in separate files. If the manuscript, figures or tables are difficult for you to read, they will also be difficult for the editors and reviewers, and the editorial office will send it back to you for revision. Your manuscript may also be sent back to you for revision if the quality of English language is poor.

An ORCID ID, freely available at https://orcid.org. (Why is this important? Your article, if accepted and published, will be attached to your ORCID profile. Institutions and funders are increasingly requiring authors to have ORCID IDs.)

The title page of the manuscript, including:

- Data availability statement
- Funding statement
- Conflict of interest disclosure
- Ethics approval statement and document
- Patient consent statement
- Relevant reporting guidelines paperwork (see 5.2 Reporting Guidelines below)
- Permission to reproduce material from other sources
- Clinical trial registration
- Your co-author details, including affiliation and email address. (Why is this important? We need to keep all co-authors informed of the outcome of the peer review process.)
- Statements relating to our ethics and integrity policies, which may include any of the following (Why are these important? We need to uphold rigorous ethical standards for the research we consider for publication):

Important: the journal operates a double-blind peer review policy. Please anonymise your manuscript and supply a separate title page file.

Open Access

This journal is a subscription journal that offers an open access option. You will have the option to choose to make your article open access after acceptance, which will be subject to an APC, unless a waiver applies.

Preprint policy:

This journal accepts articles previously published on preprint servers.

International Endodontic Journal will consider for review articles previously available as preprints. You may also post the submitted version of a manuscript to a preprint server at any time. You are requested to update any pre-publication versions with a link to the final published article.

This journal operates a double-blind peer review process. Authors are responsible for anonymizing their manuscript in order to remain anonymous to the reviewers throughout the peer review process (see "Main Text File" above for more details). Since the journal also encourages posting of preprints, however, please note that if authors share their manuscript in preprint form this may compromise their anonymity during peer review.

Data Sharing and Data Availability
This journal encourages data sharing. Review Wiley's Data Sharing policy where you will be able to see and select the data availability statement that is right for your submission.

Data Protection
If you are submitting a manuscript to the journal, or alternatively if you are reviewing for the International Endodontic Journal, your name, email address, and affiliation, and other contact details might be required for the regular operations of the publication. Please review Wiley's Data Protection Policy to learn more.

Funding
You should list all funding sources in the Acknowledgments section or in abstract of systematic review. You are responsible for the accuracy of their funder designation. If in doubt, please check the Open Funder Registry for the correct nomenclature.

Authorship
All listed authors should have contributed to the manuscript substantially and have agreed to the final submitted version. Review editorial standards and scroll down for a description of authorship criteria. An author contribution section must be completed for a submitted manuscript.

Conflict of Interest
The journal requires that all authors disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or directly related to the work that the authors describe in their manuscript. The existence of a conflict of interest does not preclude publication. If the authors have no conflict of interest to declare, they must also state this at submission. It is the responsibility of the corresponding author to review this policy with all authors and collectively to disclose with the submission ALL pertinent commercial and other relationships. Please fill out the below form if any authors have a conflict of interest.

Conflict of Interest Disclosure Form
ORCID
As part of the journal's commitment to supporting authors at every step of the publishing process, the International Endodontic Journal encourages authors to

provide an ORCID iD when submitting a manuscript. Please see Wiley's resources on ORCID here.

Reproduction of Copyright Material
If excerpts from copyrighted works owned by third parties are included, credit must be shown in the contribution. It is your responsibility to also obtain written permission for reproduction from the copyright owners. For more information visit Wiley's Copyright Terms & Conditions FAQ.

The corresponding author is responsible for obtaining written permission to reproduce the material "in print and other media" from the publisher of the original source, and for supplying Wiley with that permission upon submission.

Style and Formate
While this journal operates free format, this is the preferred style:

Double space the text;
Blank out the funding information at this stage of the review process;
Remove the keywords from the abstract they should be on the title page only;
Put a page break between the abstract and the introduction;
Remove the numbering of the sections in the main document;

Title Page
The title page should contain:

- A brief informative title containing the major key words. The title should not contain abbreviations (see Wiley's best practice SEO tips);
- A short running title of less than 30 characters;
- The full names of the authors;
- The author's institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
- Acknowledgements.

Important: the journal operates a double-blind peer review policy. Please anonymise your manuscript and prepare a separate title page containing author details.

Main Text File
Please ensure that all identifying information such as author names and affiliations, acknowledgements or explicit mentions of author institution in the text are on a separate page.

All manuscripts submitted to the International Endodontic Journal should include Title Page (separate file), Abstract, Main Text, References, Acknowledgements, Funding, Conflict of Interest, Tables, Figures, Checklists and Figure Legends as appropriate.

Manuscripts can be uploaded either as a single document (containing the main text, tables and figures), or with figures and tables provided as separate files. Should your manuscript reach revision stage, figures and tables must be provided as separate files. The main manuscript file can be submitted in Microsoft Word (.doc or .docx) or LaTex (.tex) format.

Reference Style

This journal uses Harvard reference style; as the journal offers Free Format submission, however, this is for information only and you do not need to format the references in your article. This will instead be taken care of by the typesetter.

It is the policy of the journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

Tools such as EndNote or Reference Manager for reference management and formatting can be useful. The EndNote reference style can be obtained upon request to the editorial office (iej.office@wiley.com). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp. You may also wish to review your reference style guidelines prior to submission.

Figures and Supporting Information

Figures, supporting information, and appendices should be supplied as separate files. You should review the basic figure requirements for manuscripts for peer review, as well as the more detailed post-acceptance figure requirements. View Wiley's FAQs on supporting information.

Peer Review

This journal operates under a double-blind peer review model. Except where otherwise stated, manuscripts are peer reviewed by at least two anonymous reviewers and an Associate Editor. Papers will only be sent to review if the Editor-in-Chief determines that the paper meets the appropriate quality and relevance requirements.

In-house submissions, i.e. papers authored by Editors or Editorial Board members of the title, will be sent to Editors unaffiliated with the author or institution and monitored carefully to ensure there is no peer review bias.

Wiley's policy on the confidentiality of the review process is available here.

Refer and Transfer Program

Wiley believes that no valuable research should go unshared. This journal participates in Wiley's Refer & Transfer program. If your manuscript is not accepted, you may receive a recommendation to transfer your manuscript to another suitable Wiley journal, either through a referral from the journal's editor or through our Transfer Desk Assistant.

Guidelines on Publishing and Research Ethics in Journal Articles

The journal requires that you include in the manuscript (and submit a hard copy) details of IRB approvals, ethical treatment of human and animal research participants, and gathering of informed consent, as appropriate. You will be expected to declare all conflicts of interest, or none, on submission. Please review Wiley's policies surrounding human studies, animal studies, clinical trial registration, biosecurity, and research reporting guidelines.

This journal follows the core practices of the Committee on Publication Ethics (COPE) and handles cases of research and publication misconduct accordingly (https://publicationethics.org/core-practices).

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study. The authors MUST upload a copy of the ethical approval letter when submitting their manuscript and a separate English translation.

Author Contributions

For all articles, the journal mandates the CRediT (Contribution Roles Taxonomy) more information is available on our Author Services site.

Reporting Guidelines

Animal studies

Animal studies should be written using the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13477).

When submitting manuscripts that have been written using the PRIASE 2021 guidelines, authors should include the following statement in the beginning of "Materials and Methods" section: ''The manuscript of this animal study has been written according to Preferred Reporting Items for Animal studies in Endodontology (PRIASE) 2021 guidelines."

A PRIASE 2021 checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRIASE 2021 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/priase/

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the PRIASE 2021 guidelines and their importance:

Nagendrababu V, Kishen A, Murray PE, Nekoofar MH, de Figueiredo JA, Priya E, Jayaraman J, Pulikkotil SJ, Camilleri J, RM S, Dummer PMH (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: a consensus-based development. International Endodontic Journal 54, 848-57. (https://onlinelibrary.wiley.com/doi/10.1111/iej.13477)

Nagendrababu V, Kishen A, Murray PE, Nekoofar MH, de Figueiredo JA, Priya E, Jayaraman J, Pulikkotil SJ, Jakovljevic A, Dummer PMH (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: Explanation and Elaboration. International Endodontic Journal 54, 858-86. (https://onlinelibrary.wiley.com/doi/10.1111/iej.13481)

ANEXO D - Guidelines for Authors on Submission to the *Archives of Oral Biology*

Preparation

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

Peer review

This journal operates a single anonymized review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. Editors are not involved in decisions about papers which they have written themselves or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Any such submission is subject to all of the journal's usual procedures, with peer review handled independently of the relevant editor and their research groups. More information on types of peer review.

Revised submissions

When submitting the revised manuscript, please make sure that you upload the final version of the paper with the changes highlighted. Please remove the old version(s) of the manuscript before submitting the revised version.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

To minimize unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Manuscript Structure

Follow this order when typing manuscripts: Title, Authors, Affiliations, Abstract, Keywords, Main text (Introduction, Materials & Methods, Results, Discussion for an original paper), Acknowledgments, Appendix, References, Tables and then Figure

Captions. Do not import the Figures or Tables into your text. The corresponding author should be identified with an asterisk and footnote. All other footnotes (except for table footnotes) should be identified with superscript Arabic numbers.

Introduction

This should be a succinct statement of the problem investigated within the context of a brief review of the relevant literature. Literature directly relevant to any inferences or argument presented in the Discussion should in general be reserved for that section. The introduction may conclude with the reason for doing the work but should not state what was done nor the findings.

Materials and Methods

Enough detail must be given here so that another worker can repeat the procedures exactly. Where the materials and methods were exactly as in a previous paper, it is not necessary to repeat all the details but sufficient information must be given for the reader to comprehend what was done without having to consult the earlier work.

Authors are requested to make plain that the conditions of animal and human experimentation are as outlined in the "Ethics" and "Studies on Animals" sections above

Results or Findings

These should be given clearly and concisely. Care should be taken to avoid drawing inferences that belong to the Discussion. Data may be presented in various forms such as histograms or tables but, in view of pressure on space, presentation of the same data in more than one form is unacceptable.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is occasionally appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion section.

Essential title page information

• Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

• Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.

• Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

As titles frequently stand alone in indexes, bibliographic journals etc., and indexing of papers is, to an increasing extent, becoming computerized from key words in the titles, it is important that titles should be as concise and informative as possible. Thus the animal species to which the observations refer should always be given and it is desirable to indicate the type of method on which the observations are based, e.g. chemical, bacteriological, electron-microscopic, histochemical, etc. A "running title" of not more than 40 letters and spaces must also be supplied. A keyword index must be supplied for each paper.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Structured abstract

The paper should be prefaced by an abstract aimed at giving the entire paper in miniature. Abstracts should be no longer than 250 words and should be structured as per the guidelines published in the Journal of the American Medical Association (JAMA 1995; 273: 27-34). In brief, the abstract should be divided into the following sections: (1) Objective; (2) Design - if clinical, to include setting, selection of patients, details on the intervention, outcome measures, etc.; if laboratory research, to include details on methods; (3) Results; (4) Conclusions.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

As Archives of Oral Biology is a journal with a multidisciplinary readership, abbreviations, except those universally understood such as mm, g, min. u.v., w/v and those listed below, should be avoided if possible. Examples of abbreviations which may be used without definition are: ADP, AMP, ATP, DNA, RNA, EDTA, EMG, tris.

Other abbreviations used to improve legibility should be listed as a footnote on the title page as well as being defined in both the abstract and the main text on first usage. Chemical symbols may be used for elements, groups and simple compounds, but excessive use should be avoided. Abbreviations other than the above should not be used in titles and even these should be avoided if possible.

Statistical analysis

Authors should ensure that the presentation and statistical testing of data are appropriate and should seek the advice of a statistician if necessary. A number of common errors should be avoided, e.g.: -

• Use of parametric tests when non-parametric tests are required

• Inconsistencies between summary statistics and statistical tests such as giving means and standard deviations for data which were analysed with non-parametric tests.

• Multiple comparisons undertaken with multiple t tests or non-parametric equivalents rather than with analysis of variance (ANOVA) or non-parametric equivalents.

• Post hoc tests being used following an ANOVA which has yielded a non-significant result.

• Incomplete names for tests (e.g. stating "Student's t test" without qualifying it by stating "single sample", "paired" or "independent sample")

• n values being given in a way which obscures how many independent samples there were (e.g. stating simply n=50 when 10 samples/measurements were obtained from each of 5 animals/human subjects).

• Stating that P=0.000 (a figure which is generated by some computer packages). The correct statement (in this case) is P<0.0005.

• Bar charts should only be used to show frequencies (absolute or relative/%); for means and SD, use dot plots and/or error bars.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.) but who did not meet all the criteria for authorship (see Authorship section above).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Bacterial nomenclature

Organisms should be referred to by their scientific names according to the binomial system. When first mentioned the name should be spelt in full and in italics. Afterwards the genus should be abbreviated to its initial letter, e.g. 'S. aureus' not 'Staph. aureus'. If abbreviation is likely to cause confusion or render the intended meaning unclear, the names of microbes should be spelt in full. Only those names which were included in the Approved List of Bacterial Names, Int J Syst Bacteriol 1980; 30: 225-420 and those which have been validly published in the Int J Syst Bacteriol since 1 January 1980 have standing in nomenclature. If there is good reason to use a name that does not have standing in nomenclature, the names should be enclosed in quotation marks and an appropriate statement concerning the nomenclatural status of the name should be made in the text (for an example see Int J Syst Bacteriol 1980; 30: 547-556). When the genus alone is used as a noun or adjective, use lower case Roman not italic, e.g.'organisms were staphylococci' and 'streptococcal infection'. If the genus is specifically referred to use italics e.g. 'organisms of the genus Staphylococcus'. For genus in plural, use lower case roman e.g. 'salmonellae'; plurals may be anglicized e.g.'salmonellas'. For trivial names, use lower case Roman e.g. 'meningococcus'

Artwork

Image manipulation

Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.

• Embed the used fonts if the application provides that option.

• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.

• Number the illustrations according to their sequence in the text.

• Use a logical naming convention for your artwork files.

• Provide captions to illustrations separately.

• Size the illustrations close to the desired dimensions of the published version.

• Submit each illustration as a separate file.

• Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.

Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings, embed all used fonts.

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.

TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.

TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;

• Supply files that are too low in resolution;

• Submit graphics that are disproportionately large for the content.

Tables

Please submit tables as editable text and not as images. Tables should be placed on separate page(s) towards the end of the manuscript (see Manuscript Structure, above). Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

Data references

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Preprint references

Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

Reference management software

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Reference style

Text: Citations in the text should follow the referencing style used by the American Psychological Association. You are referred to the Publication Manual of the American Psychological Association, Seventh Edition, ISBN 978-1-4338-3215-4, copies of which may be ordered online.

List: references should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:

Van der Geer, J., Hanraads, J. A. J., & Lupton, R. A. (2010). The art of writing a scientific article. Journal of Scientific Communications, 163, 51–59. https://doi.org/10.1016/j.sc.2010.00372.

Reference to a journal publication with an article number:

Van der Geer, J., Hanraads, J. A. J., & Lupton, R. A. (2018). The art of writing a scientific article. Heliyon, 19, Article e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:

Strunk, W., Jr., & White, E. B. (2000). The elements of style (4th ed.). Longman (Chapter 4).

Reference to a chapter in an edited book:

Mettam, G. R., & Adams, L. B. (2009). How to prepare an electronic version of your article. In B. S. Jones, & R. Z. Smith (Eds.), Introduction to the electronic age (pp. 281–304). E-Publishing Inc.

Reference to a website:

Powertech Systems. (2015). Lithium-ion vs lead-acid cost analysis. Retrieved from http://www.powertechsystems.eu/home/tech-corner/lithium-ion-vs-lead-acid-cost-analysis/. Accessed January 6, 2016

Reference to a dataset:

[dataset] Oguro, M., Imahiro, S., Saito, S., & Nakashizuka, T. (2015). Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Reference to a conference paper or poster presentation:

Engle, E.K., Cash, T.F., & Jarry, J.L. (2009, November). The Body Image Behaviours Inventory-3: Development and validation of the Body Image Compulsive Actions and Body Image Avoidance Scales. Poster session presentation at the meeting of the Association for Behavioural and Cognitive Therapies, New York, NY.

Reference to software:

Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S. (2020, March 25). Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/zenodo.3727209.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article.

When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page. For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data

This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to Mendeley Data. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.

Research Elements

This journal enables you to publish research objects related to your original research – such as data, methods, protocols, software and hardware – as an additional paper in a Research Elements journal.

Research Elements is a suite of peer-reviewed, open access journals which make your research objects findable, accessible and reusable. Articles place research objects into context by providing detailed descriptions of objects and their application, and linking to the associated original research articles. Research Elements articles can be prepared by you, or by one of your collaborators.

During submission, you will be alerted to the opportunity to prepare and submit a manuscript to one of the Research Elements journals.

More information can be found on the Research Elements page.

Data statement

To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.