UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA

VANESSA DOS SANTOS BRUM

EFEITO DO CONSUMO DE ÁLCOOL 12%, VINHO TINTO, SUCO DE UVA E RESVERATROL SOBRE OS NÍVEIS DE PROTEÍNA C-REATIVA EM RATOS COM PERIODONTITE

Porto Alegre
2016

VANESSA DOS SANTOS BRUM

EFEITO DO CONSUMO DE ÁLCOOL 12%, VINHO TINTO, SUCO DE UVA E RESVERATROL SOBRE OS NÍVEIS DE PROTEÍNA C-REATIVA EM RATOS COM PERIODONTITE

Trabalho de Conclusão de Curso apresentado ao Curso de Graduação em Odontologia pela Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Cirurgião-Dentista.

Orientador: Prof. Dr. Juliano Cavagni

Porto Alegre
2016

CIP - Catalogação na Publicação

Brum, Vanessa dos Santos
EFEITO DO CONSUMO DE ÁLCOOL 12%, VINHO TINTO, SUCO DE UVA E RESVERATROL SOBRE OS NÍVEIS DE PROTEÍNA C-REATIVA EM RATOS COM PERIODONTITE / Vanessa dos Santos Brum. -- 2016.
31 f.

Orientador: Juliano Cavagni.

Trabalho de conclusão de curso (Graduação) -- Universidade Federal do Rio Grande do Sul, Faculdade de Odontologia, Curso de Odontologia, Porto Alegre, BR-RS, 2016.

1. Vinho tinto. 2. Proteína C-Reativa. 3. Perda óssea alveolar. 4. Álcool. 5. Resveratrol. I. Cavagni, Juliano, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

Aos meus pais, Darci e Rose, pelo amor, carinho e apoio incondicional. Obrigada por tudo que vocês fazem por mim, por sempre me mostrarem o melhor caminho e por acreditarem nos meus sonhos. Nada disso seria possível sem vocês. Vocês são meus maiores exemplos.

Aos meus irmãos, Felipe e Régis, meus melhores amigos para a vida toda. Obrigada pelo incentivo e por sempre estarem ao meu lado, comemorando cada etapa. Vocês são essenciais.

Ao Erick, pelo companheirismo e paciência ao longo de todos esses anos de faculdade. Por sempre me apoiar e me tranquilizar quando tudo parecia complicado. Tua presença me torna mais feliz.

Ao meu orientador, Juliano Cavagni, por toda dedicação e empenho nesse trabalho e durante toda minha iniciação científica. Tu és um exemplo de pessoa e profissional, o qual tive a sorte de ter cruzado ainda na metade do curso. Obrigada por todos os ensinamentos e por ter feito a diferença na minha graduação.

Ao Marcius e ao Luciano, pela parceria e disponibilidade em todas as etapas do trabalho. Obrigada por me permitirem fazer parte dessa equipe tão especial.

Aos professores Eduardo Gaio e Cassiano Rösing, por todo o apoio.

RESUMO

BRUM, Vanessa dos Santos. **Efeito do consumo de álcool 12%, vinho tinto, suco de uva e resveratrol sobre os níveis de proteína c-reativa em ratos com periodontite.** 2016. 31 f. Trabalho de Conclusão de Curso (Graduação em Odontologia) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2016.

O objetivo deste estudo foi avaliar o efeito do consumo de álcool, vinho tinto, suco de uva e resveratrol sobre a Proteína C-reativa (PCR) em ratos Wistar com doença periodontal induzida. Quanto aos materiais e métodos, 50 ratos Wistar machos (45 dias de vida) foram alocados de forma randomizada em 5 grupos de 10 animais, após estratificação por peso: água (grupo controle), vinho tinto, suco de uva, solução alcoólica a 12% e resveratrol. Todos os animais receberam ração padrão e líquidos de acordo com o grupo experimental. Após um período de 8 semanas de adaptação ao regime oferecido, foi feita a indução da doença periodontal no 2° molar superior direito, sendo o molar contralateral considerado controle intragrupo. Após 2 semanas, os animais foram mortos por meio de decapitação, foi feita a remoção das maxilas para análise morfométrica e do fígado para mensuração dos níveis hepáticos de PCR. A PCR foi mensurada por reação em cadeia da polimerase em tempo real. Os dados foram analisados por meio de ANOVA seguida por Bonferroni e o nível de significância foi de 95%. Entre os resultados, todos os animais completaram o estudo e ganharam peso ao longo do período experimental. Não houve diferença significativa no peso corporal entre os grupos em nenhum momento do estudo. Quanto ao consumo de comida e bebida, diferenças significativas foram encontradas entre o grupo controle e os demais grupos ($p<0,01$). Considerando a perda óssea alveolar, o lado com ligadura exibiu significativamente maior perda óssea comparado ao lado sem ligadura. Não foram observadas diferenças estatisticamente significativas na perda óssea alveolar entre os grupos experimentais. Em relação aos níveis de PCR (mmol/µL), os maiores níveis foram observados em animais que consumiram solução alcoólica 12% (0,53±0,05) comparados ao grupo controle (0,45±0,03) ($p\leq0,05$). Animais que consumiram vinho tinto, suco de uva e resveratrol apresentaram diminuição significativa nos níveis de PCR comparados ao grupo controle, sendo que os animais que consumiram vinho tinto apresentaram os menores níveis de PCR (0,29±0,04) entre todos os grupos experimentais ($p\leq0,05$). Conclui-se, então, que o consumo de vinho tinto diminui os níveis de proteína C-reativa em ratos Wistar com doença periodontal.

Palavras-chave: Resveratrol. Perda óssea alveolar. Ratos. Periodontite. Álcool. Vinho tinto. Proteína C-Reativa.

ABSTRACT

BRUM, Vanessa dos Santos. **Effect of 12% alcohol, red wine, grape juice and resveratrol exposure on C-reactive protein in rats with periodontitis.** 2016. 31 p. Final Paper (Graduation in Dentistry) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2016.

The objective was to evaluate the effect of alcohol consumption, red wine, grape juice and resveratrol on C-reactive protein (CRP) in Wistar rats with induced periodontal disease. In relation to material and methods, fifty male Wistar rats (45 days old) were randomly assigned to 5 groups of 10 animals stratified by weight: Water (control group), Red Wine, Grape Juice, 12% Alcohol Solution and Resveratrol. All the animals were fed with laboratory rat chow and solutions according to each experimental group. After an 8 week adaptation period to the offered regimen, periodontal disease was induced on the maxillary right second molars, the left second molars were considered intragroup control. After 2 weeks, animals were killed by decapitation, specimens prepared for morphometric analysis and the liver was used to measure CRP by real time PCR. Data were analyzed by ANOVA followed by Bonferroni and the significance level was 95%. All animals completed the experiment and presented weight gain throughout the experimental period. No statistically significant differences were found in body weight between groups at any time point. To food and liquid intake, statistically significant differences were found between control and other groups ($p<0.01$). Considering alveolar bone loss (ABL), sides with ligature exhibited significant higher alveolar bone loss when compared to sides without ligature. No statistically significant differences on ABL were found between experimental groups. In relation to CRP levels (mmol/µL), higher levels were found in animals that consumed 12% alcohol solution (0.53±0.05) compared to control group (0.45±0.03) ($p\leq0.05$). Animals that consumed red wine, grape juice and resveratrol exhibited significant decrease in CRP levels compared to control group, and the animals that consumed red wine had the lowest CRP levels (0.29±0.04) in all experimental groups ($p\leq0.05$). The conclusion of this study is that red wine consumption decreases C-reactive protein levels in Wistar rats with periodontal disease.

Keywords: Resveratrol. Alveolar bone loss. Rats. Periodontitis. Alcohol. Red wine. C-Reactive Protein.

SUMÁRIO

1 INTRODUÇÃO

De acordo com o II Levantamento Nacional de Álcool e Drogas (LARANJEIRA et al., 2013), que analisou dados de 2006 e 2012, o número de brasileiros que ingerem bebida alcoólica com frequência (uma vez por semana ou mais) aumentou 20%. Além disso, dados de 2005 mostram que o consumo per capita mundial anual é de 6,13 litros de álcool puro, considerando pessoas com mais de 15 anos que consomem algum tipo de bebida alcoólica, e que no Brasil esse valor é ainda maior: 9,12 litros de álcool puro (OMS, 2013). Esse é um dado bastante preocupante, uma vez que o consumo excessivo de álcool é responsável por 2,5 milhões de mortes no mundo, além de ser o terceiro maior fator de risco mundial para doenças e condições incapacitantes (OMS, 2011).

Sabe-se que o consumo elevado de álcool causar complicações no fígado, problemas cardiovasculares e problemas comportamentais (MCCLAIN et al., 1999; MEYERHOFF et al., 2005; MUKAMAL et al., 2006). Entretanto, o consumo moderado de álcool tem sido relacionado a menores índices de marcadores inflamatórios no organismo, como diminuição nos níveis de proteína C-reativa, interleucina-6 e fator de necrose tumoral alfa, o que está relacionado também a um menor risco para doenças coronarianas (PAI et al., 2006; MARQUES-VIDAL et al., 2012).

A literatura sugere um efeito protetor através da diminuição na incidência de infarto do miocárdio em consumidores de álcool (MUKAMAL et al., 2001). Em concordância, um consumo leve a moderado tem sido associado a uma diminuição na mortalidade por doença cardiovascular (REHM; BONDY, 1998). Entretanto, segundo Doll (1994) e Gaziano (2000), os mecanismos da associação entre mortalidade cardiovascular e consumo de álcool ainda são incertos. As prováveis explicações para o efeito do álcool em eventos vasculares têm focado primeiramente em lipoproteínas e fatores sanguíneos (LANGER; CRIQUI; REED, 1992; GAZIANO et al., 1993). Porém, o consumo de álcool mantém uma associação significativa com risco cardiovascular mesmo após o controle de muitos desses fatores de risco. Além disso, o álcool promove uma modificação complexa dos parâmetros sanguíneos, sugerindo que pode influenciar o risco cardiovascular através de vias alternativas (MUKAMAL; JADHAV; D'AGOSTINO, 2001).

Seguindo essa linha, um estudo realizado nos Estados Unidos mostrou que o consumo de álcool está associado com uma diminuição da probabilidade do indivíduo apresentar altos níveis de proteína C-reativa (PCR). A PCR se trata de um marcador inflamatório, que aumenta drasticamente em resposta à infecção, inflamação e lesão. Dessa forma, estudos mostram que níveis elevados de PCR são um fator de risco para infarto do miocárdio e doença vascular periférica (LAGRAND et al., 1999).

Essa associação suporta um mecanismo anti-inflamatório onde o consumo moderado de álcool pode ser um fator de proteção para morte por doença cardiovascular (STEWART; MAINOUS; GILBERT, 2002). Em outro estudo, o consumo moderado de álcool também foi associado com baixas concentrações de PCR quando comparadas a nenhuma ingestão ou ingestão eventual de álcool. Esse efeito foi independente dos efeitos relacionados com o álcool sobre os lipídios. O álcool poderia atenuar a mortalidade cardiovascular, em parte, através de um mecanismo anti-inflamatório (ALBERT; GLYNN; RIDKER, 2003).

Quanto às doenças periodontais, não está clara a relação entre o consumo de álcool e o estabelecimento ou progressão da doença periodontal. Estudos mostram que o álcool pode modificar o processo saúde-doença periodontal pela interferência com a resposta do hospedeiro (MCCLAIN et al., 1999; SZABO, 1999). A literatura mostra que o um consumo moderado de vinho tinto pode ser considerado um fator de proteção para as doenças periodontais (BOUCHARD et al., 2006; SUSIN et al., 2015) e que, o consumo de vinho tinto pode estar associado inversamente à perda de inserção periodontal quando comparados a outros tipos de bebidas alcoólicas (KONGSTAD, 2008). Entretanto, alguns estudos em humanos mostram que, independente da quantidade consumida, há uma relação positiva entre o consumo de álcool e progressão de doença periodontal (TEZAL et al. 2001, 2004; PITIPHAT et al., 2003; NISHIDA et al., 2004; SHIMAZAKI et al., 2005; AMARAL; RONIR; LEÃO, 2008; AMARAL, VETTORE; LEÃO, 2009).

Esse comportamento é explicado pelo fenômeno conhecido na bioestatística como curva “U” ou “J”, ou seja, até certo ponto, o álcool se comporta como um fator de proteção e, a partir deste ponto, passa a ser um fator de risco para doenças periodontais, sendo difícil determinar esse ponto de corte (DIAZ et al., 2002). A dificuldade na determinação do ponto de corte da curva “J” também pode estar relacionado a fatores como gênero (masculino e feminino) e biodisponibilidade do

álcool no organismo, fatores estes que podem aumentar ou diminuir a susceptibilidade individual. Um levantamento epidemiológico realizado em Porto Alegre mostra que, para homens, não há relação entre consumo de álcool e progressão de doença periodontal até um consumo de aproximadamente 4g de álcool/dia (equivalente a 100ml de cerveja contendo 5% de álcool consumido diariamente). A partir deste ponto, existe uma relação dose-dependente entre consumo de álcool e progressão de doença periodontal (WAGNER, 2008).

Em animais, um estudo de Souza (2009), mostrou que em ratos expostos a um alto consumo de álcool (concentração igual ou maior do que 20%) havia associação entre consumo de álcool e doença. Por outro lado, em um estudo de Liberman et al. (2011), o consumo de álcool em baixa concentração (menor ou igual a 5%) está inversamente relacionado à perda óssea espontânea, se mostrando como um fator de proteção.

Adicionalmente, a respeito das doenças periodontais, estudos recentes mostram uma correlação entre doença periodontal e proteína C-reativa. A PCR é regulada por citocinas e reflete uma medida da fase aguda de resposta à inflamação, tendo associação com tabagismo, obesidade, triglicerídeos, diabetes e doença periodontal (GOMES-FILHO et al., 2011).

Sabe-se que os níveis de proteína C-reativa aumentam, subsequentemente, com a gravidade da doença periodontal (GOYAL et al., 2014). Os menores níveis de PCR foram encontrados em pacientes com recessões gengivais, aumentando em pacientes com gengivite e pacientes com periodontite crônica, com os mais altos níveis encontrados em pacientes com periodontite agressiva. O índice de sangramento à sondagem mostrou melhor correlação com os níveis de PCR em comparação com profundidade de sondagem, em ambos grupos de pacientes com periodontite, especialmente em pacientes com periodontite agressiva (PODZIMEK et al., 2015). Similarmente, pacientes portadores de periodontite agressiva tem aumento estatisticamente significativo nos níveis séricos de PCR comparados com indivíduos sem periodontite (SALZBERG et al., 2006).

Segundo estudos, pacientes com periodontite crônica apresentam altos níveis de PCR. Além disso, estudos mostram que o tratamento não-cirúrgico periodontal tem efeito positivo em relação à redução dos níveis séricos de proteína C-reativa (DE SOUZA et al., 2016; FREITAS; GOMES-FILHO; NAVES, 2012). Uma revisão sistemática de Paraskevas (2008) está em concordância, mostrando que há

fortes evidências em estudos transversais de que a proteína C-reativa é elevada na periodontite, em comparação aos indivíduos referência, mas, por outro lado, mostra que as evidências são modestas acerca do efeito de terapia periodontal na redução dos níveis da proteína C-reativa.

Uma meta análise com o objetivo de examinar o efeito do tratamento periodontal sobre os níveis de PCR sistêmicos e avaliar a qualidade da evidência disponível, concluiu que existe um grande número de evidências que indicam uma associação entre doença periodontal e inflamação sistêmica. No entanto, informações de ensaios clínicos randomizados e estudos de coorte não suportam a hipótese de que o tratamento periodontal pode reduzir os níveis de PCR sistêmicos (IOANNIDOU, 2006). Neste sentido, parece existir outros fatores que são capazes de interferir sobre os níveis séricos de PCR além do tratamento periodontal, à medida que este se expressa de maneira inespecífica. Como apontado anteriormente, o consumo de álcool poderia ser um fator que modifica a resposta inflamatória sistêmica e poderia ter algum impacto sobre o processo etiopatogênico da doença periodontal e, consequentemente, sobre os níveis séricos de PCR. Além disso, o tipo de bebida alcoólica parece influenciar a resposta do hospedeiro (KONGSTAD, 2008; SUSIN et al., 2015).

Dentre as bebidas que contém álcool, o vinho tinto tem se destacado por seus possíveis efeitos benéficos para a saúde (SALEEM; BASHA, 2010). O consumo moderado de vinho tinto está sendo relacionado como um possível fator de proteção para doenças crônicas degenerativas, incluindo as doenças periodontais (KONGSTAD et al., 2008; LEIFERT; ABEYWARDENA, 2008; MAGRONE; JIRILLO, 2010; NATELLA et al., 2011; SUSIN et al., 2015). Acredita-se que esse efeito esteja relacionado às substâncias encontradas no vinho, o ácido fenólico e os polifenóis, como os flavonoides presentes em frutas, vegetais nozes e sementes (HERTOG; KROMHOUT; ARAVANIS, 1995; COIMBRA et al., 2005). Os polifenóis apresentam um amplo espectro antimicrobiano e capacidade de suprimir alguns fatores de virulência microbiana (inibição do biofilme bacteriano, redução da adesão ao hospedeiro, e neutralização de toxinas bacterianas) além de mostrar sinergismo com antibióticos (DAGLIA, 2012). O potencial protetor dos polifenóis está atribuído às suas propriedades antioxidantes, anti-radicais livres e quelação de alguns metais, além de inibir ou reduzir a ação de algumas enzimas como a telomerase (NAASANI

et al., 2003), ciclooxigenase (O'LEARY et al., 2004; HUSSAIN et al., 2005) e lipooxogenase (SCHEWE et al., 2001; SADIK; SIES; SCHEWE, 2003).

O resveratrol é um composto naturalmente produzido pelas plantas em resposta a infecções, principalmente de fungos, a estresse ambiental como clima, ozônio, luz UV, entre outras situações (HARIKUMAR; AGGARWAL, 2008). Trata-se de um polifenol encontrado no vinho tinto, no suco de uva, em grãos como o amendoim e em raízes de algumas plantas e tem recebido maior destaque devido aos seus benefícios para a saúde. A literatura mostra ação do resveratrol como antioxidante, anti-inflamatório, antiviral, cardioprotetor (LIN et al., 2008), neuroprotetor, quimiopreventivo, proteção contra infecções e isquemia, redução da obesidade e doenças metabólicas associadas ao envelhecimento (BAUR et al., 2006). A ingestão de suco de uva rico em flavonoides diminui o estresse oxidativo associado às doenças cardiovasculares e inflamatórias (DAVALOS et al., 2009).

Quanto à relação entre a administração de resveratrol e as doenças periodontais, a literatura tem mostrado esta substância como fator de proteção tanto em nível antibacteriano como na resposta tecidual produzida pelo organismo. Estudos *in vitro* mostram que o resveratrol age contra a formação de colônias bacterianas de *Aggregatibacter actinomycetemcomitans* (Aa) e de *Porphyromonas gingivalis* (Pg), diminuindo a quantidade de colônias viáveis após 1 hora, resultando em nenhuma unidade formadora de colônias após 24 horas (O'CONNOR; WONG; RABIE, 2011). Estudos em ratos mostram que o resveratrol foi responsável por menor destruição periodontal e níveis de Interleucina 17 (IL-17) quando comparados a um grupo controle que recebeu placebo (CASATI et al., 2013).

Estudos em humanos mostram que, em relação à resposta tecidual induzida pelo *Porphyromonas gingivalis,* o resveratrol diminuiu a expressão de óxido nítrico e a produção de citocinas pró-inflamatórias como Interleucina 1β, 6, 8 e 12 e Fator de Necrose Tumoral-α (TNF-α) (RIZZO et al., 2012), além de ser responsável pela ativação da Sirtuína 1, substância que interfere na diferenciação de células do ligamento periodontal em células tipo osteoblastos, cuja implicação clínica pode auxiliar na regeneração do osso alveolar (LEE et al., 2011).

Considerando que tanto consumo de vinho tinto e seus componentes, quanto doenças periodontais tem interfaces com os níveis de PCR, o presente estudo se justifica à medida que avalia de maneira isolada os possíveis efeitos de

cada um dos componentes do vinho tinto, bem associados à presença de doença periodontal.

2 ARTIGO

Effect of 12% alcohol red wine, grape juice and resveratrol exposure on C-reactive protein in rats with periodontitis

INTRODUCTION

Alcohol consumption represents one of the major health problems, resulting in approximately 2.5 millions of deaths worldwide (1). Studies demonstrate that heavy alcohol consumption (≥40g/day for men and ≥20g/day for women) (2) can lead to liver complications, cardiovascular diseases and behavioral handicap (3-5). On the other hand, moderate consumption of red wine is known to have a protective effect in chronic diseases such cardiovascular, ischemic, circulatory, blindness and periodontitis (6-11). In addition, moderate alcohol consumption seems to decrease the levels of C-reactive protein (12). The anti-inflammatory potential might be either related to the presence of alcohol or to other substances in the composition of the wine. Resveratrol is one of the supposed beneficial substances, acting like an important antioxidant, anti-inflammatory, cardioprotector (12, 13) and has a role in weight loss and metabolic diseases associated to the aging proces (14).

Periodontitis is an infecto-inflammatory disease that affects the supporting dental tissues and is primarily caused by bacterial biofilm (15). Moreover, there are risk factors that can alter this relation, increasing the probability of an individual to present periodontal disease, such as smoking (16), diabetes, obesity (17, 18) and, more recently, alcohol consumption (6,19-20). However, the relationship between alcohol consumption and the progression of periodontitis is unclear. Studies demonstrate that alcohol may modify periodontal disease by interfering with the host response (5,19). Epidemiological evidence shows that moderate alcohol consumption can be considered a protective factor for periodontal disease when compared to heavy consumers and non-consumers (20) and that red wine consumption may be inversely associated with periodontal attachment loss when compared to other types of alcoholic beverages (6,21). On the other hand, a study shows that 20% alcohol consumption may increase alveolar bone loss in rats (22).

C-reactive protein (CRP) is a plasma protein that reflects a measure of acute phase response in inflammation and high levels are associated with smoking, obesity, triglycerides, diabetes and periodontal disease (23, 24). Studies

demonstrate an increase in CRP levels in periodontal disease (25,26,27) also related to disease severity, whereas lower levels is found in patients with gingival health and higher in patients with aggressive periodontitis (28). In relation to alcohol, a study demonstrated that moderate consumption is associated with lower CRP levels when compared to occasional or no alcohol intake, and this effect is independent of alcohol-related effects on lipids. In this way, alcohol may attenuate cardiovascular mortality in part through an anti-inflammatory mechanism (29).

The aim of this study was to evaluate the influence of red wine, 12% alcohol, grape juice and resveratrol on CRP levels in Wistar rats with ligature induced periodontal disease.

METHODS

Study design

This is a prospective, randomized, controlled, and blinded animal model study.

Ethical considerations

The present study was approved by Institutional Review Board both of the Universidade Federal do Rio Grande do Sul (number 26632) and Hospital de Clínicas de Porto Alegre (project number 14/0335). This study was funded by the Hospital de Clínicas de Porto Alegre. This study was supported by the Coordination for Enhancement of Higher Education Personnel, Brazil (CAPES – Grant PROCAD NF – 2008) and Incentive Fund for Research and Events of the Hospital de Clínicas de Porto Alegre, Brazil (FIPE).

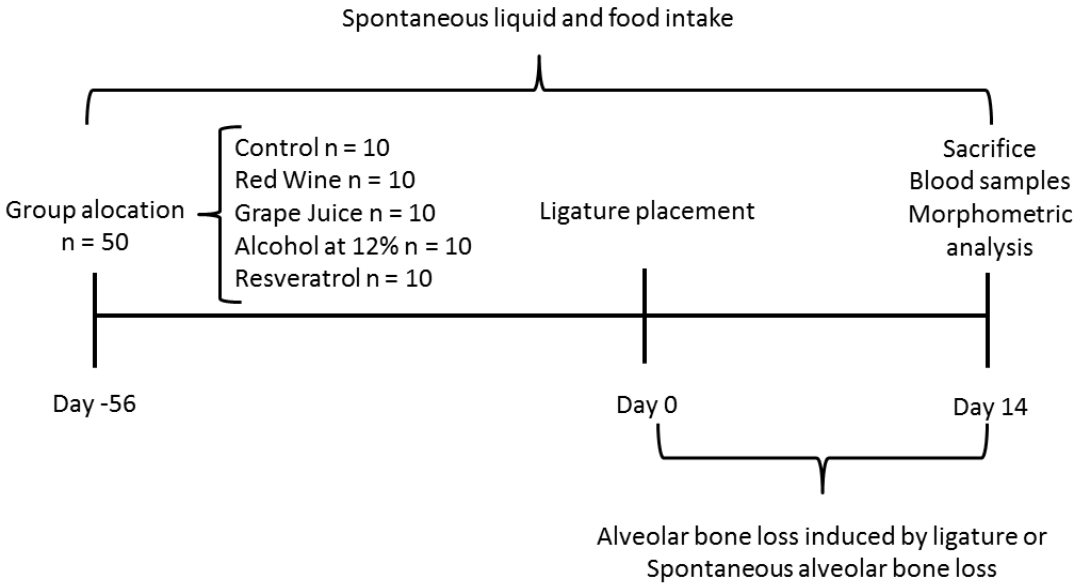
Sample size calculation

To calculate the sample size, a previous study (22) was used where a difference of 0.7mm in alveolar bone loss was found between groups submitted to 10% and 20% alcohol. Assuming alpha and beta errors of 0.05 and 0.10 respectively, 8 animals per group were considered necessary. Considering the possibility of an attrition rate of 20%, 10 animals per group were used.

Experimental procedures

Fifty male Wistar rats, 45 days old with a mean body weight of 308.75g (±25.26g) were randomly assigned to 5 groups of 10 animals stratified by weight, by means of draw: Control (tap water), Red Wine (Almaden® Cabernet Sauvignon, Bento Gonçalves, Brazil), Grape Juice (Sunny Days®, Bento Gonçalves, Brazil), 12% Alcohol (same concentration of red wine, Vetec®, Rio de Janeiro, Brazil) and 0.04-0.05mg/L Resveratrol (the same concentration found in the red wine, Equilibrium Pharmacy®, Porto Alegre, Brazil). All animals were fed with laboratory rat chow and solutions according to each experimental group. Solutions and food intake were spontaneous and *ad libitum*. Animals were housed in boxes of 5 animals each in a controlled environment (temperature 22°C ± 2°C and dark/light cycle of 12 hours) during the whole experimental period. Food and liquid intake were measured daily and body weight measured once a week. Figure 1 shows the study flowchart.

Figure 1 - Study flowchart



Spontaneous and ligature-induced alveolar bone loss

General anesthesia was obtained by inhalation of Isoflurane in all animals. Alveolar bone loss was induced by placement of 4-0 cotton ligatures (Ethicon®, Johnson & Johnson®, São Paulo, Brazil) around the maxillary right second molars. The contra-lateral tooth remained as intra-group control (30-33) and used for the

analysis of spontaneous periodontitis. After 14 days of ligature placement, animals were sacrificed, blood samples collected and specimens prepared for morphometric analysis.

Morphometric analysis

After sacrifice, the right and the left segments of the maxillae were immersed in sodium hypochlorite with 9% active chlorine (Mazzarollo®, Gravataí, Brazil) for 2 hours. Then, the specimens were washed, dried and stained with 1% methylene blue (Quinta Essência, Porto Alegre, Brazil) for better viewing of cement-enamel junction (29-31).

Standardized digital pictures were taken from the buccal and palatal aspects of each specimen using a millimetric ruler and a Nikon D5100® camera coupled with medical lenses and minimal focal distance. Each specimen was placed with the occlusal surface parallel to the floor. Linear measurements were performed with Adobe Photoshop CS6® (Adobe Systems Software Ireland Ltd). These measurements resulted in a pixel value, which was converted into millimeters. Periodontal bone loss was defined as the distance between the cement-enamel junction (CEJ) and the alveolar bone crest. Buccal and palatal measurements were made at five points and a mean of these values was considered as the bone loss.

The examiner was unaware of the group distribution as well as of the ligature presence or absence. A trained and calibrated examiner performed all the measurements. Prior to measurements, calibration was performed by double measurement of randomly chosen pictures (n=20) with one-week interval. The intra-class correlation coefficient (ICC) between measurements was 0.99. During the experiment a new calibration was performed with 10% of the sample and the ICC between measurements was also 0.99.

C-Reactive protein

C-reactive protein was measured in the liver because is where it is synthesized (34). In addition, a serological analysis was previously performed and the marker was not detected, probably by the method used.

RNA extraction

Tissue samples were fragmented and mixed with Trizol reagent (Invitrogen) using a tissue homogenizer (MA102, Marconi). The concentration and quality of RNA was estimated by reading on a spectrophotometer at 260 nm and calculating the ratio 260/280, respectively (BioPhotometer, Eppendorf).

cDNA Synthesis

RNA samples were treated with Dnase (Deoxyribonuclease I, Invitrogen) for removal of possible genomic DNA residues. Next, reverse transcription reaction was performed using 2mg of total RNA, reverse transcriptase (Superscript III, Invitrogen) and Oligo dT primer (Invitrogen) in the presence of Rnase inhibitor (RNase OUT, Invitrogen) in a final volume of 20 ml.

Real time PCR

PCR amplifications were performed in duplicate and using 50ng cDNA per reaction. The reactions were prepared with standardized reagents for Real Time PCR (TaqMan Universal PCR Master Mix, Applied Biosystems) plus the primer sets (Mm00432680_g1 - C-reactive protein and Mm99999915_g1 - internal control GAPDH) and specific probe for each gene. The reaction conditions were 50°C for 2 minutes, 95 °C for 10 minutes and 40 times of 95 °C for 15 seconds and 60 °C for 1 minute. The fluorescence readings were performed by 7500 Real-Time PCR equipment (Applied Biosystems) to each amplification cycle and then analyzed by the Sequence Detection Software (SDS) v1.3 (Applied Biosystems).

All reactions were submitted to the same conditions of analysis and normalized by dye signal of ROX passive reference for correction of fluctuations in reading due to changes in volume and evaporation throughout the reaction. The result, expressed in TC value, refers to the number of PCR cycles required to fluorescent signal reach the detection threshold.

Individual results expressed in CT values were assembled according to the experimental group for statistical analysis.

Statistical analysis

For each evaluated parameter the normality was tested by means of Shapiro-Wilk test and the appropriate statistical test was selected according to this assumption. Mean and standard deviation of body weight, liquid/caloric intake, alveolar bone loss and CRP (main outcome) for each experimental group were generated and compared by repeated measures one-way ANOVA, followed by Bonferroni or Turkey multiple comparison test. Analysis was performed with Stata (StataCorp, College Station, USA) for Macintosh and Prism GraphPad and the significance level was set at 0.05.

RESULTS

All animals completed the experimental protocol and presented significant weight gain throughout the study (Figure 3). No statistically significant differences were observed in body weight between groups during the study.

Figure 2 - Body weight (g) throughout study. No statistically differences were observed between groups along the study (ANOVA, p>0.05).

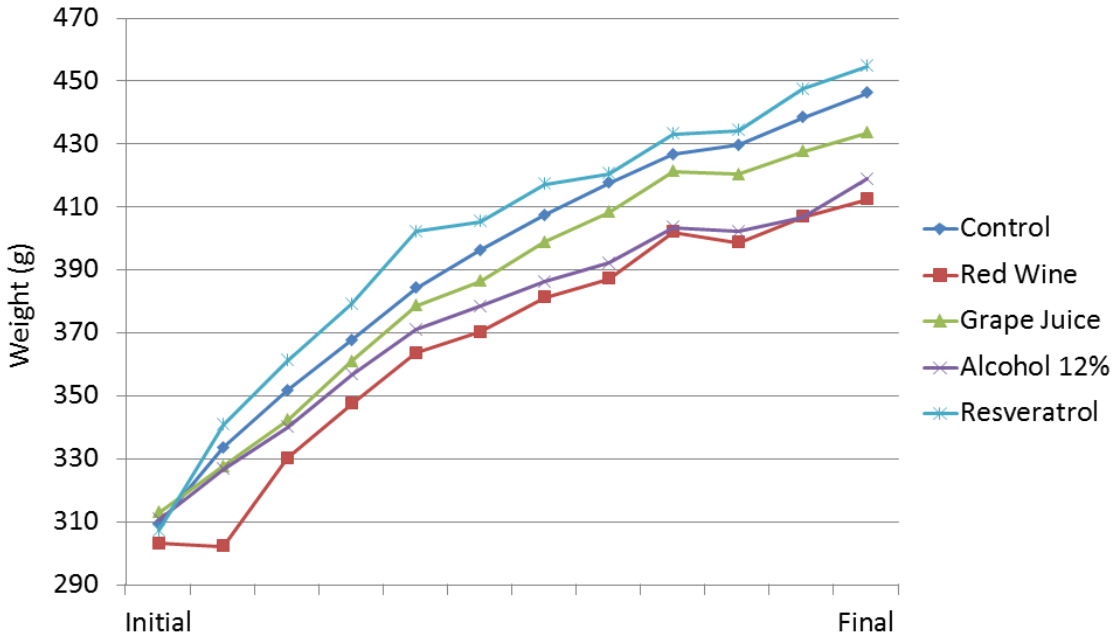


Table 1 summarizes the mean daily food (g), liquid (mL) and calories (cal) intake per animal. For both food and liquid consumption, statistically significant differences were observed between control and all groups (p<0.01). Animals that

consumed red wine and 12% alcohol exhibited a significantly lower liquid intake and groups exposed to red wine, grape juice and 12% alcohol showed lower food intake, when compared to resveratrol and control groups. In respect to daily calories intake, grape juice and 12% alcohol demonstrated higher and lower calories intake, respectively, compared to control group ($p<0.01$).

Table 1 - Mean daily liquid (mL), food (g) and calories intake (cal) for each group (one-way ANOVA, Bonferroni).

	Control	Red Wine	Grape Juice	Alcohol 12%	Resveratrol	P
Liquid	32.51 (±1.91)	22.78 (±1.07)*	38.17 (±1.44)*	23.44 (±0.42)*	38.50 (±1.14)*	< 0.01
Food	25.24 (±0.60)	19.05 (±0.24)*	19.55 (±0.42)*	19.43 (±0.17)*	25.83 (±0.24)*	< 0.01
Calories	74.46 (±1.77)	73.74 (±1.54)	84.41 (±2.27)*	72.09 (±0.83)*	76.18 (±0.70)	< 0.01

* Significant difference compared to control group

Table 2 shows the average alveolar bone loss (mm ± SD) for all groups. No statistically significant differences were observed between groups both in sides with and without ligature, but the side with ligature had significantly higher bone loss**.**

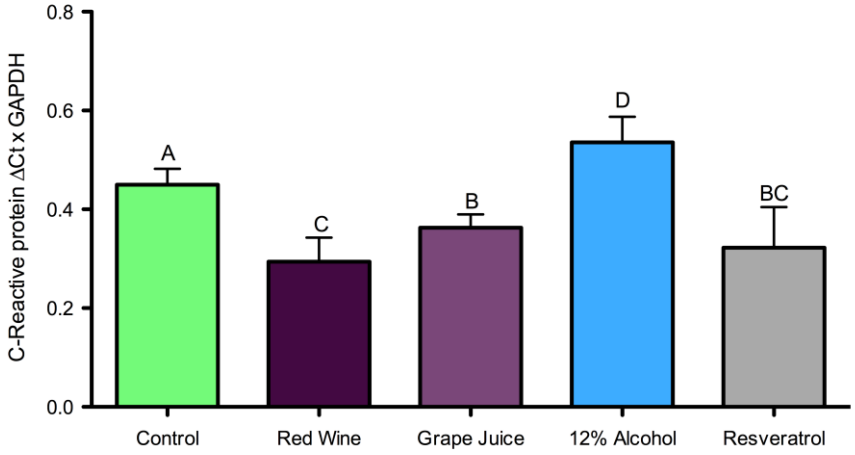
Table 2 - Alveolar bone loss (mm, ±SD) in teeth with and without ligature according the experimental group (one-way ANOVA, Bonferroni).

	Control	Red Wine	Grape Juice	Alcohol 12%	Resveratrol	p*
With ligature	0.60 (±0.07)	0.59 (±0.04)	0.64 (±0.10)	0.59 (±0.09)	0.67 (±0.08)	0.12
Without ligature	0.36 (±0.04)	0.35 (±0.04)	0.39 (±0.05)	0.35 (±0.04)	0.36 (±0.03)	0.17

Figure 3 demonstrates hepatic CRP levels in mmol/µL according to experimental groups. Significant differences were observed between control and other groups. Animals which consumed red wine presented lower levels of CRP (0.29±0.04) compared to control group (0.45±0.03) and animals which consumed alcohol had higher values of CRP (0.53±0.05) compared to control group. Animals

which consumed grape juice (0.36±0.02) and resveratrol (0.32±0.08) also presented lower levels of CRP compared to control group, however there is no significant difference between resveratrol and red wine and between resveratrol and grape juice.

Figure 3 - Hepatic CRP levels according to groups.



Different letters indicate statistical significance among groups
One-way ANOVA - Tukey

DISCUSSION

The present study evaluated the effect of red wine, 12% alcohol, grape juice and resveratrol associated with ligature induced periodontitis in hepatic CRP levels in Wistar rats. The main results demonstrated that rats, which consumed red wine, presented lower CRP levels compared to the other groups.

The idea behind the study is that alcoholic beverages seem to have impact in biological processes. In this sense, the literature, including periodontal studies, has demonstrated a j-shaped curve in the relationship between exposure to alcoholic beverages and occurrence and severity of diseases. This means that to some extent alcohol exposure acts as a protective factor and, after a determined point, a deleterious effect is demonstrated (35).

The results of this study need to be put into the perspective of the capacity of the design to generate evidence. Animal studies are important to generate hipotheses in mechanistic understanding of disease processes. Therefore, Wistar rats were chosen since there are similarities in biological features with humans (36). In addition, the use of animals allows better control of different variables that are

present in humans and could act as confounding bias such as environmental, psychological, behavioral, among other circumstances (37-41).

Studies clearly demonstrate a protective effect of red wine consumption in important outcomes, including cardiovascular diseases (8,10). In relation to periodontal diseases, alcohol seems to act similarly (7, 20, 21). To the best of our knowledge, studies using red wine consumption and periodontal diseases are inexistent. In this sense, the present study included 5 groups of liquid intake: a control group, to be the reference, red wine, which is part of the hypothesis in this study, alcohol in the same concentration of red wine and two important components of red wine (grape juice and resveratrol). The choice of resveratrol is based on the idea that it would be the substance responsible for the beneficial effect of red wine (42,43). On the other hand, alcohol in similar concentration seems to affect systemic inflammation (44). Thus, the groups of the present study try to separate different components of red wine to shad some light into the possible mechanisms.

The experiment was performed under contemporary laboratory practices and the liquid intake was restricted to the allocated solution. Thus, both body weight and control of food/liquid intake were verified to check possible negative influences of the experiment on their general health. The results demonstrated that despite statistically significant differences in liquid, food and calories intake, no statistically significant differences were observed in body weight. This allows to assume that groups were not systemically affected by the experimental process differently. Therefore, it can be presumed that no negative systemic effect was detected in any of the groups, making them comparable and supporting the encountered results.

Hence, in the present experiment, blinding of the examiner, reproducibility analysis, sample size estimation and control of the environment of animal housing warranted a lower risk of bias, increasing the internal validity of the results.

All animals were exposed to ligature-induced periodontal breakdown, which is known to be an inflammatory challenge in the body (45). The induction model was effective, since the intra-group controls displayed lower values (statistically significant) of alveolar bone loss. This has been consistently demonstrated in the literature and the results of the present study are in line with these findings (46-48).

In alveolar bone loss, no statistically significant differences were observed between groups. This could lead to the interpretation that red wine and/or its components would have no effect on pathogenesis of periodontal diseases.

However, this outcome might be obscured by the fact that only one time-point was utilized. Fifteen days of ligature is known to be able to produce periodontal breakdown, however limit the day-by-day or less time period analyses (49).

C-reactive protein (CRP) is an acute phase plasma protein produced by hepatocytes (34), which is nonspecific and produced in response to various stimuli. As previously described, increased levels of CRP may be related, for example, to smoking, obesity, triglycerides, diabetes and periodontal disease (23, 24, 25). CRP levels increase considerably in response to infection, inflammation and injury, being one of the markers of choice in the control of this response. In this study, the results showed what was expected, that red wine and resveratrol could decrease CRP levels.

Among the polyphenols studied in the literature, resveratrol has received greater attention due to the health benefits. Resveratrol is found in red wine, grape juice and peanuts (50) and acts as antioxidant, anti-inflammatory, antiviral and cardioprotective (13) through many different pathways. In relation to resveratrol and periodontal diseases, the literature has shown this substance as an antibacterial protection and a protetor in the tissue response produced by the organism (51, 52).

In the present study, CRP was used as a proxy of systemic inflammation. The results indicated that both red wine and resveratrol exposures lead to lower levels of CRP. In this sense, it could be assumed that resveratrol is one of the important components of the wine that would be responsible for the beneficial effects. The presence of ligature-induced periodontal disease, with confirmed higher degrees of alveolar bone loss warrants that a true inflammatory challenge was present in all animals and that the results in CRP reveal degree of inflammatory process.

The results of the present study are of great interest as part of the necessary understanding of pathogenesis of periodontal diseases and the potential of red wine as a modulator of the process. The model utilized in the present study allows that this hypothesis is further pursued.

CONCLUSION

This study was probably the first to analyze the different components of wine and its effects on CRP and periodontal disease. The conclusion is that, for alveolar bone loss, there is no statistically significant differences between groups. However,

red wine and resveratrol consumption decreases CRP levels and is a potential protective factor for systemic inflammation in Wistar rats.

REFERENCES

1. World Health Organization. Global status report on alcohol and health. Geneva; 2011.

2. World Health Organization. International guide for monitoring alcohol consumption and related harm. Geneva; 2000.

3. Mukamal KJ, Chung H, Jenny NS, Kuller LH, Longstreth WT, Jr., Mittleman MA, et al. Alcohol consumption and risk of coronary heart disease in older adults: the Cardiovascular Health Study. J Am Geriatr Soc. 2006;54(1):30-7.

4. Meyerhoff DJ, Bode C, Nixon SJ, de Bruin EA, Bode JC, Seitz HK. Health risks of chronic moderate and heavy alcohol consumption: how much is too much? Alcohol Clin Exp Res. 2005;29(7):1334-40.

5. McClain CJ, Barve S, Deaciuc I, Kugelmas M, Hill D. Cytokines in alcoholic liver disease. Semin Liver Dis. 1999;19(2):205-19.

6. Kongstad J, Hvidtfeldt UA, Gronbaek M, Jontell M, Stoltze K, Holmstrup P. Amount and type of alcohol and periodontitis in the Copenhagen City Heart Study. J Clin Periodontol. 2008;35(12):1032-9.

7.Leifert WR, Abeywardena MY. Cardioprotective actions of grape polyphenols. Nutr Res. 2008;28(11):729-37.

8.Mannari C, Bertelli AA, Stiaccini G, Giovannini L. Wine, sirtuins and nephroprotection: not only resveratrol. Med Hypotheses. 2010;75(6):636-8.

9. Sheu SJ, Liu NC, Chen JL. Resveratrol protects human retinal pigment epithelial cells from acrolein-induced damage. J Ocul Pharmacol Ther. 2010;26(3):231-6.

10. Natella F, Macone A, Ramberti A, Forte M, Mattivi F, Matarese RM, et al. Red wine prevents the postprandial increase in plasma cholesterol oxidation products: a pilot study. Br J Nutr. 2011:1-6.

11. Magrone T, Jirillo E. Polyphenols from red wine are potent modulators of innate and adaptive immune responsiveness. Proc Nutr Soc. 2010;69(3):279-85.

12. Pai JK, Hankinson SE, Thadhani R, Rifai N, Pischon T, Rimm EB. Moderate alcohol consumption and lower levels of inflammatory markers in US men and women. Atherosclerosis. 2006; 186 (1): 113-20.

13. Lin JF, Lin SM, Chih CL, Nien MW, Su HH, Hu BR, et al. Resveratrol reduces infarct size and improves ventricular function after myocardial ischemia in rats. Life Sci. 2008;83(9-10):313-7.

14. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature. 2006;444(7117):337-42.

15. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol.1998; 25(2):134-44.

16. Bergström J, Eliasson S, Dock, J. A 10-Year prospective study of tobacco smoking and periodontal health. J Periodontol. 2000; 71(8):1338-47

17. Al-Zahrani MS, Bissada NF, Borawskit EA. Obesity and periodontal disease in young, middle-aged, and older adults. J Periodontol. 2003; 74(5):610-5.

18. Dalla Vecchia CF, Susin C, Rosing CK, Oppermann RV, Albandar JM. Overweight and obesity as risk indicators for periodontitis in adults. J Periodontol. 2005; 76(10):1721-8.

19. Szabo G. Consequences of alcohol consumption on host defence. Alcohol Alcohol. 1999;34(6):830-41

20. Bouchard P, Boutouyrie P, Mattout C, Bourgeois D. Risk assessment for severe clinical attachment loss in an adult population. J Periodontol. 2006;77(3):479-89.

21. Susin C, Wagner MC, Haas AN, Oppermann RV, Albandar JM. The association between alcohol consumption and periodontitis in southern Brazilian adults. J Periodont Res. 2015; 50:622-28.

22. Souza DM, Ricardo LH, Kantoski KZ, Rocha RF. Influence of alcohol consumption on alveolar bone level associated with ligature-induced periodontitis in rats. Braz Oral Res. 2009, 23(3):326-332.

23. Salto M, Ishimitsu T, Minami J, Ono H, Ohrui M, Matsuoka H. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. Atherosclerosis. 2013;167:73-9.

24.Tüter G, Kurtis B, Serdar M. Evaluatuion of gingival crevicular fluid and serum levels of high-sensitivity C-reactive protein in chronic periodontitis patients with and without coronary artery disease. J Periodontol. 2007;78:2319-24.

25. Gomes-Filho IS, Coelho JM, da Cruz SS, Passos JS, Freitas CO, Farias NS, et al. Chronic periodontitis and C-reactive protein levels. JPeriodontol. 2011,82(7):969–78.

26. Freitas CO, Gomes-Filho IS, Naves RC. Influence of periodontal therapy on C-reactive protein level: a systematic review and meta-analysis. J Appl Oral. 2012,20(1):1-8.

27. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol. 2008, 35(4):277–290.

28. Podzimek S, Mysak J, Janatova T, Duskova J. C-Reactive Protein in peripheral blood of patients with chronic and aggressive periodontitis, gingivitis, and gingival recessions. Mediators Inflamm. 2015, 2015:1-8.

29. Albert MA, Glynn RJ, Ridker PM. Alcohol consumption and plasma concentration of C-reactive protein. Circulation 2003, 107:443-337.

30. Galvao MP, Chapper A, Rosing CK, Ferreira MB, de Souza MA. Methodological considerations on descriptive studies of induced periodontal diseases in rats. Pesqui Odontol Bras. 2003;17(1):56-62.

31. Simch RP, Gaio EJ, Rosing CK. Effect of body weight in the pathogenesis of ligature-induced periodontal disease in Wistar rats. Acta Odontol Scand. 2008;66(3):130-134.

32. Soletti AC, Gaio EJ, Rosing CK. Effect of neonatal clomipramine in the pathogenesis of ligature-induced periodontitis in Lewis rats. Acta Odontol Scand. 2009;67(2):94-98.

33. Liberman DN, Pilau RM, Gaio EJ, Orlandini LF, Rösing CK. Low concentration alcohol intake may inhibit spontaneous alveolar bone loss in Wistar rats. Arch Oral Biol. 2011;56(2):109-113.

34. Black, S, Kushner I, Samols D. C-reactive protein. J Biol Chem. 2004,279(47):48487-48490.

35. Díaz LE, Montero A, González-Gross M, Vallejo AI, Romeo J, Marcos A. Influence of alcohol consumption on immunological status: a review. Eur J Clin Nutr. 2002;56(3):50-3.

36. Klausen B. Microbiological and immunological aspects of experimental periodontal disease in rats: a review article. J Periodontol. 1991;62(1):59-73.

37. Fine DH. Of mice and men: animal models of human periodontal disease. J Clin Periodontol. 2009;36(11):913-4.

38. Oz HS, Puleo DA. Animal models for periodontal disease. J Biomed Biotechnol. 2011;2011:754857.

39. Struillou X, Boutigny H, Soueidan A, Layrolle P. Experimental animal models in periodontology: a review. Open Dent J. 2010;4:37-47.

40. Weinberg MA, Bral M. Laboratory animal models in periodontology. J Clin Periodontol. 1999;26(6):335-40.

41. Listgarten MA. Similarity of epithelial relationships in the gingiva of rat and man. J Periodontol.1975;46(11):677-80.

42. Coimbra SR, Lage, SH, Brandizzi L, Yoshida Y, Da Luz, PL. S. The action of red wine and purple grape juice on vascular reactivity is independent of plasma lipids in hypercholesterolemic patients. Braz J Med Biol Res. 2005;38:1339-47.

43. Hertog MG; Kromhout D; Aravanis C. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. Arch Int Med. 1995;155: 381-6.

44. Marques-Vidal P, Bochud M, Bastardot F, Von Känel R, Ferrero F, Gaspoz JM, et al. Associations between alcohol consumption and selected cytokines in a Swiss population-based sample (CoLaus study). Atherosclerosis 2012;222(1): 245-50.

45. Tomofuji T, Sanbe T, Ekuni D, Azuma T, Irie K., Maruyama, et al. Oxidative damage of rat liver induced by ligature-induced periodontitis and chronic ethanol consumption. Arch Oral Biol. 2008;53(12): 1113-18.

46. Wagner MC, Rocha JM, Gaio EJ, Cavagni J, Carrard VC, Rösing CK. Effect of 15% Alcohol Dependence on Alveolar Bone Loss and TNF-α Secretion in Wistar Rats. Braz Dent J. 2016 Mar-Apr;27(2):135-40

47. Cavagni J, de Macedo IC, Gaio EJ, Souza A, de Molon RS, Cirelli JA, et al. Obesity and Hyperlipidemia Modulate Alveolar Bone Loss in Wistar Rats. J Periodontol. 2016 Feb;87(2):e9-17.

48. Cavagni J, Soletti AC, Gaio EJ, Rösing CK. The effect of dexamethasone in the pathogenesis of ligature-induced periodontal disease in Wistar rats. Braz Oral Res. 2005 Oct-Dec;19(4):290-4.

49. Bjornsson MJ, Velschow S, Stoltze K, Havemose-Poulsen A, Schou S, Holmstrup P. The influence of diet consistence, drinking water and bedding on periodontal disease in Sprague-Dawley rats. Journal of periodontal research 2003;38(6):543-550.

50. Harikumar KB, Aggarwal BB. Resveratrol: a multitargeted agent for age-associated chronic diseases. Cell Cycle. 2008;7(8):1020-35.

51. O'Connor DJ, Wong RW, Rabie AB. Resveratrol inhibits periodontal pathogens in vitro. Phytother Res. 2011;25(11):1727-31.

52. Rizzo A, Bevilacqua N, Guida L, Annunziata M, Romano Carratelli C, Paolillo R. Effect of resveratrol and modulation of cytokine production on human periodontal ligament cells. Cytokine. 2012;60(1):197-204.

3 CONSIDERAÇÕES FINAIS

O consumo excessivo de álcool é um problema de saúde pública que afeta milhões de pessoas no mundo inteiro, porém as consequências deste consumo variam de acordo com o gênero, tipo, quantidade e frequência de bebida ingerida. A literatura mostra que, dentre as bebidas que contém álcool, o vinho tinto tem se destacado como um fator de proteção para doenças crônicas degenerativas como doença cardiovascular, doenças isquêmicas e acidente vascular cerebral. Entretanto, restam dúvidas se o responsável por esta melhora é o vinho tinto em si ou substâncias derivadas da uva, como os flavonoides ou o resveratrol.

Os resultados desse trabalho demonstram que o consumo de vinho tinto, suco de uva e resveratrol diminuiram os níveis de proteína C-reativa (PCR) em ratos Wistar. Considerando que o consumo de vinho tinto foi responsável pelos menores níveis PCR, acredita-se num possível efeito antiinflamatório da bebida como um todo. Entretanto, esse foi possivelmente um dos primeiros trabalhos a relacionar os diferentes componentes do vinho (álcool 12%, suco de uva e resveratrol) com doença periodontal induzida em ratos e proteína C-reativa, portanto mais estudos devem ser conduzidos para confirmação desses resultados.

REFERÊNCIAS

ALBERT, M.A.; GLYNN, R.J.; RIDKER, P.M. Alcohol consumption and plasma concentration of C-reactive protein. **Circulation**, Baltimore, v. 107, p. 337-443, Jan. 2003.

AMARAL, C. D. S. F.; RONIR, R. L.; LEAO, A. T. T. The relationship between alcohol dependence and periodontal disease. **J. Periodontol.**, Chicago, v. 79, no. 6, p. 993-998, 2008.

AMARAL, C. D. S. F.; VETTORE, M. V.; LEÃO, A. The relationship of alcohol dependence and alcohol consumption with periodontitis: a systematic review. **J. Dent.**, Bristol, v. 37, no. 9, p. 643-651, 2009.

BAUR, J. A. et al. Resveratrol improves health and survival of mice on a high-calorie diet. **Nature**, Boston, v. 444, no. 7117, p. 337-342, Nov. 2006.

BOUCHARD, P. et al. Risk assessment for severe clinical attachment loss in an adult population. **J. Periodontol.**, Chicago, v. 77, no. 3, p. 479-489, Mar. 2006.

CASATI, M. Z. et al. Resveratrol decreases periodontal breakdown and modulate local levels of cytokines during periodontitis in rats. **J. Periodontol.**, Chicago, v. 84, no. 10, p. 58-64, Mar. 2013.

COIMBRA, S. R. et al. The action of red wine and purple grape juice on vascular reactivity is independent of plasma lipids in hypercholesterolemic patients. **Braz. J . Med. Biol. Res.**, Ribeirão Preto, v. 38, p. 1339-1347, 2005.

DAGLIA, M. Polyphenols as antimicrobial agents. **Curr. Opin. Biotechnol.**, London, v. 23, no. 2, p. 174-181, Apr. 2012.:

DAVALOS, A. et al. Effects of grape red juice polyphenols in NADPH oxidase subunit expression in human neutrophils and mononuclear blood cells. **Br. J. Nutr.**, Cambridge, v. 102, p. 1125-1135, 2009.

DE SOUZA, A.B. et al. Short-term changes on C-reactive protein (CRP) levels after non-surgical periodontal treatment in systemically healthy individuals. **Clin. Oral Investig.**, Berlin, v. 20, no. 784, Apr. 2016.

DIAZ, L. E. et al. Influence of alcohol consumption on immunological status: a review. **Eur. J. Clin. Nutr.**, London, v. 56, no. 3, p. 50-53, Aug. 2002.

DOLL, R.; PETO, R.; HALL, E. et al. Mortality in relation to consumption of alcohol: 13years' observations on male British doctors. **BMJ**, London, v. 309, no. 6959, p. 911-918, 1994.

FREITAS, C.O.; GOMES-FILHO, I.S.; NAVES R.C. Influence of periodontal therapy on C-reactive protein level: a systematic review and meta-analysis. **J Appl. Oral Sci.**, Bauru, v. 20, no. 1, p. 1-8, 2012.

GOMES-FILHO, I.S. et al. Chronic periodontitis and C-reactive protein levels. **J. Periodont.**, Chicago, v. 82, no. 7, p. 969–978, July 2011.

GOYAL, L. et al. Comparative evaluation of serum C-reactive protein levels in chronic and aggressive periodontitis patients and association with periodontal disease severity. **Contemp. Clin. Dent.**, Mumbai, v. 5, no. 4, p. 484–488, 2014.

GAZIANO, J.M.; GAZIANO, T.A., GLYNN, R. et al. Light to moderate alcohol consumption and mortality in the Physicians' Health Study enrollment cohort. **J. Am. Coll. Cardiol.**, v. 35, no. 1, p. 96-105, Jan. 2000.

GAZIANO, J.M.; BURING, J.R.; BRESLOW, J.L. et al. Moderate alcohol intake increased levels of high-density lipoproteins and its subfractions, and decreased risk of myocardial infarction. **N. Engl. J. Med.**, Boston, v. 329, no. 25, p 1829–1834, Dec. 1993.

HARIKUMAR, K. B.; AGGARWAL, B. B. Resveratrol: a multitargeted agent for age-associated chronic diseases. **Cell Cycle,** Georgetown, v. 7, no. 8, p. 1020-1035, Apr. 2008.

HERTOG, M. G.; KROMHOUT, D.; ARAVANIS, C. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. **Arch. Int. Med.**, Chicago, v. 155, p. 381-386, 1995.

HUSSAIN, T. et al. Green tea constituent epigallocatechin-3-gallate selectively inhibits COX-2 without affecting COX-1 expression in human prostate carcinoma cells. **Int. J. Cancer**, New York, v. 113, no. 4, p. 660-669, Feb. 2005.

IOANNIDOU, E.; MALEKZADEH, T.; DONGARI-BAGTZOGLOU, A. Effect of periodontal treatment on serum C-reactive protein levels: A systematic review and meta-analysis. **J. Periodontol.**, Chicago, v. 77, n. 10, p. 1635–1642, Oct. 2006.

KONGSTAD, J. et al. Amount and type of alcohol and periodontitis in the Copenhagen City Heart Study. **J. Clin. Periodontol.**, Chicago, v. 35, no. 12, p. 1032-1039, Dec. 2008.

KULISCH, C.; ECKERS, N.; ALBRECHT, D. Method of euthanasia affects amygdala plasticity in horizontal brain slices from mice. **J. Neurosci. Methods**, Amsterdam, v. 201, no. 2, p. 340-345, Oct. 2011.

LAGRAND, W.K. et al. Creactive protein as a cardiovascular risk factor: more than an epiphenomenon? **Circulation**, Baltimore, v. 100, no. 1, p. 96-102, July 1999.

LANGER, R.D.; CRIQUI, M.H.; REED, D.M. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. **Circulation**, Baltimore, v. 85, p. 910-915, 1992.

LARANJEIRA, R. et al. **II LENAD**: Levantamento Nacional de Álcool e Drogas (LENAD). São Paulo: UNIFESP. Instituto Nacional de Ciencia e Tecnologia para Políticas Públicas de Álcool e Outras Drogas (INPAD), 2013. v. 2, p. 34-43.

LEE, Y. M. et al. The role of sirtuin 1 in osteoblastic differentiation in human periodontal ligament cells. **J. Periodontal Res.**, Copenhagen, v. 46, no. 6, p. 712-721, Dec. 2011.

LEIFERT, W. R.; ABEYWARDENA, M. Y. Cardioprotective actions of grape polyphenols. **Nut. Res.,** Tarrytown, v. 28, p. 729-737, 2008.

LIBERMAN, D. N. et al. Low concentration alcohol intake may inhibit spontaneous alveolar bone loss in Wistar rats. **Arch. Oral Biol.**, Oxford, v. 56, no. 2, p. 109-113, Feb. 2011.

LIN, J. F. et al. Resveratrol reduces infarct size and improves ventricular function after myocardial ischemia in rats. **Life Sci.**, Oxford, v. 83, no. 9-10, p. 313-317, Aug. 2008.

MAGRONE, T.; JIRILLO, E. Antioxidants and the immune system Polyphenols from red wine are potent modulators of innate and adaptative immune responsiveness. **Proc. Nut. Soc**, London, v. 69, p. 279-285, 2010.

MANNARI, C. et al. Wine, sirtuins and nephroprotection: Not only resveratrol. **Med. Hypotheses**, New York, v. 75, p. 636-8, 2010.

MARQUES-VIDAL, P. et al. Associations between alcohol consumption and selected cytokines in a Swiss population-based sample (CoLaus study). **Atherosclerosis,** Limerick, v. 222, no. 1, p. 245-250, May 2012.

MCCLAIN, C. J. et al. Cytokines in alcoholic liver disease. **Semin. Liver. Dis.**, New York, v. 19, no. 2, p. 205-219, 1999.

MEYERHOFF, D. J. et al. Health risks of chronic moderate and heavy alcohol consumption: how much is too much? **Alcohol Clin. Exp. Res.**, Oxford, v. 29, no. 7, p. 1334-1340, July 2005.

MUKAMAL, K. J. et al. Alcohol consumption and risk of coronary heart disease in older adults: the Cardiovascular Health Study. **J. Am. Geriatr. Soc.**, v. 54, no. 1, p. 30-73, Jan. 2006.

MUKAMAL, K.J.; JADHAV, P.P.; D'AGOSTINO, R.B. et al. Alcohol consumption and hemostatic factors: analysis of the Framingham Offspring Cohort. **Circulation**, Baltimore, v.104, p. 1367-73, 2001.

NAASANI, I. et al. Blocking telomerase by dietary polyphenols is a major mechanism for limiting the growth of human cancer cells in vitro and in vivo. **Cancer Res.**, Baltimore, v. 63, no. 4, p. 824-830, Feb. 2003.

NATELLA, F. et al. Red wine prevents the postprandial increase in plasma cholesterol oxidation products: a pilot study. **Br. J. Nutr.,** Wallingford, v. 2011, p. 1-6, 2011.

NISHIDA, N. et al. Association of ALDH(2) genotypes and alcohol consumption with periodontitis. **J. Dent. Res.**, Thousand Oaks, v. 83, no. 2, p. 161-165, Feb. 2004.

O'CONNOR, D. J.; WONG, R. W.; RABIE, A. B. Resveratrol inhibits periodontal pathogens in vitro. **Phytother. Res.**, London, v. 25, no. 11, p. 1727-1731, Nov. 2011.

O'LEARY, K. A. et al. Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription. **Mutat. Res.**, Amsterdam, v. 551, no. 1-2, p. 245-254, July 2004.

OMS. **International guide for monitoring alcohol consumption and related harm**. Geneva, 2000. 209 p.

_____. **Global status report on alcohol and health**. Geneva, 2011.

_____. **Levels of consumption**: total adult per capita consumption by country. Geneva, 2013.

PARASKEVAS, S.; HUIZINGA, J. D; LOOS, B. G. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. **J. Clin. Periodontol.,** Malden, v. 35, no. 4, p. 277–290, 2008.

PAI, J. K. et al. Moderate alcohol consumption and lower levels of inflammatory markers in US men and women. **Atherosclerosis,** Limerick, v. 186, no. 1, p. 113-120, May 2006.

PITIPHAT, W. et al. Alcohol consumption increases periodontitis risk. **J. Dent. Res.**, Thousand Oaks, v. 82, no. 7, p. 509-513, July 2003.

PODZIMEK, S. et al. C-Reactive protein in peripheral blood of patients with chronic and aggressive periodontitis, gingivitis, and gingival recessions. **Mediators Inflamm.**, Sylvania, v. 2015, p. 1-8, June 2015.

REHM J, BONDY S. Alcohol and all-cause mortality: na overview. **Novartis Found. Symp.**, Chichester, v. 216, p. 223-236, 1998.

RIZZO, A. et al. Effect of resveratrol and modulation of cytokine production on human periodontal ligament cells. **Cytokine**, Oxford, v. 60, no.1, p. 197-204, 2012.

SADIK, C. D.; SIES, H.; SCHEWE, T. Inhibition of 15-lipoxygenases by flavonoids: structure-activity relations and mode of action. **Biochem. Pharmacol.**, Oxford, v. 65, no. 5, p. 773-781, Mar. 2003.

SALEEM, T. S. M.; BASHA, S. D. Red wine: a drink to your heart. **J. Cardiovasc. Dis. Res.,** Bangalore, v. 1, no. 4, p. 171-176, 2010.

SALZBERG, T.N., et al. C-reactive protein levels in patients with aggressive periodontitis. **J. Periodontol.**, Chicago, v. 77, no. 6, p. 933–939, 2006.

SCHEWE, T. et al. Polyphenols of cocoa: inhibition of mammalian 15-lipoxygenase. **Biol. Chem**., Berlin, v. 382, no. 12, p. 1687-1696, Dec. 2001.

SHIMAZAKI, Y. et al. Relationship between drinking and periodontitis: the Hisayama Study. **J. Periodontol.**, Chicago, v. 76, no. 9, p. 1534-1541, Sept. 2005.

SOUZA, D. M. et al. Influence of alcohol consumption on alveolar bone level associated with ligature-induced periodontitis in rats. **Braz. Oral Res.**, São Paulo, v. 23, n. 3, p. 326-32, Sept. 2009.

STEWART, H.; MAINOUS, A.G.; GILBERT G. Relation between alcohol consumption and C-reactive protein levels in the adult US population. **J. Am. Board Fam. Pract.,** Lexington, v. 15, no.6, p.437-442, Dec. 2002.

SZABO, G. Consequences of alcohol consumption on host defence. **Alcohol Alcohol,** Oxford, v. 34, no. 6, p. 830-841, Dec. 1999.

SUSIN, C. et al. The association between alcohol consumption and periodontitis in southern Brazilian adults. **J. Periodont. Res.,** Copenhagen, v. 50, p. 622-628, 2015.

TEZAL, M. et al. The effect of alcohol consumption on periodontal disease. **J. Periodontol.**, Chicago, v. 72, no. 2, p. 183-189, Feb. 2001.

_____. Alcohol consumption and periodontal disease. The Third National Health and Nutrition Examination Survey. **J. Clin. Periodontol.,** Malden, v. 31, no. 7, p. 484-488, July 2004.

WAGNER, M. C. **O efeito do consumo de álcool sobre a progressão da perda de inserção periodontal:** Estudo de Porto Alegre, 2008. Trabalho de conclusão de curso (Mestrado em Odontologia) - Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2008.

WANG, W. et al. SIRT1 inhibits TNF-alpha-induced apoptosis of vascular adventitial fibroblasts partly through the deacetylation of FoxO1. **Apoptosis.,** London, v. 18, no. 6, p. 689-701, June 2013.