UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

DEIWESON DE SOUZA MONTEIRO

Efeitos do estresse crônico sobre o estado redox e tecidual das glândulas salivares parótida e submandibular: um estudo *in vivo*

BELÉM-PA

2025

UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

Efeitos do estresse crônico sobre o estado redox e tecidual das glândulas salivares parótida e submandibular: um estudo *in vivo*

Autor(a): Deiweson de Souza Monteiro

Orientador(a): Prof.ª Dr.ª Rafael Rodrigues Lima

Documento de Dissertação apresentada ao Programa de Pós-graduação em Farmacologia e Bioquímica do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito para a obtenção do título de Mestre em Farmacologia e Bioquímica.

BELÉM-PA

2025

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD
Sistema de Bibliotecas da Universidade Federal do Pará
Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

M772e Monteiro, Deiweson de Souza.
Efeitos do estresse crônico sobre o estado redox e tecidual das glândulas salivares parótida e submandibular : um estudo in vivo / Deiweson de Souza Monteiro. — 2025.
x, 13 f. : il. color.

Orientador(a): Prof. Dr. Rafael Rodrigues Lima
Dissertação (Mestrado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-graduação em Farmacologia e Bioquímica, Belém, 2025.

1. Estresse crônico. 2. Glândulas salivares. 3. Estresse oxidativo. 4. Histomorfometria. I. Título.

CDD 612.015

DEIWESON DE SOUZA MONTEIRO

Efeitos do estresse crônico sobre o estado redox e tecidual das glândulas salivares parótida e submandibular: um estudo *in vivo*

Documento de Dissertação apresentada ao Programa de Pós-graduação em Farmacologia e Bioquímica do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito para a obtenção do título de Mestre em Farmacologia e Bioquímica.

Data de aprovação: 27/03/2025

Banca Examinadora

Prof. Dr. Rafael Rodrigues Lima – Orientador
Universidade Federal do Pará
Presidente da Banca Examinadora

Prof. Dra. Bruna Romana de Souza
Universidade Estadual do Rio de Janeiro
Membro Titular da Banca Examinadora

Prof. Dra. Luanna de Melo Pereira Fernandes
Universidade Estadual do Pará
Membro Titular da Banca Examinadora

Dr. Leonardo de Oliveira Bittencourt
Universidade Federal do Pará
Membro Titular da Banca Examinadora

AGRADECIMENTOS

Agradeço a minha família por ser meu alicerce, incentivadores e financiadores dos meus estudos. Vocês me moldaram e iremos vencer juntos!

Aos meus amigos da universidade e companheiros do Laboratório de Biologia Estrutural e Funcional, obrigado por cada manhã, tarde, noite, final de semana e viagens repletas de árduos trabalhos científicos, café e risadas.

Obrigado aos professores e pesquisadores do Programa de Pós-graduação em Farmacologia e Bioquímica por enriqueceram minha formação, principalmente, meu mestre Rafael Lima. Obrigado por acreditar em mim, plantar e lapidar meu crescimento científico.

Aos meus amigos da vida, também agradeço por serem parte fundamental da minha jornada. Obrigado por cada ajuda direta ou indireta.

À UFPA e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior por todo apoio técnico e financeiro concedidos a mim e ao meu orientador.

Agradeço aos Deuses do Universo por me abençoarem.

RESUMO

O estresse é uma reação à pressão mental e emocional, ansiedade ou traumas. O estresse crônico é definido como a exposição constante a esses eventos. Ele pode afetar diversos sistemas do corpo, aumentar a pressão arterial e enfraquecer a imunidade, interferindo assim nos processos de saúde fisiológica. Sob essa perspectiva, diversos órgãos podem apresentar repostas ou alterações em estado de estresse crônico. Portanto, este estudo objetivou avaliar os efeitos do estresse crônico sobre as glândulas salivares de ratos, investigando sua bioquímica oxidativa e parâmetros histomorfológicos. Para isso, 32 ratos machos albinos da linhagem *Wistar* foram divididos aleatoriamente em dois grupos: estresse crônico e controle. Os animais submetidos ao estresse crônico passaram por um protocolo de imobilização, sendo alocados em um tubo de polivinil por 4 horas diárias durante 28 dias, o que limitava seus movimentos. Posteriormente, os animais foram eutanasiados para coleta das glândulas salivares parótida e submandibular. O estado redox das glândulas foi avaliado por meio dos ensaios de capacidade antioxidante contra radicais peroxil (ACAP) e substâncias reativas ao ácido tiobarbitúrico (TBARS). A análise histológica foi realizada por morfometria dos tecidos corados com hematoxilina e eosina e histoquímica através da técnica de PicroSirius Red. Tanto as glândulas parótida quanto as submandibulares dos ratos estressados apresentaram estresse oxidativo, caracterizado por uma redução nos níveis de ACAP e um aumento nos níveis de TBARS. No entanto, as glândulas parótidas mostraram-se mais suscetíveis a alterações prejudiciais nos tecidos, como aumento da área do estroma e da fração de área de colágeno, redução da área acinar e menor tamanho dos ácinos e ductos. Enquanto as glândulas submandibulares não apresentaram nenhuma alteração histomorfológica. Nossos resultados sugerem que o estresse crônico pode causar uma modulação prejudicial do estado redox das glândulas salivares, com diferentes repercussões histológicas entre as glândulas.

Palavras-chave: estresse crônico; glândulas salivares; estresse oxidativo; histomorfometria.

ABSTRACT

Stress is a reaction to mental and emotional pressure, anxiety, or trauma. Chronic stress is defined as constant exposure to such events. It can affect various body systems, increase blood pressure, and weaken immunity, thereby interfering with physiological health processes. From this perspective, several organs may exhibit responses or alterations under conditions of chronic stress. Therefore, this study aimed to evaluate the effects of chronic stress on the salivary glands of rats, investigating their oxidative biochemistry and histomorphological parameters. For this purpose, 32 male albino Wistar rats were randomly divided into two groups: chronic stress and control. The animals in the chronic stress group were subjected to an immobilization protocol, being placed in a polyvinyl tube for 4 hours daily for 28 days, which restricted their movement. Subsequently, the animals were euthanized for the collection of the parotid and submandibular salivary glands. The redox status of the glands was assessed using the antioxidant capacity against peroxyl radicals (ACAP) assay and thiobarbituric acid reactive substances (TBARS) assay. Histological analysis was performed through morphometry of tissues stained with hematoxylin and eosin, and histochemistry using the PicroSirius Red technique. Both the parotid and submandibular glands of the stressed rats exhibited oxidative stress, characterized by a reduction in ACAP levels and an increase in TBARS levels. However, the parotid glands proved to be more susceptible to harmful tissue alterations, such as an increase in stromal area and collagen area fraction, a reduction in acinar area, and smaller size of acini and ducts. In contrast, the submandibular glands did not show any histomorphological alterations. Our results suggest that chronic stress can cause a harmful modulation of the redox status of the salivary glands, with different histological repercussions between the glands.

Keywords: chronic stress; salivary glands; oxidative stress; histomorphometry.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE SIGLAS

ABAP	2,2´-azobis 2 methylpropionamidine dihydrochloride
ACAP	Antioxidant Capacity Against Peroxyl Radicals
ACTH	Adrenocorticotrophic Hormones
ARRIVE	Animal Research: Reporting of In Vivo Experiments
BDNF	Brain-Derived Neurotrophic Factor
CEUA	Comissão de Ética no Uso de Animais
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
H2DCF-DA	2´,7´-dichlorofluorescein diacetate
HE	Hematoxilina e Eosina
HPA	Hipotálamo-Hipófise-Adrenal
IL-1b	Interleukin-1 Beta
LPO	Lipid Peroxidation
OMS	Organização Mundial da Saúde
ROS	Reactive Oxygen Species
SAM	Sistema Simpato-Adrenomedular
TBARS	Thiobarbituric Acid Reactive Substances
TGF-beta	Transforming Growth Factor-beta
TNF-alpha	Tumor Necrosis Factor-alpha
Tris-HCl	Tris(hidroximetil)aminometano com cloreto de sódio
UFPA	Universidade Federal do Pará
WHO	World Health Organization

Sumário

1. VISÃO INTEGRADORA DO PROBLEMA

O estresse é uma condição amplamente conhecida na sociedade contemporânea, caracterizada por ser uma resposta fisiológica e psicológica desencadeada pela exposição a agentes estressores, como situações adversas ou eventos desafiadores (Alotiby, 2024). Ao longo da evolução humana, a exposição a fatores estressores como fome, temperaturas extremas, medo, exigências laborais e doenças tem sido constante, configurando o estresse como um mecanismo adaptativo essencial à sobrevivência (Boff & Oliveira, 2021). A habilidade do organismo em redirecionar energia e modular funções fisiológicas diante desses estímulos constitui a base funcional do estresse como um fenômeno fisiológico (Yaribeygi et al., 2017). Os primeiros registros conceituais do estresse foram estabelecidos por Hans Selye, em 1936, o qual já destacava a importância da capacidade adaptativa e da resistência ao estresse como elementos fundamentais para a manutenção da vida, com a participação integrada de todos os órgãos e sistemas vitais nesse processo (Selye, 1936).

Os mecanismos biológicos relacionados ao estresse envolvem principalmente a ativação do sistema endócrino, com destaque para os eixos simpato-adrenomedular (SAM) e hipotálamo-hipófise-adrenal (HPA), que são responsivos a estímulos estressores de natureza física ou psicológica (Russell & Lightman, 2019). A ativação desses eixos leva à liberação de diversas moléculas sinalizadoras e hormônios, como adrenalina e cortisol, que promovem diversas alterações fisiológicas. O cortisol, em especial, exerce papel crucial na modulação desses processos, como resposta inflamatória, gliconeogênese, e funções cognitivas e emocionais. No entanto, o problema se instala quando a exposição a episódios estressores aumenta ou se intensifica, o que pode resultar em disfunção regulatória desses sistemas, comprometendo o equilíbrio hormonal e molecular. A ativação crônica do eixo HPA promove um aumento dos níveis de cortisol, o que pode comprometer a homeostase, especialmente nos sistemas imunológico e inflamatório, levando à produção exacerbada de citocinas pró-inflamatórias (Zefferino, Di Gioia & Conese, 2021). A inflamação prolongada, por sua vez, pode induzir um estado de estresse oxidativo, desencadeado pela ativação de células imunocompetentes, como neutrófilos e macrófagos, que liberam espécies reativas de oxigênio (Dinh et al., 2014). Esse conjunto de alterações fisiopatológicas caracteriza o que se denomina estresse crônico (Roberts & Karatsoreos, 2021).

Evidências científicas demonstram que o estresse crônico está amplamente disseminado no contexto contemporâneo, manifestando-se em situações como a síndrome de *burnout* associada ao ambiente de trabalho, ou em eventos de escala global, como catástrofes climáticas, atos de terrorismo e transtornos de estresse pós-traumático (Gradus, 2017). No cenário atual, a prevalência do estresse crônico tem aumentado de forma expressiva, impulsionada por fatores como o ritmo acelerado da vida moderna, instabilidade econômica e exigências profissionais excessivas (Shchaslyvi et al., 2024). De acordo com a Organização Mundial da Saúde (OMS), milhões de pessoas em todo o mundo são afetadas por essa condição, a qual exerce impacto direto sobre a saúde física e mental, aumentando o risco de doenças

cardiovasculares, depressão e disfunções imunológicas (WHO, 2007). No contexto brasileiro, o quadro é alarmante, considerando que uma parcela expressiva da população enfrenta elevados níveis de estresse, potencializados por fatores socioeconômicos adversos, como violência urbana, dificuldades financeiras e pressões no ambiente de trabalho. Tais fatores impactam negativamente a saúde pública, resultando em maior incidência de enfermidades relacionadas ao estresse, sobrecarga dos serviços de saúde e redução da qualidade de vida da população (Souza et al., 2021).

O estresse crônico representa um fator de risco significativo para a saúde humana, com impactos tanto em níveis sistêmicos quanto locais, afetando diversos órgãos, tecidos e funções fisiológicas (O'Connor et al., 2021). No contexto da saúde bucal, evidências apontam a associação do estresse crônico com três principais condições clínicas: o bruxismo, a doença periodontal e a xerostomia. O bruxismo, por apresentar etiologia multifatorial, é frequentemente correlacionado a distúrbios emocionais e de hábitos, como comportamentos ansiogênicos e estresse. A doença periodontal, por sua vez, é uma doença com forte envolvimento imunológico, sistema que faz parte do processo de estresse, e assim também pode apresentar relação com modulações referentes ao estresse crônico (Castro et al., 2020). Já a xerostomia, caracterizada pela sensação de boca seca, é comumente relatada por indivíduos que relatam apresentar estresse psicológico. Diante da natureza sistêmica do estresse crônico e sua associação com distúrbios orais, torna-se plausível a hipótese de que as glândulas salivares também estejam entre os alvos comprometidos por esse desequilíbrio. Essa relação pode ser evidenciada pelo aumento da concentração de cortisol salivar durante episódios de estresse, demonstrando uma ligação direta entre o estresse e as glândulas salivares (Bozovic et al., 2013).

As glândulas salivares, responsáveis pela síntese e excreção da saliva, desempenham funções críticas para a manutenção da integridade da cavidade oral. A produção e o fluxo salivar é regulada por mecanismos neuroendócrinos complexos, que envolvem tanto o sistema nervoso autônomo quanto a mediação hormonal, tornando essas glândulas particularmente vulneráveis a alterações causadas por estímulos crônicos, como o estresse. A importância da saliva vai além da lubrificação oral, visto que a mesmo contribui com funções imunológicas, digestivas, remineralizadoras e reguladoras do pH, sendo sua relação, portanto, fundamental para outras doenças orais, principalmente para as doenças periodontais e cárie (Chibly et al., 2022; Saruta et al., 2020).

Neste cenário, o estudo dos efeitos do estresse crônico sobre as glândulas salivares apresenta relevância científica e clínica, considerando-se o possível comprometimento estrutural e funcional dessas glândulas frente a um estado persistente de ativação neuroendócrina. Sob esta perspectiva, o presente estudo utilizou um modelo experimental animal para investigar os efeitos do estresse crônico sobre as glândulas salivares maiores, com foco em parâmetros bioquímicos oxidativos e nas alterações morfológicas teciduais decorrentes da exposição contínua ao estressor.

2. ARTIGO DA DISSERTAÇÃO: O ESTRESSE CRÔNICO DESENCADEIA COMPROMETIMENTOS NO STATUS REDOX DAS GLÂNDULAS SALIVARES ASSOCIADOS A DIFERENTES RESPOSTAS HISTOLÓGICAS EM RATOS

STRESS
2025, VOL. 28, NO. 1, 2447114
https://doi.org/10.1080/10253890.2024.2447114

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Chronic stress triggers impairments of the redox status of salivary glands associated with different histological responses in rats

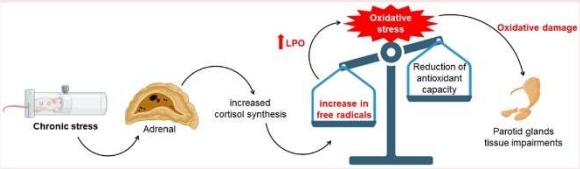
Deiweson Souza-Monteiro[a], Walessa Alana Bragança Aragão[a], Yago Gecy de Sousa Né[a], Paulo Fernando Santos Mendes[a], Antonio Hernandes Chaves-Neto[b], Lílian Lund Amado[c] and Rafael Rodrigues Lima[a]

[a]Laboratory of Functional and Structural Biology, Biological Sciences Institute, Federal University of Pará, Belém, Brazil; [b]Department of Basic Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba, Brazil; [c]Laboratory of Ecotoxicology, Biological Sciences Institute, Federal University of Pará, Belém, Brazil

ABSTRACT

Stress occurs as a reaction to mental and emotional pressure, anxiety, or scarring. Chronic stress is defined as constant submission to these moments. It can affect several body systems, increase blood pressure, and weaken immunity, thereby interfering with physiological health processes. Thus, this study aims to evaluate the effects of chronic stress on the redox status and histomorphological parameters of salivary glands. Thirty-two albino Wistar male rats were randomly divided into two groups: chronic stress and control. Chronically stressed animals were subjected to a restraint protocol by introducing them into a polyvinyl tube for 4 hours daily for 28 days, allowing immobilization of their movements. Subsequently, the animals were euthanized for further collection of the parotid and submandibular salivary glands. The redox state of the glands was evaluated using the antioxidant capacity against peroxyl radicals (ACAP) and thiobarbituric acid reactive substances (TBARS) assays. Histological analysis was performed through morphometry of the tissues stained with hematoxylin and eosin and histochemical through picrosirius red staining. Both the parotid and submandibular glands of stressed rats exhibited oxidative stress due to a decrease in ACAP and an increase in TBARS levels. However, the parotid glands are more susceptible to harmful changes in the tissue, such as an increase in the stromal area and in the collagen area fraction, decrease in the acinar area, and smaller size of the acinus and ducts. Our results suggest that chronic stress may cause harmful modulation of the redox state of the salivary glands, with different histological repercussions.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 7 May 2024
Accepted 8 December 2024

KEYWORDS
Chronic stress; salivary glands; oxidative stress; histomorphometric; PicroSirius Red; physical immobilization

Introduction

Stress is defined as a pattern of physiological, emotional, cognitive, and behavioral reactions to certain situations (Houtman et al., 2007). Financial, educational, security, work, violence, and medical problems are some of the major stressors in society, along with relationships and problems with basic amenities (Singh et al., 2019). Exposure of the body to external or internal negative factors promotes nonspecific reactions that activate the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (Hong et al., 2021). The activation of the HPA axis begins with the release of corticotropin from the hypothalamus, which in turn stimulates the release of adrenocorticotrophic hormones (ACTH) by the pituitary gland (Marin et al., 2011). These hormones act on the adrenal gland, releasing increased levels of glucocorticoids, such as cortisol, which trigger physiological reactions throughout the body as an adaptive response to stressors (Iob & Steptoe, 2019).

A prolonged increase in cortisol levels promotes an imbalance in the homeostasis of immune functions and inflammatory processes, resulting in the excessive release of

CONTACT Rafael Rodrigues Lima rafalima@ufpa.br Laboratory of Functional and Structural Biology, Institute of Biological Sciences, Federal University of Pará, Augusto Corrêa Street n. 1, Campus do Guamá, Belém, Pará, 66075-110, Brazil.
Supplemental data for this article can be accessed online at https://doi.org/10.1080/10253890.2024.2447114.

proinflammatory cytokines (Zefferino et al., 2021). The establishment of a prolonged inflammatory process can trigger an oxidative imbalance by the activation of immune cells, such as neutrophils and macrophages, which produce reactive oxygen species (ROS) (Dinh et al., 2014). Besides, cortisol intensifies catabolism, leading to an increase in the activity of the electron transport chain, increasing the generation of ROS (Zefferino et al., 2021). This homeostatic imbalance causes oxidative stress, which promotes alterations in several biomolecules such as lipids, proteins, carbohydrates, and DNA (Mendes et al., 2014).

In this context, chronic stress compromises physiological functioning at the systemic level and may reach the oral cavity, as evidenced by previous studies on periodontal tissues (Castro, Ferreira et al., 2020, Castro, Nascimento et al., 2020). Thus, considering the systemic reach of stress, it can be suggested that salivary glands are also affected by this homeostatic imbalance (Keremi et al., 2017). Salivary gland function is coordinated by the sympathetic and parasympathetic nervous systems, which control salivary flow and protein secretion (Saruta et al., 2020). Almost all stressors have the ability to activate both the sympathetic nervous system and the HPA axis and, as mentioned previously, can release molecules that contribute to the excessive release of free radicals, contributing to the generation of oxidative stress (Juszczyk et al., 2021).

Upon chronic stress, cortisol levels can also be measured in salivary contents and are correlated with blood cortisol levels, reflecting activation of the HPA axis (Blair et al., 2017). However, salivary gland investigations need to be advanced beyond the issue of stress biomarkers in saliva; thus, the aim of this study was to investigate the possible redox status and histomorphological changes in salivary glands.

Materials & methods

Ethics statement and experimental groups

To conduct this study, the license of the Ethics Committee for the Use of Experimental Animals from the Federal University of Pará was obtained under protocol number CEUA-UFPA 9109300420. Albino Wistar rats (*Rattus norvegicus albinus*) weighing 200–250 g ($n = 32$, 90 days old) were obtained from the Central Bioterium of Federal University of Pará. They were housed in the Bioterium of Pharmacology and Biochemistry Post-graduation Program and allocated to plastic cages (41 cm × 34 cm × 18 cm) appropriate to the species following the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (Kilkenny et al., 2010). Before selection, all animals were subjected to a one-day immobilization protocol to ensure their suitability for this behavioral experiment. All animals exhibited similar behavior during this initial phase; therefore, 32 rats were selected. Subsequently, the animals were randomly assigned into two experimental groups: control group and chronic stress group ($n = 16$ per group). Eight animals of each group were used for the biochemistry assays while the remaining eight animals per group were used for the histological analysis. The sample size was based on a previous study with the same immobilization protocol and redox state assays (Castro, Nascimento et al., 2020).

The chronic stress group was subjected to a previously established protocol for chronic stress induced by physical immobilization (Castro, Nascimento et al., 2020; Semenoff-Segundo et al., 2012). The animals were placed in size-compatible polyvinyl tubes (28 cm, internal diameter: 50 cm). These tubes were closed with a polyvinyl cap that allowed adaptation of the full animal body inside the tube, with holes to allow breathing, and were subjected to physical restraint for 4 hours daily for 28 days. The control group was maintained in the same environment without any intervention. Both groups received controlled pelleted food (Presence, Neovia, Brazil), filtered water *ad libitum* and the room was under standard conditions with 12h light/dark cycle and controlled temperature (25 °C). Figure 1 illustrates the methodological design and it was based on a methodological figure from a previously study published by our group (Castro, Nascimento et al., 2020).

Biological samples resection

The salivary glands were collected for further analysis evaluating the following outcomes: oxidative biochemistry essays (Antioxidant capacity against peroxyl radicals and Determination of Thiobarbituric Acid Reactive Substances) and histological analysis (morphometric evaluation and collagen analysis). From this perspective, eight animals from each group were intraperitoneally anesthetized with a solution of ketamine

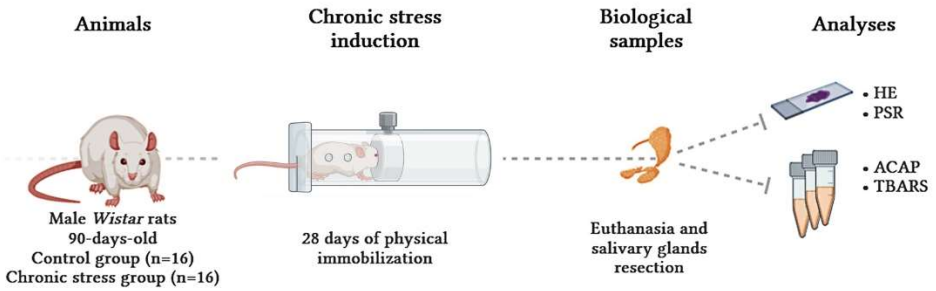


Figure 1. Methodological description of the study. Rats were submitted to a chronic stress induction protocol of 4 daily hours of physical immobilization during 28 days. The salivary glands were collected and submitted to histological analyses by hematoxylin & eosin (HE) and PicroSirius Red (PSR) staining and to biochemical analyses by antioxidant capacity against peroxyl radicals (ACAP) and determination of thiobarbituric acid reactive substances (TBARS) assays.

hydrochloride (90 mg/kg, 10%) and xylazine hydrochloride (2 mg/kg, 2%). After complete loss of the corneal and limb reflexes, the animals were euthanized by cardiac exsanguination, and their parotid and submandibular glands were resected. The organs were immediately frozen in liquid nitrogen and stored at −80° for further biochemistry assays. The remaining 16 animals were intraperitoneally anesthetized using the protocol described above. The animals were then perfused with heparinized (1%) saline solution (0.9%) and formaldehyde (4%) for fixation. The salivary glands of these animals were collected and post-fixed in a 4% formaldehyde solution until histological analysis. There was no need to euthanize any animal prior to the planned end. Thus, all the 32 animals were euthanized at the planned end and included in this study.

Oxidative biochemistry assays

As a pretreatment for the biochemical assays, the samples were thawed and resuspended in Tris-HCl solution (20 mM, pH 7.4) for sonic disintegration (~1 g/mL). The supernatants were used for the antioxidant capacity against peroxyl radical (ACAP) and lipid peroxidation (LPO) assays.

Antioxidant capacity against peroxyl radicals

ACAP was analyzed using the reactive oxygen species (ROS) quantitation produced by the equally-concentrated samples (2.5 µg proteins/µL) after exposed to a peroxyl radical generator (Amado et al., 2008). Peroxyl radicals were produced by the thermal (35 °C) decomposition of 2, 2′-azobis 2 methylpropionamidine dihydrochloride (ABAP; 4 mM; Sigma-Aldrich, St. Louis, MI, USA). For ROS determination, the compound 2′,7′-dichlorofluorescein diacetate (H2DCF-DA, Invitrogen™, Whaltam, MA) was used at a final concentration of 40 nM. Readings were performed using a fluorescence microplate reader (Victor X3, Perkin Elmer, Waltham, MA) every 5 min for 1 h. The relative difference between the ROS areas with and without ABAP was considered as a measure of antioxidant capacity. The results were converted to the inverse of the relative area.

Determination of Thiobarbituric Acid Reactive Substances (TBARS)

The samples were incubated with a thiobarbituric acid solution at 94 °C in a water bath for 60 min. After cooling to room temperature, n-butyl alcohol was added and the mixture was vortexed and centrifuged (2500 rpm for 10 min). Also, we performed protein content determination in the crude homogenate of the samples by Bradford's method (Bradford, 1976) to correct the TBARS results. The results are expressed as nmol/g of protein (Percário et al., 1994).

Histological analysis

The stored salivary glands were dehydrated using increasing concentrations of ethanol (70%, 80%, 90%, absolute I, and absolute II), diaphanized in xylol, and embedded in Paraplast (McCormick Scientific, Baltimore, MD). After inclusion, the materials were sliced using a microtome (Leica Biosystems, RM2125 RTS, Nussloch, Germany) to obtain sections with a thickness of 5 µm and placed on individual slides. Three slides of each animal with two sections were stained with hematoxylin and eosin (HE) while three slides of each animal with two sections were stained with PicroSirius Red. Both were photomicrographed using a digital color camera (DS-Fi3, Nikon, Tokyo, Japan) connected to an optical microscope (Nikon Eclipse CiH550s, Tokyo, Japan), however photomicrographs of sections stained with PicroSirius Red were obtained through polarized light microscope to determine the collagen content (Juengsomjit et al., 2022). For the morphometric analysis on the HE sections, the total stroma area, total acinar area, total ductal area, mean acini size and mean duct size parameters of tissue morphology were evaluated and expressed in μm^2 (Fernandes et al., 2015; Souza-Monteiro et al., 2022). Values were obtained using the ImageJ software (NIMH of Health, Bethesda, MD) digital image analyzer. The collagen content was also analyzed thorough Image J by taking the arithmetic mean of threshold percentages from five fields/section. DS-M was aware of the experimental stages and conduction of the analysis.

Statistical analysis

Data were plotted using GraphPad Prism 7.0 (GraphPad Software Inc., La Jolla, CA). To analyze the Gaussian distribution of our data, we performed the Shapiro-Wilk normality test. Parametric data were then submitted to student's *T* test, while non-parametric data was submitted to Mann Whitney test to perform comparisons between the groups, considering a statistical significance level of $p < 0.05$. Biochemical and morphometry results were expressed as mean ± standard error of the mean. Histochemical results were expressed as median and interquartile range. The test power was calculated using the difference between the groups' averages with OpenEpi software (Version 2.3.1, Emory University, Atlanta, GA), considering a type I error of 5% and a power of 80%. All the data values are available on supplemental file (Table S1).

Results

Chronic stress promotes oxidative stress on salivary glands of rats

Our results show that chronic stress reduced the ACAP levels in the parotid gland (control: 5.86 ± 0.34, chronic stress: 4.39 ± 0.39, $p = 0.01$) (Figure 2(A)) and in the submandibular gland (control: 4.85 ± 0.40, chronic stress: 3.15 ± 0.37, $p = 0.01$). Besides, the immobilization model also increased the TBARS levels in the parotid gland (control: 0.98 ± 0.18 nmol/g of protein, chronic stress: 2.12 ± 0.28 nmol/g of protein, $p = 0.007$) and in the submandibular gland (control: 0.55 ± 0.21 nmol/g of protein, chronic stress: 1.79 ± 0.23 nmol/g of protein, $p = 0.005$) (Figure 2(B)).

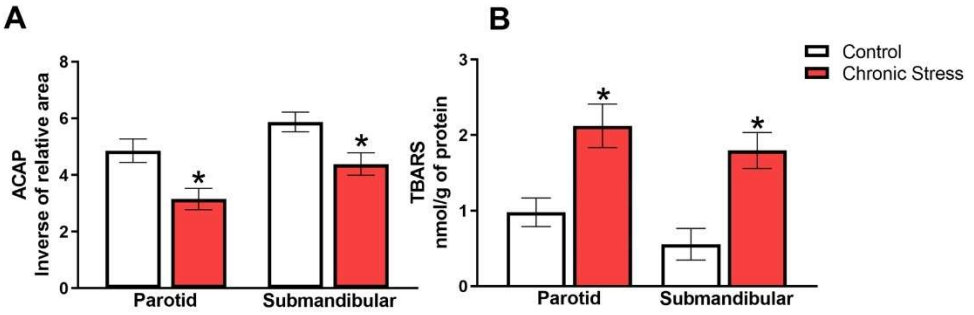


Figure 2. Effects of chronic stress on the oxidative biochemistry of salivary glands of rats. In A, Antioxidant capacity against peroxyl radicals (ACAP) and in B, Thiobarbituric Acid Reactive Substances (TBARS) levels of chronically stressed rats ($n = 8$/group). The results are expressed as the mean ± standard error of the mean (SEM). Student's t-test, $^*p < 0.05$.

Table 1. Morphometric measurements of salivary glands of rats submitted to chronic stress (mean ± SEM).

	Submandibular glands			Parotid glands		
Measures	Control group	Chronic stress group	p Value	Control group	Chronic stress group	p Value
Total stroma area (μm^2)	5020 ± 468	4166 ± 703	0.3421	7272 ± 1316	12395 ± 1327	0.0337*
Total acinar area (μm^2)	56579 ± 669	56711 ± 2265	0.9521	57135 ± 1249	52034 ± 1496	0.0397*
Total ductal area (μm^2)	5101 ± 789	3936 ± 576	0.2673	3496 ± 605	2818 ± 789	0.5212
Mean acini size (μm^2)	37.25 ± 2.60	26.07 ± 4.91	0.0795	40.84 ± 2.34	29.81 ± 0.91	0.0046*
Mean duct size (μm^2)	61.25 ± 7.63	62.86 ± 2.81	0.8498	83.6 ± 13.3	40.23 ± 4.49	0.0214*

*(Student's t test, $p < 0.05$).

The histomorphometry of parotid gland of rats suffers changes after exposure to chronic stress

Our data revealed that chronic stress affected the histomorphology of the parotid gland. The total stromal tissue area increased in rats subjected to the immobilization protocol ($p = 0.03$). In addition, our data revealed a decrease in total acinar area after stress ($p = 0.03$) and in the acini size ($p = 0.004$). The size of the ducts was reduced ($p = 0.02$); however, the total duct area was not affected by the stress protocol ($p = 0.52$). In the submandibular glands, no statistical differences were found in the parameters evaluated ($p > 0.05$) (Table 1, Figure 3).

Chronic stress altered the collagen fibers content of parotid gland

In addition to the histomorphometry results, our histochemical analysis revealed that the area fraction of collagen fibers on the parotid gland of stressed animals (median 27.85% interquartile range: 25.60–33.15%, Figure 4) was significantly higher than in salivary glands of control animals (median: 17.99%, interquartile range: 11.78–22.94%) ($p = 0.005$). On the other hands, in the submandibular glands, no difference was observed between the groups (control median: 22.35% [interquartile range: 18.24–24.91%], chronic stress median: 17.18% [interquartile range: 13.22–22.21%], $p = 0.17$, Figure 4).

Discussion

Our study showed that histomorphological impairments were related to oxidative stress in the parotid glands of chronically stressed rats. Animals subjected to stress conditions by body restraint present a decrease in the antioxidant competence and an increase in lipid peroxidation, culminating in oxidative stress in both the parotid and submandibular glands, which are the major organs responsible for the synthesis of saliva. These effects on the parotid gland also may also be related to harmful changes observed in this gland tissue.

Stress is a stimulus that evokes a biological response. Bodily responses to these stimuli are usually associated with actions that impair homeostasis (Park et al., 2019). Stress is related to the prevalence of illness in the general population because it can affect mental and physical functions that affect many bodily processes. Anxiety, nervousness, and depressive symptoms are associated with stress (Salari et al., 2020). It has been shown that stress conditions can induce disturbance on the central nervous system (CNS) by disrupting neuronal death and blood brain barrier permeability in both humans (Hanin, 1996) and rodents (Sántha et al., 2015). However, the underlying damage was not restricted to the CNS. Literature provides significant evidence of the effects of stress on the immune, cardiovascular, endocrine, and gastrointestinal systems (Yaribeygi et al., 2017). Studies have suggested that stress can affect digestive function by modifying the processes of absorption, intestinal permeability, and mucus secretion (Nabavizadeh et al., 2011).

Repeated episodes of restraint stress can sustain an increase in circulating corticosterone levels in rats (Zhang et al., 2014) and animals tend to respond to stressful events with alterations in immune homeostasis and other molecular changes (Monteiro et al., 2015). Inflammatory cytokine levels are altered after chronic stress induced by immobilization (Voorhees et al., 2013; Johnson et al., 2019), as well as the oxidative response in the blood (Castro, Nascimento et al., 2020). The systemic effects observed in the blood of

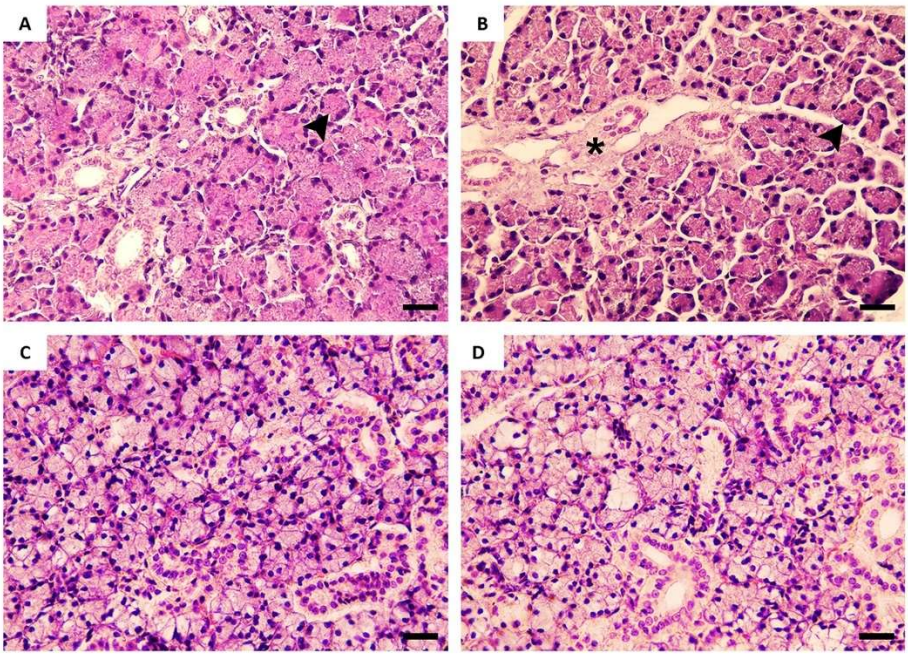


Figure 3. Effects of chronic stress on the morphology of salivary glands of rats. Representative photomicrographs of control (A) and chronic stress (B) parotid glands and control (C) and chronic stress (D) submandibular glands ($n = 8$/group). Arrowheads highlights the difference in acinus diameter between control and chronic stress parotid glands. Asterisk indicates the increase in stromal tissue observed in the parotid gland. No histomorphological changes were observed in the submandibular gland. Sections were stained with hematoxylin and eosin. Scale bar = 10 µm.

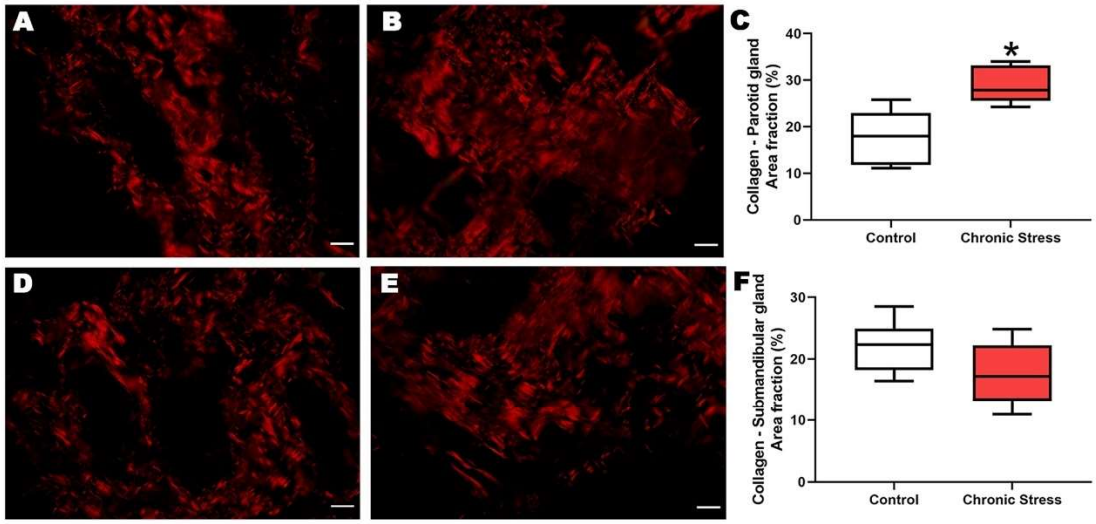


Figure 4. Effects of chronic stress on the collagen fibers of salivary glands of rats. Representative photomicrographs of control (A) and chronic stress (B) parotid glands and control (D) and chronic stress (E) submandibular glands ($n = 8$/group). Percentages of collagen areas of parotid (C) and submandibular (F) glands. Sections were stained with PicroSirius Red. Results are expressed as median and quartiles. Mann-Whitney test, $^*p < 0.05$. Scale bar: 20 µm.

chronically stressed rats include a decrease in antioxidant molecules, such as glutathione, and increase in lipid peroxidation by higher levels of TBARS, a pro-oxidant parameter that establishes oxidative stress (Castro, Nascimento et al., 2020). Furthermore, still related to the last study, chronic stress also affected the nitrosative status of the blood by increasing the nitric oxide concentration. In our study, the redox state of the salivary glands was assessed using ACAP and TBARS. During ACAP analysis, a generator of peroxyl radicals was added to the samples, and the activity of the samples toward the

interception of the harmful ROS molecules generated was measured as a parameter of their antioxidant state (Amado et al., 2009). Oxidative modulation caused by chronic stress may also be related to the local oxidative alterations observed in the glandular tissue. These systemic changes can have downstream effects on various tissues, including glandular tissues such as the salivary glands. Specifically, the systemic oxidative stress and inflammatory responses observed in the blood of chronically stressed rats could contribute to changes in the redox status and overall health of the glandular tissue. Both the parotid and submandibular glands showed impairment in their antioxidant systems, as observed by the reduction in ACAP levels after chronic stress. Our study is the first to show that chronic stress affects the antioxidant activity of the organs responsible for saliva production and synthesis. Thus, it plays an important role in understanding the modulation of salivary biomarkers used in other analyses. By understanding these antioxidant mechanisms within the salivary glands, we can gain insights into how these glands respond to stressors and how changes in their redox status might be reflected in salivary biomarkers. This knowledge is essential for interpreting salivary biomarkers accurately, particularly in the context of stress-related conditions or diseases. For instance, oxidative stress in the glandular tissue could alter the levels of oxidative biomarkers in saliva and affect the presence of antioxidants molecules.

Regarding the prooxidant parameter, the lipid peroxidation of the salivary glands was measured by the TBARS levels. The TBARS assay detects molecules related to lipid peroxidation via a facile reaction with thiobarbituric acid. These molecules are released as a result of the attack of polyunsaturated fatty acids on the lipid membrane by ROS and reactive nitrogen species (Leon & Borges, 2020). TBARS measures the levels of molecules, such as malondialdehyde, as indicators of lipid peroxidation. Studies from our group investigating salivary glands have shown that TBARS is a sensitive method for evaluating the damage caused by aggressors such as ethanol or fluoride intoxication (Fernandes et al., 2015; Lima et al., 2021). Our data revealed that, in addition to antioxidant impairment, chronic stress may also modulate an increase in lipid peroxidation, as observed by the TBARS levels, characterizing an oxidative stress state in the salivary glands. An increase in malondialdehyde levels associated with the triggering of oxidative stress after chronic stress exposure has already been shown in the brain, serum, liver, and kidneys of rats (Samarghandian et al., 2017; Almohaimeed et al., 2021). In the serum, this alteration has also been associated with an increase in inflammatory markers and alterations in the histology of the salivary glands (Almohaimeed et al., 2021), which is in accordance with our findings.

An imbalance between free radicals and antioxidant molecules observed during oxidative stress is harmful to the body. In the salivary glands, these pathogenic mechanisms are associated with the modulation of the regulation of proteins related to calcium homeostasis, mitochondrial function, cellular metabolism, and cytoskeleton constituents (Souza-Monteiro et al., 2022). Additionally, oxidative stress is associated with salivary gland DNA damage (Aragão et al., 2022). This alteration on the redox process involves the ROS oxidation of low-density protein and lipid accumulation on the formation of fibrotic tissue in other organs increasing the level of inflammatory mediators, such as TNF-α, IL-1β and TGF-β (Pizzino et al., 2017).

On the stomatognathic system, studies shows that modulation on the inflammation of inflammatory diseases, such as periodontitis and apical periodontitis, can also be related to chronic stress. Chronic unpredictable stress increases the intensity of inflammatory infiltrates, periapical lesion volume, and bone loss in rats with apical periodontitis (Minhoto et al., 2021). In the periodontium without endodontic tissue-related lesions, restraint stress alters the activity of osteoclastic and osteoblastic signaling metabolism, enhancing damage to the alveolar bone microstructure (Castro, Nascimento et al., 2020). These findings confirm the hypothesis that chronic stress can reach the oral and maxillofacial structures and thus affect the salivary glands. Hormones derived from glucocorticoids, such as corticosterone and cortisol, have been linked to generation of ROS, highlighting this possible via of mechanism of the chronic stress (Aschbacher et al., 2013; Zefferino et al., 2021).

As mentioned previously, chronic stress could also affect the integrity of the histological features of the glands, as an imbalance in the redox state could affect cellular biology and thus the integrity of its features. From this perspective, we investigated whether the harmful redox scenario could be reversed in salivary gland tissue. Interestingly, our findings showed that the salivary glands presented different responses to redox modulation. The submandibular gland was more resistant than the parotid gland, which showed alterations in both parenchymal and stromal features (Table 1, Figure 3). While the stromal area increased, and the acinar area, acini, and duct sizes decreased in the parotid glands after chronic stress, these parameters remained unaltered in the submandibular glands. Although the submandibular and parotid glands are both salivary glands, each is a separate organ with different constitutions and is regulated in different ways, which could explain the different findings observed among the glands (Kondo et al., 2015).

Parasympathetic innervation of the parotid gland begins when the auriculotemporal nerve stimulates the inferior salivatory nucleus. Subsequently, the axons travel along the ninth cranial nerve and pass through the jugular and otic ganglia. In contrast, the innervation of the submandibular gland begins in the superior salivary nucleus. It then travels through the chorda tympani branch of the facial nerve and the submandibular ganglion before reaching the gland through the postganglionic fibers (Maruyama et al., 2019). In addition, the parotid gland makes a greater contribution to the whole saliva during stimulation, whereas the submandibular gland contributes more during rest (Proctor & Carpenter, 2014). Given the distinct characteristics of the glands and the key mechanisms associated with autonomic nervous system activation due to chronic stress, the differing responses observed between the glands might be explained by each unique patterns of innervation and regulation that the different glands present. Different activation pathways are involved in the pathogenic mechanism of chronic stress in the CNS, thus promoting different effects on peripheral organs (Maruyama et al., 2019). From this perspective, parotid glands

could be more susceptible to the damage as during chronic stress the animals have behaviors that activates this gland more. This phenomenon could be involved in the increased area and size of the parenchyma components observed in the parotid gland, and impairments in the signaling pathways responsible for the regulation of these cells could trigger changes in their function, altering their morphology.

Salivary glands are primarily composed of two types of cells: fluid-secreting acinar structures and those forming the draining network of ducts. The submandibular gland comprises a mixed population of mucous and serous acini that serve as a source of antibodies, whereas the parotid gland mainly comprises serous acini that secrete a solution containing proline-rich proteins, amylase, and antibodies. Their ductal network is also different from that of the parotid gland, exhibiting longer intercalated ducts and shorter striated ducts than the submandibular. Interestingly, we observed alterations in the parotid gland's ductal system (Figure 3, Table 1). The literature provides data on ductal cell changes following stressful events in rats. Depressive animals showed an increase in cytokines such as vascular endothelial growth factor in ductal cells and Brain-derived neurotrophic factor (BDNF) in the glandular tissue of the submandibular gland. The authors discussed these changes as potential protective mechanisms by these cells against stressors, as well as the involvement of BDNF in tissue repair and remodeling processes (Eldomiaty et al., 2023). In our study, the submandibular gland's ductal system did not exhibit any alterations, which may be related to the aforementioned data, highlighting the gland's ability to develop protective mechanisms under stress. The histological differences between the submandibular and parotid glands could explain why the parotid gland exhibited ductal system alterations while the submandibular gland did not, as well as the endocrine characteristics observed in the latter gland.

Parotid gland has a large amount of connective tissue with septa containing numerous fat cells (Kondo et al., 2015; Saruta et al., 2010). Our histological analyses showed that the stromal tissue was enhanced after stress only in the parotid glands, which could be explained by a more sensitive response to the activation of fat cells, which are more abundant in the parotid connective tissue. The authors noted that prominent stroma might portend an increased propensity for malignant changes (Ito et al., 2009), highlighting the damage caused by chronic stress.

Additionally, we examined the collagen content within the stromal tissue to ascertain whether chronic stress might impact the collagen fibers of this component (Figure 4). Consistent with the histomorphometric findings, our data indicated that the proportion of PicroSirius Red staining area was greater in the collagen fibers of the parotid gland in stressed animals compared to control animals, a phenomenon not observed in the submandibular gland. PicroSirius Red selectively binds to collagens by interacting through its sulfonic acid groups with the basic groups found within the collagen molecules. When this staining technique is employed alongside a polarized microscope, it enables an accurate identification and characterization of tissue components containing collagen (Manjunatha et al., 2015). Research employing this method has indicated that varied lesions within distinct salivary glands can yield divergent outcomes, as they are influenced by pathology mechanisms, histogenetic factors, and proliferation rates (Manjunatha et al., 2015; Junqueira et al., 1979). Inflammatory salivary gland diseases exhibiting higher invasiveness tend to produce increased areas of orange-red collagen compared to less aggressive lesions (Allon et al., 2006). These findings suggest potential associations with fibrosis production for tissue healing, protective mechanisms, or the process of epithelial-mesenchymal interaction facilitating nutrient and waste exchange during lesion progression (Junqueira et al., 1979; Juengsomjit et al., 2022). Consequently, our data indicates that chronic stress could impair the parotid gland, affecting the connective stroma tissue and resulting in heightened collagen content, possibly indicative of a fibrotic response to the aggression.

Conclusions

Chronic stress in rats triggers the modulation of the antioxidant competence and pro-oxidant functions of salivary glands, culminating in the harmful events of oxidative stress. This harmful state promotes a response in the morphology of the parotid glands, affecting important cells related to the functioning of the main salivary glands responsible for whole saliva production during stimulation. In addition, oxidative stress did not affect the morphology of the submandibular glands, highlighting the differences in autonomy between the salivary glands. From a translational perspective, chronic stress could be dangerous to oral health, as it has been shown to modulate the harmful effects on oral homeostasis. Our research demonstrated the evident implications of chronic stress on the salivary glands. However, our data is limited to provide a total comprehensive understanding of how chronic stress influences these organs, necessitating further studies. Moreover, the biochemical and histological alterations observed in our data may be linked to additional impairments, which could be the focus of future investigations, including proteomic alterations, functional changes, and modulation in saliva composition.

Acknowledgements

The authors thank the Federal University of Pará and the São Paulo State University for technical and scientific support. We also acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Pró-Reitoria de Pesquisa e Pós-graduação from Federal University of Pará (PROPESP-UFPA).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

RRL is a researcher from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and received grant under number 312275/2021-8. D.S-M received support by Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil), Finance Code 001. The APC was funded by Pró-Reitoria de Pesquisa e Pós-graduação from Federal University of Pará (PROPESP-UFPA).

Notes on contributors

Deiweson Souza-Monteiro is a Master student affiliated with the Laboratory of Functional and Structural Biology at Federal University of Pará, Brazil. His research interest is oral biology and stress, with focus on the effects of stress on oral diseases and organs.

Walessa Alana Bragança Aragão is a PhD candidate affiliated with the Laboratory of Functional and Structural Biology at the Federal University of Pará, Brazil. Her research interests focus on oral biology and dentistry.

Yago Gecy de Sousa Né is a PhD candidate affiliated with the Laboratory of Functional and Structural Biology at the Federal University of Pará, Brazil. His research interest focus on oral biology.

Paulo Fernando Santos Mendes is a PhD candidate affiliated with the Laboratory of Functional and Structural Biology at the Federal University of Pará, Brazil. His research interests include pharmacology and biochemistry.

Antonio Hernandes Chaves-Neto is a professor at the School of Dentistry, São Paulo State University (Unesp), Araçatuba campus, Brazil. His research interests focus on salivary glands and saliva biochemistry.

Lílian Lund Amado is a professor at the Biological Sciences Institute of the Federal University of Pará, Brazil. Her research interests focus on ecotoxicology and oxidative stress.

Rafael Rodrigues Lima is a professor at the Biological Sciences Institute of the Federal University of Pará, Brazil. His research interests focus on oral biology, chronic stress, and toxicology.

ORCID

Rafael Rodrigues Lima http://orcid.org/0000-0003-1486-4013

References

Allon, I., Vered, M., Buchner, A., & Dayan, D. (2006). Stromal differences in salivary gland tumors of a common histopathogenesis but with different biological behavior: A study with picrosirius red and polarizing microscopy. *Acta Histochemica*, *108*(4), 259–264. https://doi.org/10.1016/j.acthis.2006.05.007

Almohaimeed, H. M., Albadawi, E. A., Mohammedsaleh, Z. M., Alghabban, H. M., Seleem, H. S., Ramadan, O. I., & Ayuob, N. N. (2021). Brain-derived Neurotropic factor (BDNF) mediates the protective effect of *Cucurbita pepo* L. on salivary glands of rats exposed to chronic stress evident by structural, biochemical and molecular study. *Journal of Applied Oral Science: revista FOB*, *29*, e20201080. https://doi.org/10.1590/1678-7757-2020-1080

Amado, L. L., Garcia, M. L., Ramos, P. B., Freitas, R. F., Zafalon, B., Ferreira, J. L. R., Yunes, J. S., & Monserrat, J. M. (2009). A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: Application to evaluate microcystins toxicity. *The Science of the Total Environment*, *407*(6), 2115–2123. https://doi.org/10.1016/j.scitotenv.2008.11.038

Aragão, W. A. B., Bittencourt, L. O., Lima, L. A. D O., de Souza, M. P. C., Nogueira, L. S., Dionizio, A., Buzalaf, M. A. R., de Oliveira, E. H. C., Crespo-Lopez, M. E., & Lima, R. R. (2022). DNA damage and proteomic profile changes in rat salivary glands after chronic exposure to inorganic mercury. *Biological Trace Element Research*, *200*(9), 3983–3995. https://doi.org/10.1007/s12011-021-02986-7

Aschbacher, K., O'Donovan, A., Wolkowitz, O. M., Dhabhar, F. S., Su, Y., & Epel, E. (2013). Good stress, bad stress and oxidative stress: Insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology*, *38*(9), 1698–1708. https://doi.org/10.1016/j.psyneuen.2013.02.004

Blair, J., Adaway, J., Keevil, B., & Ross, R. (2017). Salivary cortisol and cortisone in the clinical setting. *Current Opinion in Endocrinology, Diabetes, and Obesity*, *24*(3), 161–168. https://doi.org/10.1097/MED.0000000000000328

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Dinh, Q. N., Drummond, G. R., Sobey, C. G., & Chrissobolis, S. (2014). Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. *BioMed Research International*, *2014*, 406960–406911. https://doi.org/10.1155/2014/406960

Eldomiaty, M. A., A Hassan, Z., Halawa, A. M., Elnajar, A. M., & Almohamadi, N. (2023). Structural changes and neurotrophic factors upregulation in submandibular gland in a rat model of depression: Proposed correlation with stress indicators during and after the relief of depression. *Anatomical Science International*, *98*(2), 185–195. https://doi.org/10.1007/s12565-022-00686-3

Fernandes, L. M., Teixeira, F. B., Alves-Junior, S. M., Pinheiro, J. J., Maia, C. S., & Lima, R. R. (2015). Immunohistochemical changes and atrophy after chronic ethanol intoxication in rat salivary glands. *Histology and Histopathology*, *30*(9), 1069–1078. https://doi.org/10.14670/HH-11-604

Hanin, I. (1996). The Gulf War, stress and a leaky blood-brain barrier. *Nature Medicine*, *2*(12), 1307–1308. https://doi.org/10.1038/nm1296-1307

Hong, H., Ji, M., & Lai, D. (2021). Chronic stress effects on tumor: Pathway and mechanism. *Frontiers in Oncology*, *11*, 738252. https://doi.org/10.3389/fonc.2021.738252

Houtman, I., Jettinghof, K., & Cedillo, L. (2007). *Raising awareness of stress at work in developing countries: Advice to employers and worker representatives*. World Health Organization.

Iob, E., & Steptoe, A. (2019). Cardiovascular disease and hair cortisol: A novel biomarker of chronic stress. *Current Cardiology Reports*, *21*(10), 116. https://doi.org/10.1007/s11886-019-1208-7

Ito, F. A., Jorge, J., Vargas, P. A., & Lopes, M. A. (2009). Histopathological findings of pleomorphic adenomas of the salivary glands. *Medicina oral, patologia oral y cirugia bucal*, *14*(2), E57–E61.

Johnson, J. D., Barnard, D. F., Kulp, A. C., & Mehta, D. M. (2019). Neuroendocrine regulation of brain cytokines after psychological stress. *Journal of the Endocrine Society*, *3*(7), 1302–1320. https://doi.org/10.1210/js.2019-00053

Juengsomjit, R., Meesakul, O., Arayapisit, T., Larbcharoensub, N., & Janebodin, K. (2022). Polarized microscopic analysis of picrosirius red stained salivary gland pathologies: An observational study. *European Journal of Dentistry*, *16*(4), 930–937. https://doi.org/10.1055/s-0042-1743145

Junqueira, L. C., Bignolas, G., & Brentani, R. R. (1979). Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *The Histochemical Journal*, *11*(4), 447–455. https://doi.org/10.1007/BF01002772

Juszczyk, G., Mikulska, J., Kasperek, K., Pietrzak, D., Mrozek, W., & Herbet, M. (2021). Chronic stress and oxidative stress as common factors of the pathogenesis of depression and Alzheimer's disease: The role of antioxidants in prevention and treatment. *Antioxidants (Basel, Switzerland)*, *10*(9), 1439. https://doi.org/10.3390/antiox10091439

Keremi, B., Beck, A., Fabian, T. K., Fabian, G., Szabo, G., Nagy, A., & Varga, G. (2017). Stress and salivary glands. *Current Pharmaceutical Design*, *23*(27), 4057–4065. https://doi.org/10.2174/1381612823666170215110648

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology*, *8*(6), e1000412. https://doi.org/10.1371/journal.pbio.1000412

Kondo, Y., Nakamoto, T., Jaramillo, Y., Choi, S., Catalan, M. A., & Melvin, J. E. (2015). Functional differences in the acinar cells of the murine major salivary glands. *Journal of Dental Research*, *94*(5), 715–721. https://doi.org/10.1177/0022034515570943

Leon, J. A. D., & Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *Journal of Visualized Experiments: JoVE*, 159, Published 2020 May 12. https://doi.org/10.3791/61122

Lima, L. A. D O., Miranda, G. H. N., Aragão, W. A. B., Bittencourt, L. O., Dos Santos, S. M., de Souza, M. P. C., Nogueira, L. S., de Oliveira, E. H. C., Monteiro, M. C., Dionizio, A., Leite, A. L., Pessan, J. P., Buzalaf, M. A. R.,

& Lima, R. R. (2021). Effects of fluoride on submandibular glands of mice: Changes in oxidative biochemistry, proteomic profile, and genotoxicity. *Frontiers in Pharmacology*, *12*, 715394. https://doi.org/10.3389/fphar.2021.715394

Castro, M. M. L., Ferreira, R. O., Fagundes, N. C. F., Almeida, A. P. C., Maia, L. C., & Lima, R. R. (2020). Association between psychological stress and periodontitis: A systematic review. *European Journal of Dentistry*, *14*(1), 171–179. https://doi.org/10.1055/s-0039-1693507

Castro, M. M. L., Nascimento, P. C., Souza-Monteiro, D., Santos, S. M., Arouck, M. B., Garcia, V. B., Araújo, R. F. D., de Araujo, A. A., Balbinot, G. D S., Collares, F. M., Rosing, C. K., Monteiro, M. C., Ferraz Maia, C. S., & Lima, R. R. (2020). Blood oxidative stress modulates alveolar bone loss in chronically stressed rats. *International Journal of Molecular Sciences*, *21*(10), 3728. https://doi.org/10.3390/ijms21103728

Manjunatha, B. S., Agrawal, A., & Shah, V. (2015). Histopathological evaluation of collagen fibers using picrosirius red stain and polarizing microscopy in oral squamous cell carcinoma. *Journal of Cancer Research and Therapeutics*, *11*(2), 272–276. https://doi.org/10.4103/0973-1482.154061

Marin, M. F., Lord, C., Andrews, J., Juster, R. P., Sindi, S., Arsenault-Lapierre, G., Fiocco, A. J., & Lupien, S. J. (2011). Chronic stress, cognitive functioning and mental health. *Neurobiology of Learning and Memory*, *96*(4), 583–595. https://doi.org/10.1016/j.nlm.2011.02.016

Maruyama, C. L., Monroe, M. M., Hunt, J. P., Buchmann, L., & Baker, O. J. (2019). Comparing human and mouse salivary glands: A practice guide for salivary researchers. *Oral Diseases*, *25*(2), 403–415. https://doi.org/10.1111/odi.12840

Mendes, A. A., Souza, L. F., Walz, R., & Dafre, A. L. (2014). Perspectives on molecular biomarkers of oxidative stress and antioxidant strategies in traumatic brain injury. *BioMed Research International*, *2014*, 723060–723018. https://doi.org/10.1155/2014/723060

Minhoto, G. B., Khoury, R. D., Orozco, E. I. F., Prado, R. F., & Valera, M. C. (2021). Effect of chronic unpredictable stress on the progression of experimental apical periodontitis in rats. *International Endodontic Journal*, *54*(8), 1342–1352. https://doi.org/10.1111/iej.13515

Monteiro, S., Roque, S., de Sá-Calçada, D., Sousa, N., Correia-Neves, M., & Cerqueira, J. J. (2015). An efficient chronic unpredictable stress protocol to induce stress-related responses in C57BL/6 mice. *Frontiers in Psychiatry*, *6*, 6. https://doi.org/10.3389/fpsyt.2015.00006

Nabavizadeh, F., Vahedian, M., Sahraei, H., Adeli, S., & Salimi, E. (2011). Physical and psychological stress have similar effects on gastric acid and pepsin secretions in rat. *Journal of Physiology and Biochemistry*, *7*, 164–174.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. (2011). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press (US).

Park, C., Rosenblat, J. D., Brietzke, E., Pan, Z., Lee, Y., Cao, B., Zuckerman, H., Kalantarova, A., & McIntyre, R. S. (2019). Stress, epigenetics and depression: A systematic review. *Neuroscience and Biobehavioral Reviews*, *102*, 139–152. https://doi.org/10.1016/j.neubiorev.2019.04.010

Percário, S., Vital, A. C. C., & Jablonka, F. (1994). Dosagem do malondialdeído. *Newslab*, *2*(6), 46–50.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, *2017*(1), 8416763. https://doi.org/10.1155/2017/8416763

Proctor, G. B., & Carpenter, G. H. (2014). Salivary secretion: Mechanism and neural regulation. *Monographs in Oral Science*, *24*, 14–29. https://doi.org/10.1159/000358781

Salari, N., Hosseinian-Far, A., Jalali, R., Vaisi-Raygani, A., Rasoulpoor, S., Mohammadi, M., Rasoulpoor, S., & Khaledi-Paveh, B. (2020). Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: A systematic review and meta-analysis. *Globalization and Health*, *16*(1), 57. https://doi.org/10.1186/s12992-020-00589-w

Samarghandian, S., Azimi-Nezhad, M., Farkhondeh, T., & Samini, F. (2017). Anti-oxidative effects of curcumin on immobilization-induced oxidative stress in rat brain, liver and kidney. *Biomedicine & Pharmacotherapy=Biomedecine & pharmacotherapie*, *87*, 223–229. https://doi.org/10.1016/j.biopha.2016.12.105

Sántha, P., Veszelka, S., Hoyk, Z., Mészáros, M., Walter, F. R., Tóth, A. E., Kiss, L., Kincses, A., Oláh, Z., Seprényi, G., Rákhely, G., Dér, A., Pákáski, M., Kálmán, J., Kittel, Á., & Deli, M. A. (2015). Restraint stress-induced morphological changes at the blood-brain barrier in adult rats. *Frontiers in Molecular Neuroscience*, *8*, 88. https://doi.org/10.3389/fnmol.2015.00088

Saruta, J., Sato, S., & Tsukinoki, K. (2010). The role of neurotrophins related to stress in saliva and salivary glands. *Histology and Histopathology*, *25*(10), 1317–1330. https://doi.org/10.14670/HH-25.1317

Saruta, J., To, M., Sakaguchi, W., Kondo, Y., & Tsukinoki, K. (2020). Brain-derived neurotrophic factor is related to stress and chewing in saliva and salivary glands. *The Japanese Dental Science Review*, *56*(1), 43–49. https://doi.org/10.1016/j.jdsr.2019.11.001

Semenoff-Segundo, A., Porto, A. N., Semenoff, T. A. D. V., Cortelli, J. R., Costa, F. O., Cortelli, S. C., & Bosco, A. F. (2012). Effects of two chronic stress models on ligature-induced periodontitis in Wistar rats. *Archives of Oral Biology*, *57*(1), 66–72. https://doi.org/10.1016/j.archoralbio.2011.07.014

Singh, A., Arora, M., Sharma, V., & Kotwal, A. (2019). Stress: Prevalence and correlates among residents of a suburban area. *Industrial Psychiatry Journal*, *28*(1), 98–102. https://doi.org/10.4103/ipj.ipj_33_18

Souza-Monteiro, D., Guerra, M. C. D. S., Bittencourt, L. O., Aragão, W. A. B., Dionizio, A., Silveira, F. M., Buzalaf, M. A. R., Martins, M. D., Crespo-Lopez, M. E., & Lima, R. R. (2022). Salivary glands after prolonged aluminum exposure: Proteomic approach underlying biochemical and morphological impairments in rats. *International Journal of Molecular Sciences*, *23*(4), 2251. https://doi.org/10.3390/ijms23042251

Voorhees, J. L., Tarr, A. J., Wohleb, E. S., Godbout, J. P., Mo, X., Sheridan, J. F., Eubank, T. D., & Marsh, C. B. (2013). Prolonged restraint stress increases IL-6, reduces IL-10, and causes persistent depressive-like behavior that is reversed by recombinant IL-10. *PloS One*, *8*(3), e58488. https://doi.org/10.1371/journal.pone.0058488

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T. P., & Sahebkar, A. (2017). The impact of stress on body function: A review. *EXCLI Journal*, *16*, 1057–1072. https://doi.org/10.17179/excli2017-480

Zefferino, R., Di Gioia, S., & Conese, M. (2021). Molecular links between endocrine, nervous and immune system during chronic stress. *Brain and Behavior*, *11*(2), e01960. https://doi.org/10.1002/brb3.1960

Zhang, W., Hetzel, A., Shah, B., Atchley, D., Blume, S. R., Padival, M. A., & Rosenkranz, J. A. (2014). Greater physiological and behavioral effects of interrupted stress pattern compared to daily restraint stress in rats. *PloS One*, *9*(7), e102247. https://doi.org/10.1371/journal.pone.0102247

3. CONCLUSÃO

O estresse crônico em ratos induz alterações no equilíbrio redox celular, impactando funções antioxidantes e pró-oxidantes e resultando em estresse oxidativo. Esse desequilíbrio provoca modificações na morfologia das glândulas parótidas, comprometendo células essenciais para a produção de saliva. De forma interessante, a morfologia das glândulas submandibulares não foi afetada, evidenciando diferenças na resposta entre as glândulas salivares à um evento estressor. Do ponto de vista translacional, esses achados sugerem que o estresse crônico pode representar um risco à saúde bucal, influenciando negativamente a homeostase oral.

Embora nossa pesquisa demonstre claramente os impactos do estresse crônico nas glândulas salivares, ainda há lacunas na compreensão completa desse processo. Estudos adicionais são necessários para elucidar os mecanismos envolvidos e suas possíveis consequências. Além disso, as alterações bioquímicas e histológicas observadas podem estar associadas a outros prejuízos, como modificações proteômicas, alterações funcionais e variações na composição da saliva, que devem ser exploradas em investigações futuras.

4. REFERÊNCIAS

ALOTIBY, Amna. Immunology of stress: a review article. **Journal of Clinical Medicine**, v. 13, n. 21, p. 6394, 2024.

BOFF, Sérgio Ricardo; OLIVEIRA, Alexandre Gabarra. Aspectos fisiológicos do estresse: uma revisão narrativa. **Research, Society and Development**, v. 10, n. 17, p. e82101723561-e82101723561, 2021.

BOZOVIC, Djordje; RACIC, Maja; IVKOVIC, Nedeljka. Salivary cortisol levels as a biological marker of stress reaction. **Med Arch**, v. 67, n. 5, p. 374-377, 2013.

CASTRO, Micaele ML et al. Association between psychological stress and periodontitis: a systematic review. **European journal of dentistry**, v. 14, n. 01, p. 171-179, 2020.

CHIBLY, Alejandro M. et al. Salivary gland function, development, and regeneration**. Physiological reviews**, v. 102, n. 3, p. 1495-1552, 2022.

DINH, Quynh N. et al. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. **BioMed research international**, v. 2014, n. 1, p. 406960, 2014.

GRADUS, Jaimie L. Prevalence and prognosis of stress disorders: a review of the epidemiologic literature. **Clinical epidemiology**, p. 251-260, 2017.

O'CONNOR, Daryl B.; THAYER, Julian F.; VEDHARA, Kavita. Stress and health: A review of psychobiological processes. **Annual review of psychology**, v. 72, n. 1, p. 663-688, 2021.

ROBERTS, Brandon L.; KARATSOREOS, Ilia N. Brain–body responses to chronic stress: a brief review. **Faculty reviews**, v. 10, 2021.

RUSSELL, Georgina; LIGHTMAN, Stafford. The human stress response. **Nature reviews endocrinology**, v. 15, n. 9, p. 525-534, 2019.

SARUTA, Juri et al. Brain-derived neurotrophic factor is related to stress and chewing in saliva and salivary glands. **Japanese Dental Science Review**, v. 56, n. 1, p. 43-49, 2020.

SELYE, Hans. A syndrome produced by diverse nocuous agents. **Nature**, v. 138, n. 3479, p. 32-32, 1936.

SHCHASLYVYI, Aladdin Y.; ANTONENKO, Svitlana V.; TELEGEEV, Gennadiy D. Comprehensive review of chronic stress pathways and the efficacy of behavioral stress reduction programs (BSRPs) in managing diseases. **International Journal of Environmental Research and Public Health**, v. 21, n. 8, p. 1077, 2024.

SOUZA, Alex Sandro Rolland et al. Factors associated with stress, anxiety, and depression during social distancing in Brazil. **Revista de saude publica**, v. 55, p. 5, 2021.

WHO (World Health Organization), HOUTMAN, I., CEDILLO, L. Raising awareness of stress at work in developing countries: advice to employers and worker representatives, 2007.

YARIBEYGI, Habib et al. The impact of stress on body function: A review. **EXCLI journal**, v. 16, p. 1057, 2017.

ZEFFERINO, Roberto; DI GIOIA, Sante; CONESE, Massimo. Molecular links between endocrine, nervous and immune system during chronic stress. **Brain and behavior**, v. 11, n. 2, p. e01960, 2021.