UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Rayara Nogueira de Freitas

Avaliação dos parâmetros bioquímicos, funcionais, histomorfométricos e estado redox das glândulas salivares de ratos Wistar tratados com o anticonvulsivante valproato de sódio

Araçatuba - SP

2023

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Rayara Nogueira de Freitas

Avaliação dos parâmetros bioquímicos, funcionais, histomorfométricos e estado redox das glândulas salivares de ratos Wistar tratados com o anticonvulsivante valproato de sódio

Dissertação apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Mestre em Ciências, área de concentração Saúde Bucal da Criança.

Orientador: Prof. Ass. Dr. Antonio Hernandes Chaves Neto

Araçatuba - SP

2023

Catalogação-na-Publicação (CIP)

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

Freitas, Rayara Nogueira de.
F866a Avaliação dos parâmetros bioquímicos, funcionais, histomorfométricos e estado redox das glândulas salivares de ratos Wistar tratados com o anticonvulsivante valproato de sódio / Rayara Nogueira de Freitas. - Araçatuba, 2023
37 f. : il. ; tab.

Dissertação (Mestrado) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba
Orientador: Prof. Antonio Hernandes Chaves Neto

1. Anticonvulsivantes 2. Ácido valproico 3. Saliva 4. Glândula parótida 5. Glândula submandibular 6. Estresse oxidativo I. T.

Black D27
CDD 617.645

Claudio Hideo Matsumoto – CRB-8/5550

AGRADECIMENTOS

A **Deus** pela saúde e oportunidade que me deu de conhecer as pessoas que fizeram com que esta caminhada fosse especial.

À minha família, em especial minha mãe **Rejane de Nogueira de Souza,** que sempre esteve e estará ao meu lado, amparando, amando e cuidando, e que sempre torceu pelo meu sucesso. Muito obrigada por sempre me incentivar e acreditar que eu seria capaz de superar os obstáculos que a vida me apresentou.

Ao meu namorado, **Gladiston William Lobo Rodrigues** por sempre me incentivar, motivar, e vibrar a cada conquista minha, e sempre me encorajando há nunca desistir dos meus sonhos, e que sempre esteve presente nos momentos mais difíceis. Sou eternamente grata a você.

Ao meu orientador, Prof. Dr. **Antonio Hernandes Chaves Neto,** que confiou em mim, me concedendo a oportunidade de desenvolver este estudo. Muito obrigada, pelos ensinamentos, conselhos, mas principalmente, pela paciência com a qual guiaram o meu aprendizado, e por desempenhar tal função com dedicação e amizade. Foi uma honra ter tido a oportunidade de trabalhar com um educador tão comprometido, capaz e habilidoso quanto o senhor.

Aos meus amigos, **Gabriela Alice Fiais, Lucas Guilherme Leite da Silva, Douglas Sadrac de Biagi Ferreira** e **Marcelo Kawaguchi,** por compartilharem tantos momentos de descobertas e por todo o companheirismo ao longo deste percurso. Foi um prazer imenso poder conhecê-los. Sempre me lembrarei de vocês com muito carinho. Não há palavras que possam exprimir a minha gratidão a vocês.

À **Universidade Estadual Paulista "Júlio de Mesquita Filho"**, na pessoa do diretor da Faculdade de Odontologia de Araçatuba Prof. **Glauco Issamu Miyahara** e do vice-diretor Prof. **Alberto Carlos Botazzo Delbem**.

À **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)**, pelo apoio financeiro (CAPES Código de financiamento 001).

À **Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – Processo: 2021/10656-7)**, pelo apoio financeiro, concedida ao aluno de iniciação cientifica **Lucas Guilherme Leite da Silva.**

“Não existem sonhos impossíveis para aqueles que realmente acreditam que o poder realizador reside no interior de cada ser humano. Sempre que alguém descobre esse poder, algo antes considerado impossível, se torna realidade.”

Albert Einstein

FREITAS, RN. **Avaliação dos parâmetros bioquímicos, funcionais, histomorfométricos e estado redox das glândulas salivares de ratos Wistar tratados com o anticonvulsivante valproato de sódio**. 2023. 37 f. Dissertação (Mestrado em Ciências, área de concentração Saúde Bucal da Criança) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2023.

RESUMO

O presente estudo investigou os efeitos do anticonvulsivante valproato de sódio (VPA) nas glândulas salivares por meio de parâmetros bioquímicos, funcionais, histomorfométricos e estado redox. Para tanto, vinte e quatro ratos Wistar (5 semanas de idade) foram divididos aleatoriamente em três grupos (n=08/grupo): grupo (Controle) tratados com solução salina 0,9% (m/v); grupo (VPA100) tratados com 100 mg/kg de VPA; grupo (VPA400) tratados com 400 mg/kg de VPA na forma de xarope (Depakene®, Abbott Laboratórios do Brasil, Ltda.). Após 21 dias consecutivos de tratamento por gavagem intragástrica, a saliva total induzida pela pilocarpina foi coletada para determinação do fluxo salivar, pH, capacidade tamponante, proteína total, amilase, fosfato, cálcio, potássio, sódio e cloreto. Nas glândulas salivares foram realizadas análises histomorformétricas, capacidade oxidante total, peroxidação lipídica, proteína carbonilada, capacidade antioxidante total, ácido úrico, glutationa reduzida (GSH), superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx). Os resultados foram submetidos análise de variância (One-Way ANOVA) seguido do teste *post hoc* de Tukey ($p < 0,05$). O fluxo salivar, pH, capacidade tamponante, proteína total, potássio, sódio e cloreto foram semelhantes entre os grupos. Contudo, fosfato e cálcio reduziram no VPA400, enquanto a amilase foi maior nos grupos VPA100 e VPA400. Não detectamos nas glândulas salivares diferenças significantes nas áreas de ácinos, ductos e tecido conjuntivo entre os grupos. Não houve mudanças significativas no estado redox das glândulas submandibulares. Por sua vez, nas glândulas parótidas detectamos redução da capacidade oxidante total e peroxidação lipídica nos grupos VPA100 e VPA400, aumento da SOD no VPA400, além da maior concentração de ácido úrico nos grupos VPA100 e VPA400. Conclui-se que o tratamento crônico com VPA modificou a composição bioquímica da saliva total e causou distúrbios do estado redox da glândula parótida em ratos.

Palavras-chave: Anticonvulsivantes. Ácido valproico. Saliva. Glândula parótida. Glândula submandibular. Estresse oxidativo.

FREITAS, RN. **Evaluation of biochemical, functional, histomorphometric parameters and redox status of the salivary glands of male Wistar rats treated with the anticonvulsant sodium valproate.** 2023. 37 f. Dissertação (Mestrado em Ciências, área de concentração Saúde Bucal da Criança) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2023.

ABSTRACT

The present study investigated the effects of the anticonvulsant sodium valproate (VPA) on the salivary glands through biochemical, functional, histomorphometric parameters and redox status. For this, twenty-four Wistar rats (5 weeks old) were randomly divided into three groups (n=08/group): group (Control) treated with 0.9% saline solution (w/v); group (VPA100) treated with 100 mg/kg of VPA; group (VPA400) treated with 400 mg/kg of VPA in the form of syrup (Depakene®, Abbott Laboratórios do Brasil, Ltda.). After 21 consecutive days of treatment via intragastric gavage, total saliva induced by pilocarpine was collected to determine salivary flow, pH, buffering capacity, total protein, amylase, phosphate, calcium, potassium, sodium, and chloride. In the salivary glands, histomorphometric analysis, total oxidant capacity, lipid peroxidation, carbonyl protein, total antioxidant capacity, uric acid, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were performed. The results were subjected to analysis of variance (One-Way ANOVA) followed by Tukey's *post hoc* test ($p < 0.05$). Salivary flow, pH, buffering capacity, total protein, potassium, sodium and chloride were similar between groups. However, phosphate and calcium were reduced in VPA400, while amylase was higher in VPA100 and VPA400 groups. We did not detect significant differences in the areas of acini, ducts and connective tissue in the salivary glands between the groups. There were no significant changes in the redox state of the submandibular glands. In turn, in the parotid glands, we detected a reduction in the total oxidant capacity and lipid peroxidation in the VPA100 and VPA400 groups, an increase in SOD in the VPA400, in addition to a higher concentration of uric acid in the VPA100 and VPA400 groups. It is concluded that chronic treatment with VPA modified the biochemical composition of whole saliva and caused disturbances in the redox state of the parotid gland in rats.

Keywords: Anticonvulsants. Valproic acid. Saliva. Parotid gland. Submandibular gland. Oxidative stress.

LISTA DE FIGURAS

LISTA DE TABELA

SUMÁRIO

Evaluation of biochemical, functional, and histomorphometric parameters and redox status of the salivary glands of male Wistar rats treated with the anticonvulsant sodium valproate

Rayara Nogueira de Freitas[1], Lucas Guilherme Leite da Silva[2], Gabriela Alice Fiais[1,4], Douglas Sandrac de Biagi Ferreira[2], Allice Santos Cruz Veras[3,4], Giovana Rampazzo Teixeira[3,4], Sandra Helena Penha Oliveira[2,4], Rita Cássia Menegati Dornelles[2,4], Ana Cláudia de Melo Stevanato Nakamune[2], Walid D. Fakhouri[5], Antonio Hernandes Chaves-Neto[1,2,4*].

[1]Programa de Pós-Graduação em Ciências - Saúde Bucal da Criança, Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araçatuba, São Paulo, Brazil.

[2]Departamento de Ciências Básicas, Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araçatuba, São Paulo, Brazil.

[3]Departamento de Educação Física, Universidade Estadual Paulista (Unesp), Faculdade de Ciências e Tecnologia, Presidente Prudente, SP, Brazil.

[4]Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas - SBFis, Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araçatuba, São Paulo, Brazil.

[5]Center for Craniofacial Research, Department of Diagnostic and Biomedical Sciences, School of Dentistry, University of Texas Health Science Center at Houston, Houston, TX, USA.

Correspondence to:

Professor Antonio Hernandes Chaves-Neto

Address: Department of Basic Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba, Rodovia Marechal Rondon, km 527/528, CEP 16018-805, Araçatuba, SP, Brazil.

Phone: +55 18 3636 2869

E-mail: antonio.hernandes@unesp.br

*Artigo redigido conforme as diretrizes de submissão da revista *Clinical Oral Investigation*: https://www.springer.com/journal/784/submission-guidelines

Abstract

Objective: To investigate the effects of the anticonvulsant sodium valproate (VPA) on salivary glands using biochemical, functional, histomorphometric, and redox parameters.

Materials and methods: Male Wistar rats were randomized into three groups (n=08/group): Control (NaCl 0.9%), VPA100 (100 mg/kg), and VPA400 (400 mg/kg). After 21 consecutive days of treatment with VPA by intragastric gavage, pilocarpine-induced total saliva was collected to determine salivary flow and biochemical composition. Analyses of histomorphometric parameters and redox balance markers were performed on the parotid and submandibular glands.

Results: Salivary flow, pH, buffering capacity, total protein, potassium, sodium, and chloride were similar between groups. However, phosphate and calcium were reduced in VPA400, while amylase was increased in both VPA100 and VPA400. We did not detect significant differences in the areas of acini, ducts, and connective tissue in the salivary glands between the groups. There were no significant changes in the redox status of the submandibular glands. In turn, in the parotid glands we detected reduced total oxidizing capacity and lipid peroxidation and higher uric acid concentration in both the VPA100 and VPA400 groups, and increased SOD in the VPA400 group.

Conclusion: Chronic treatment with VPA modified the salivary biochemical composition and caused disturbances in the redox state of the parotid gland in rats.

Clinical relevance: Electrolyte imbalance associated with increased salivary amylase is a potential risk to oral homeostasis when exposed to VPA-induced salivary gland dysfunction.

Keywords: Anticonvulsants, Valproic acid, Parotid gland, Submandibular gland, Oxidative stress.

Introduction

Epilepsy is a chronic brain pathology, characterized by spontaneous and recurrent seizures, resulting from excessive, irregular, or synchronous electrical activity in the brain, and can be focal or generalized in origin [1, 2]. It is estimated that approximately 50 million people worldwide are diagnosed with epilepsy [3], of which the highest incidence rate is during childhood, specifically during the first 10 years of life [4, 5]. Most of these children live in underdeveloped countries, with a prevalence of 3.6-44 / 1000 [6]. In Brazil, data on the prevalence of epilepsy in children are still scarce, but based on research carried out in the city of São Paulo, the prevalence is 9.7 / 1000 in children aged between zero and sixteen years [7].

The epileptic and convulsive crisis resulting from the disease can almost always be controlled with the appropriate use of anticonvulsant drugs [8, 9], including sodium valproate (VPA), indicated as a frontline treatment for seizure disorders and epileptic syndromes [10]. Its mechanism of action has not yet been fully consolidated, however, evidence reports that VPA acts by increasing the synthesis and release of γ-aminobutyric acid (GABA), enhancing GABAergic transmission in specific regions of the brain, reducing neuronal excitation of receptors of N-methyl-D-aspartate glutamate (NMDA), dampening high-frequency neural firing, and blocking voltage-sensitive sodium channels [11, 12]. In addition, VPA is also used as a neuroprotector in cases of Alzheimer's disease [13], migraine [14], and bipolar disorders [15, 16]. Although VPA has an acceptable level of side effects, epidemiological research suggests that it can induce hepatotoxicity [17], pancreatitis [18], and teratogenicity [19]. Regarding oral health, clinical evidence has shown that chronic use of VPA may be associated with a higher prevalence of gingival enlargement, periodontal disease, and an increased risk of developing caries disease [20-23]. However, to date, the mechanisms are still little known. Thus, research is needed to investigate the effects of the anticonvulsant sodium valproate on oral health.

Saliva is a fluid composed of 99% water, and the remainder is made up of organic and inorganic compounds, which play important roles in maintaining oral health, such as eliminating pathogens, acting as a lubricating barrier and buffering capacity, and maintaining tooth integrity, tissue repair, and digestion [24, 25]. Saliva is produced primarily by the major salivary glands, composed of the parotid, submandibular, and sublingual glands [26]. The salivary glands also have a broad defense system of enzymatic antioxidants: superoxide dismutase (SOD), catalase (CAT), and salivary glutathione peroxidase (GPx), and non-enzymatic antioxidants: uric acid, reduced glutathione (GSH), albumin, and lactoferrin [27-29]. The set of antioxidants is important for the homeostasis of the redox balance and neutralization of the production of reactive oxygen species that, when in excess, promote oxidation biomolecules, and, consequently, the development of tissue dysfunction [30, 31]. In this context, drug-induced oxidative stress is implicated in the toxicity mechanisms that lead to salivary gland dysfunction. In addition, 5-Fluorouracil, a chemotherapeutic agent, has been shown to have a pro-oxidant effect by increasing lipid peroxidation and causing an imbalance in SOD and CAT enzymatic

antioxidant defenses, with negative impacts on salivary flow rate and composition [32]. Likewise, rats treated with sibutramine hydrochloride, an anti-obesity drug, presented reduced SOD, CAT, and GPx antioxidant defenses, which was associated with increased lipid peroxidation, demonstrating increased free radicals and oxidative stress, leading to a decline in α -amylase, total protein, and morphometric alterations in the salivary glands [33].

Evidence points to disturbances of the redox state as part of the toxicological mechanism of VPA in different tissues. In the kidneys, VPA has been shown to reduce SOD, CAT, and GPx activity as well as total antioxidant capacity, compromising glomerular filtrate [34], while in the brain, VPA increased lipid peroxidation and carbonyl protein, generating instability of the GSH, SOD, CAT, and GPx antioxidant defenses, demonstrating the involvement of oxidative stress in the imbalance of the redox state of neural tissue [35]. Although the mechanism is not fully elucidated, it is suggested that VPA amplifies biochemical stress in the body, generating an imbalance between reactive oxygen species and antioxidants, inducing oxidative stress, and inhibiting several groups of enzymes [36, 37]. Despite the evidence demonstrating that VPA can modulate oxidative stress in a tissue-specific way, it has not yet been described whether this anticonvulsant can alter the oxidative state in the salivary glands. Taking into account that VPA is captured and secreted by the salivary glands [38, 39] it is pertinent to analyze its possible effect on the function of the salivary glands.

Therefore, considering the association of oxidative stress caused by this anticonvulsant in different tissues, we hypothesized that chronic treatment with VPA impairs salivary flow and biochemical composition, through increased oxidative damage in the salivary glands. To this end, the current study aimed to investigate the effects of chronic treatment with valproic acid on the biochemical, functional, and histomorphometric parameters, and redox state of the parotid and submandibular glands of male Wistar rats.

Materials and methods

Animals

All experimental procedures were carried out in accordance with the instructions of the Brazilian College of Animal Care (COBEA) and approved by the Ethics Committee on the Use of Animals of Unesp, Araçatuba School of Dentistry (Authorization Protocol No. 0215-2021). Twenty-four male Wistar rats (*Rattus norvegicus albinus*, 5 weeks old) were housed in a temperature-controlled room (22 ± 2 °C), with a 12/12-hour light-dark cycle. The animals had access to feed and water *ad libitum*. The same batch of standard rodent feed (Neovia Nutrição e Saúde Animal LTDA, Paulínia, SP, Brazil) was used from the acclimatization period until the end of the experiment to avoid variation in antioxidant intake. Body mass, water, and feed intake were measured twice a week from the start of the treatments.

Study design

The animals were randomized by the draw method (based on a sequence of numbers generated by computer), and distributed into three independent groups (n= 08/group): group (Control) treated with 0.9% saline solution (w/ v); group (VPA100) treated with 100 mg/kg of VPA in the form of syrup; group (VPA400) treated with 400 mg/kg of VPA in the form of syrup (Depakene®, Abbott Laboratórios do Brasil, Ltda.). VPA100 and VPA400 are approximately equivalent to the initial therapeutic dose and the maximum daily dose in humans, respectively, adapted for rats. The saline solution administered corresponds to the maximum daily volume of VPA. To calculate the doses, the translational dose formula was used based on the surface area of the body (mg/m^2) [40], from information provided in the syrup leaflet [41]. These doses were also selected due to the results obtained in different toxicity studies [34, 42-45]. In all groups, the treatment occurred via intragastric gavage, with the substances administered once a day, always in the morning, for 21 consecutive days [46-48].

Collection and measurement of salivary flow rate

After the treatment period (twenty-one days), the animals were weighed and anesthetized by a combination of xylazine hydrochloride (10 mg/kg - Coopers, Brasil, Ltda.) and ketamine hydrochloride (75 mg/kg - Fort Dodge, Animal Health Ltd.). After anesthesia, pilocarpine nitrate (5 mg/kg, IP, Sigma-Aldrich, St. Louis, MO, USA), diluted in 0.9% NaCl (w/v), was applied intraperitoneally to stimulate salivary flow. Total saliva was collected for 10 minutes counting from the first drop of saliva. For this purpose the animals were placed on an inclined support with their mouths over a previously weighed container. Salivary volume was calculated by the difference in weight between the full and empty bottle, considering saliva density as 1 g/mL [49]. The salivary flow rate was determined by the amount of saliva collected per minute, divided by the animal's weight, expressed in mL/min/kg of body mass. The saliva samples were then fractionated and stored (-80 °C) until the analysis was carried out. In sequence, after saliva collection, pH and buffering capacity were evaluated. The salivary pH value was determined with a specific electrode (Analyzer®, São Paulo, SP, Brazil) connected to a pH meter (Thermo Fischer, Orion 720A, MA, USA). The buffering capacity was determined using the titulometric method, by measuring the volume of lactic acid (0.1 mol/L) necessary to reduce salivary pH to 4.0 and expressed in mL of lactic acid [50].

Collection and storage of tissue and blood samples

The animals were euthanized by exsanguination via cardiac puncture, always in the morning (9:00 – 11:00 am) to minimize the effects of the circadian rhythm. The blood obtained was centrifuged at 1000 x g for 15 min at 4 °C and stored in a -80 °C freezer. Afterward, the parotid and submandibular glands were immediately removed and cleaned of adipose tissue and blood elements, and then dried and weighed. The salivary gland index was used as a relative mass

index (organ/body mass ratio) [50]. The glands on the right side were destined for biochemical analysis, while the glands on the left side were histologically processed for histomorphometric analysis.

Biochemical parameters

For the biochemical assays, reagents from Sigma-Aldrich, Germany/USA were used. Absorbance was measured using a microplate reader spectrophotometer (PowerWave 340, BioTek, USA) and a UV-Vis spectrophotometer (U-2000, Hitachi, Japan). All analyses were performed in duplicate and normalized by the total protein content, making it possible to evaluate differences in the proportionality of biochemical analytes present in both saliva and salivary gland homogenate [50].

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity assays

ALT and AST activity was determined in blood serum using standard commercial kits (Bioclin®, Quibasa, Minas Gerais, Brazil).

Saliva biochemical analysis

Total protein concentration was measured in all samples by the Hartree-Lowry method [51]. Salivary amylase activity was evaluated with a commercial kit (Bioclin®, Quibasa, Minas Gerais, Brazil) where the saliva sample is incubated with the substrate starch and heated to 37°C. When adding the iodinated solution, starch not yet hydrolyzed acquires a color that decreases proportionally to enzymatic activity. One unit (U) of amylase activity was established as the amount of enzyme needed to hydrolyze 10 mg of starch in 30 minutes of reaction under the described assay conditions [52]. Calcium, phosphate, and chloride levels in saliva samples were determined using commercial colorimetric kits (Bioclin®, Quibasa, Minas Gerais, Brazil). For sodium and potassium, an ion-selective electrolyte analyzer (Roche 9180®, Electrolyte Analyzer, Austria) was used. Salivary electrolyte levels were expressed in mmol/L.

Histomorphometric analysis

Parotid and submandibular salivary gland samples were fixed in Bouin's solution for 24 hours, and preserved in 70% alcohol, subsequently dehydrated in ethanol, clarified in xylene, and included in paraplast. Five micrometer cuts of the salivary glands were stained with hematoxylin and eosin. The histomorphometric study was performed by a certified histologist blinded to the treatments. The pictures were taken using a digital camera (Olympus XC50, Tokyo, Japan) that was connected to a light microscope (Olympus BX53, Tokyo, Japan) with an original magnification of 400x. Six photomicrographs were obtained of two histological sections per gland. To demarcate the areas occupied by acini and ducts, the “hands-free” tool in Image J software (ImageJ, National Institutes of Health, USA, version 1.50i) was used, in which the total area of the salivary glands was measured in (μm^2). After averaging the areas of the six photos with the

total area set to 100%, the findings were plotted by calculating the proportion of each structure still present in each image using the rule of three. Five animals per group were used in this investigation [33, 53].

Biochemical analysis of the salivary gland

Preparation of salivary gland homogenates

The parotid and submandibular glands were homogenized at 5% (w/v) in 50 mmol/L sodium phosphate buffer (pH 7.4), containing 0.2% v/v Triton X-100, 10 mmol/L of diethylenetriaminepentaacetic acid (DTPA), and 2 mmol/L of phenylmethanesulfonyl fluoride (PMSF). For both, Potter-Elvehjem homogenizer (Model MA 099®, Marconi, São Paulo, Brazil) was used. After 10 min of centrifugation at 10,000 x g at 4°C, the supernatant was fractionated and stored at -80°C until use. A portion of the homogenate was used for the determination of GSH, prepared in 5% (w/v) trichloroacetic acid containing 2 mmol/L of diethylenetriaminepentaacetic acid (EDTA) and centrifuged for 10 min at 4,000 x g, 4°C.

Oxidative damage product assays

The total oxidative capacity was evaluated with the Erel method [54], this test is based on oxidants present in the sample that oxidize the ferrous ion-o-dianisidine complex to ferric ion. The ferric ion produces an orange xylenol complex in acid medium. Color intensity was measured spectrophotometrically at 560/800 nm. The results were expressed in H_2O_2 per gram of protein. Thiobarbituric acid reactive compounds (TBARs) have been used as a technique for lipid peroxidation [55]. The extinction coefficient of the formed aldehydes (ε_{532} = 1.56 105 M^{-1} cm^{-1}) was used to determine how many were produced. Carbonylated protein was determined spectrophotometrically (450 nm), using the simplified alkaline 2,4-dinitrophenylhydrazine (DNPH) method [56]. The carbonyl concentration was calculated by the molar absorption coefficient (ε_{450} = 22,000 M^{-1} cm^{-1}).

Assay of non-enzymatic antioxidant defenses

The ferric reducing antioxidant power (FRAP) assay, as reported by Benzie and Strain [57], was used for spectrophotometric analysis of the total antioxidant capacity. A standard curve constructed using solutions with various concentrations of ferrous sulfate was used to calculate the results. Uric acid levels were determined using a commercial kit (Bioclin®, Quibasa, Minas Gerais, Brazil). GSH concentration was measured using 5,5'-dithiobis-(2-nitrobenzoate) (DTNB) according to the spectrophotometric method described by Beutler et al. [58]. The amount of GSH was determined by the GSH molar extinction coefficient (ε_{412} = 1.36×104 M^{-1} cm^{-1}). GSH levels were expressed as nmol per gram of total protein.

Assay of enzymatic antioxidant defenses

SOD activity was determined by evaluating the autoxidation rate of pyrogallol in 50 mmol/L of Tris-HCl buffer (pH 8.2) containing 1 mmol/L of diethylenetriaminepenta acetic acid. One SOD unit was defined as the amount of enzyme needed to inhibit the auto-oxidation of pyrogallol by 50%. The oxidation of pyrogallol forms a colored product, detected spectrophotometrically at 420 nm [57]. The activity of CAT in the tissue homogenate was analyzed through the H_2O_2 degradation rate, spectrophotometrically (240 nm) [58]. Enzyme activity was determined using the H_2O_2 molar extinction coefficient (ε_{240} = 0.0394 mM^{-1} cm^{-1}). The analysis of the GPx enzyme was carried out based on the measure of the decrease in absorbance at 340 nm, promoted during the reduction of oxidized glutathione (GSSG). The reduction is catalyzed by glutathione reductase (GR) in the presence of NADPH. *Tert-butyl* hydroperoxide [59] was used as a substrate. The results were expressed using the NADPH molar extinction coefficient (ε_{340} = 6.2 mM^{-1} cm^{-1}), in which a unit of GPx activity was considered as 1 mmol of NADPH consumed per minute.

Statistical analyses

Data were processed using GraphPad Prism 8 (GraphPad Software, Inc.; La Jolla, CA, USA). The Shapiro-Wilk test showed normal distribution of the results obtained, so one-way ANOVA parametric analysis was used, followed by Tukey's multiple comparisons test. Statistical significance was defined as $p < 0.05$. Results are expressed as mean ± standard deviation. All analyses were performed in duplicate.

Results

After 21 days of treatment with VPA, the levels of enzymatic activities of the plasma markers of liver injury ALT and AST were similar between groups (Table 1). The initial and final body mass did not show significant differences between the animals in the Control, VPA100, and VPA400 groups. There were also no differences in feed and water intake between groups (Table 1). The mass and index of the parotid and submandibular glands did not differ in either group, and we did not detect significant differences in the total protein concentration between the groups in either salivary gland (Table 1).

The results of the histomorphometric analyses showed that VPA treatments did not cause structural alterations in the parotid gland (Fig. 1A, B, and C), since the area of acini (Fig. 1D), ducts (Fig. 1E), and connective tissue (Fig. 1F) showed no abnormalities compared to the Control group. In the submandibular gland (Fig. 1G, H, and I) no significant structural changes were observed in the area of acini (Fig. 1J), ducts (Fig. 1K), and connective tissue (Fig. 1L) compared to the Control group.

In the present study, neither dose of VPA altered flow rate (Fig. 2A), pH (Fig. 2B), buffering capacity (Fig. 2C), and total salivary protein content (Fig. 2D) when compared to the Control group. However, salivary amylase activity significantly increased in the VPA100 ($p < 0.01$) and VPA400 ($p < 0.05$) groups compared to the Control group (Fig.

2E). There was also a reduction in salivary concentrations of phosphate in the VP400 group compared to the Control ($p < 0.01$) and VPA100 groups ($p < 0.01$) (Fig. 2F), and calcium (Fig. 2G) compared to the Control ($p < 0.05$) and VPA100 groups ($p < 0.05$). No significant differences were observed in salivary concentrations of potassium (Fig. 2H), sodium (Fig. 2I), and chloride (Fig. 2J) between the experimental groups.

In turn, the parotid glands from both groups treated with VPA showed lower concentrations of total oxidant capacity (VPA100, $p < 0.01$; VPA400, $p < 0.01$) and oxidative damage to lipids (VPA100, $p < 0$ .0001; VPA400, $p < 0.0001$) in relation to the Control group (Fig. 3A and 3B), while in the submandibular gland the total oxidant capacity and oxidative damage to lipids remained similar between groups (Fig. 3A and 3B). Concentrations of oxidative protein damage, as determined by protein carbonyl, also did not differ between groups in either salivary gland (Fig. 3C).

The analysis of non-enzymatic antioxidant defense parameters showed that the doses of VPA did not significantly influence the concentrations of total antioxidant capacity (Fig. 4A) and GSH (Fig. 4C) in either salivary gland. However, in the parotid gland, uric acid (Fig. 4B) increased in both the VPA100 ($p < 0.05$) and VPA400 ($p < 0.05$) groups compared to the Control group, while in the submandibular gland, uric acid remained unchanged among the groups (Fig. 4B).

The set of enzymatic antioxidant defenses are represented in the study by SOD, CAT, and GPx. At the end of treatment, only the VPA400 group promoted a significant increase in SOD compared to the Control ($p < 0.05$) and VPA100 ($p < 0.05$) in the parotid gland (Fig. 5A), while in the submandibular gland the activity of SOD remained similar between groups (Fig. 5A). In contrast, we did not find significant differences in CAT and GPx activities between the experimental groups in either salivary gland (Fig. 5B and C).

Discussion

The present study investigated the potential adverse effects of the anticonvulsant VPA, on the biochemical and functional parameters and redox state of the parotid and submandibular salivary glands in rats, after 21 days of treatment. The main findings were: 1. VPA does not change flow rate, pH, buffering capacity, and total salivary protein concentration; 2. VPA increased amylase and decreased salivary phosphate and calcium; 3. VPA does not influence the area of acini, ducts, and connective tissue in the salivary glands; 4. Total oxidant capacity and lipid oxidative damage were lower in both VPA-treated groups; 5. VPA induced an increase in uric acid and SOD in the parotid gland.

Our study showed that VPA therapy at doses of 100 mg/kg/day and 400 mg/kg/day, which are approximately equivalent to the initial therapeutic dose and the maximum daily dose in humans, respectively [41], did not cause liver damage, given that serum ALT and AST aminotransferases remained similar between experimental groups. Similar results were found in male Wistar rats treated with 400 mg/kg of VPA via intragastric gavage for 28 [43] and 60

consecutive days [60], as well as at a dose of 500 mg/kg for 14 days [61]. On the other hand, it is described that VPA has toxic metabolites that can increase markers of oxidative stress and consequent hepatotoxicity [62]. In fact, a study with rats demonstrated an increase in plasma AST and ALT activity after intraperitoneal administration with 700 mg/kg/day [63], however, histopathological damage was observed from intraperitoneal administration of the dose of 300 mg/kg/day [64]. Furthermore, our results must be analyzed with caution, since in rats the histopathological alterations induced by high doses of VPA may occur independently of the increase in plasma AST and ALT [65]. In humans, VPA-induced hepatotoxicity is dependent on comorbidities, dose, polytherapy with other anticonvulsants, age, dietary habits, and genetic factors [66, 67].

VPA treatments also did not alter the final body mass of the animals in this study. These results are in line with other findings in the literature, which showed that rats treated with VPA via intragastric gavage at doses of 500 mg/kg for 14 days [65] and 400 mg/kg for 28 days [43] presented no changes in body mass. Likewise, male and female Wistar rats treated daily with VPA at 400 mg/kg for 90 days also showed body masses similar to untreated animals [45]. On the other hand, the chronic treatment of rats with VPA at a dose of 300 mg/kg for 10 weeks decreased the animals' body mass [68]. In another study, rats treated intraperitoneally with 250 mg/kg for 14 days showed an increase in body mass after treatment [36]. The divergences found between the studies may be associated with the variability of the doses used and administration protocols, which may influence the metabolism of VPA. In turn, water and feed intake was not impaired by VPA treatment in our experimental model. Contrary to our results, mice treated with 4 mg/kg of VPA intraperitoneally for 13 weeks presented increased feed intake [69]. In turn, rats treated with a dose of 120 mg/kg/day of VPA presented a reduction in body weight and feed intake [70], while another study showed that rats fed a diet containing VPA (1%) maintained body weight gain and feed intake similar to the control [71]. Therefore, there is no consensus on the effects of VPA on water and feed intake, making further investigation necessary.

The impairment in the size, mass, and index of the salivary glands of rodents demonstrated a correlation with the dysfunctions of the salivary glands, especially with the reduction in salivary flow in case of intoxication with the chemotherapeutic 5-fluorouracil [72] and also in experimental models of diabetes [73, 74], hypothyroidism [75], and radiotherapy [76], and also in situations of dietary imbalances and malnutrition [77]. In this context, VPA did not affect the mass and index of the salivary glands, which is consistent with the fact that the salivary flow rate remained similar between the treated groups. In the case of VPA, studies have shown that the toxicological effects in rats can occur independently of changes in the mass and index of organs, as observed in organs such as ovaries [45], testicles [43, 45], prostate [78], and liver [65]. These results must be considered with caution, as the dysfunctions of the salivary glands are not restricted to changes in salivary flow, but also to changes in the composition and function of saliva.

Rare clinical cases in humans have reported the potential of chronic treatment with VPA to induce sialadenitis. [79-81], whose pathological mechanism has been associated with damage to the intraglandular nervous system [79, 81], in particular increased sympathetic nervous system excitation threshold and inhibition of protein secretion caused by VPA [79]. The findings of histopathological examinations of sialadenitis describe the presence of enlarged acini [81] with hydropic degeneration [80], plus enlarged secretory ducts in the parotid glands [79], while for the submandibular gland they showed fibrous patterns with single glandular acini without atypical cells [79]. Despite the clinical findings of sialadenitis, our experimental model in rats did not identify the alterations described above, bearing in mind that the acinar, ductal, and connective tissue structures were similar between the groups. This discrepancy could be explained by predisposing factors, such as eating and endocrine disorders, malnutrition, and prolonged use of sympathomimetic drugs, conditions in which sialadenitis is more prevalent [81]. Furthermore, the total set of our salivary gland function results corroborates the histomorphometric findings, since the effects of VPA were restricted to partial modification of salivary composition.

Pilocarpine is a parasympathomimetic that stimulates salivary flow through activation of M1 and M3 muscarinic acetylcholine receptors present in the salivary glands. [82]. Therefore, we can consider that the VPA had no anticholinergic effects, since salivary flow, pH, and buffering capacity were not compromised in the treated groups. This result contradicts our expectations, as studies have shown that the salivary glands of rats have GABA receptors, an ionotropic receptor that, when activated, allows the influx of chloride [83, 84], in addition to GABA biosynthetic and metabolic enzymes [85, 86]. The GABAergic system can interact with the cholinergic system, exerting an inhibitory effect on salivary flow [85, 87]. However, the maintenance of salivary concentrations of chloride, sodium, and potassium in the treated groups supports the salivary flow results, since these electrolytes are essential in the flow of water during salivary secretion. [88]. Our results suggest that the effect of VPA on electrolyte balance may be tissue-specific, since rats of both sexes treated with a single dose of VPA (300 mg/kg) showed increased urinary excretion of sodium, chloride, and potassium [89, 90].

Although salivary flow and pH remained unchanged, the salivary concentration of phosphate and calcium ions showed a decline in VPA400. Transcellular transport of calcium during salivary secretion is mediated by proteins that conduct influx, uptake, and subsequent efflux towards the acinar lumen. [91]. This process in rats is subject to regulation of the autonomic nervous system in the submandibular [92] and parotid glands [93, 94]. In turn, phosphate is taken up against its electrochemical gradient by type II sodium-phosphate cotransporter proteins, which are driven by the sodium gradient across the basolateral membrane, suggesting that a number of proteins expressed by the salivary glands are involved in phosphate reabsorption/absorption [95]. The reason why salivary calcium and phosphate are lower in animals treated with the highest dose of VPA is not yet clear, however it may be related to changes in the function and expression

of transport proteins, as well as disturbances in the regulation of induced secretion by the autonomic nervous system. Our findings also demonstrate the specific tissue effect of this anticonvulsant, since VPA 500 mg/kg/day/IP did not change the urinary excretion of calcium and phosphate in rats [96].

Salivary glands are composed of more than one type of protein-secreting cell, in which salivary amylase constitutes a proportion of approximately 50% of proteins synthesized and secreted [97]. It is well described that pilocarpine, a cholinergic agonist, stimulates the salivary secretion of amylase in rats, mainly due to the discharge of this enzyme by the parotid gland. [98]. In contrast, amylase exocytosis by the submandibular and sublingual glands constitutes an unregulated constitutive phenomenon, independent of extracellular or intracellular signals. [99]. This leads us to suggest that the increase in amylase secretion in VPA400 was basically due to the effects of the anticonvulsant on the parotid glands. However, our results do not allow us to define whether this effect is due to the action of VPA at the central and peripheral nervous system level. In addition, the local toxic effect on the salivary glands should not be ruled out, as VPA is captured and secreted by the salivary glands and demonstrates a positive correlation between salivary and plasmatic concentrations. [38, 39]. Our findings also corroborate clinical studies with VPA that detected an increase in amylase in the blood [100], urine [101], and saliva [102]. In turn, the maintenance of total salivary protein concentration after the end of treatment suggests compensation of protein synthesis and secretion by the submandibular and sublingual glands. Therefore, we suggest that research should be conducted to promote better understanding of the mechanisms of action of VPA on the synthesis and secretion of amylase by the salivary glands.

It is well evidenced that the chronic use of certain classes of drugs can generate oxidative stress, which is strongly associated with dysfunctions of the salivary glands, resulting in changes in the quality and quantity of saliva secreted in the oral cavity. In our study, after 21 days, the submandibular gland was apparently more tolerant to the adverse effects of VPA, since the enzymatic and non-enzymatic antioxidant defense system and the markers of oxidative damage remained unchanged in the treated groups. In fact, the submandibular glands have been shown to be less vulnerable to oxidative damage compared to the parotid glands in experimental models of diabetes [103], a high-sucrose diet [104], and chronic alcohol intake [105]. On the other hand, treatment with VPA400 induced changes in the redox status of the parotid gland, characterized by higher concentrations of uric acid and SOD activity, which coincided with the reduction in markers of total oxidant capacity and lipid oxidative damage, while the total antioxidant capacity, GSH, CAT, and GPx remained similar between experimental groups. Studies demonstrate the relevance of GSH in the detoxification of lipid hydroperoxides induced by VPA intoxication in the liver, kidneys [106], heart [107], testicles [108], and rat brain [109]. In these tissues, the reduction in GSH concentration was associated with increased lipid oxidative damage. Contrary to these studies, the parotid and submandibular glands maintained GSH concentrations after treatment with VPA, which leads us to suggest that VPA may have a tissue-specific toxic effect.

Despite the slight alterations in the redox state of the salivary glands induced by VPA, the salivary biochemical analyses concomitantly evidenced disturbances in the secretion of calcium, phosphate, and amylase. These findings lead us to suggest a lack of temporal correlation between the results of the redox state and the dysfunction of salivary composition induced by VPA. A similar profile was identified in the liver of rats treated with VPA 500 mg/kg/IP, where the significant increase in TBARs occurred late in relation to hepatic necrosis, denoting the lack of temporal correlation between biomarkers of oxidative damage and tissue injury [110]. Furthermore, the reduction in lipid peroxidation in the parotid glands may be a reflection of the adaptive response to low-level chronic oxidative stress during the 21 days of treatment and also due to the increase in antioxidant defense, especially SOD and uric acid. Lower concentrations of lipid peroxidation are also found in the salivary glands of streptozotocin-induced diabetic rats in late periods as a consequence of increased uric acid [111], CAT, and SOD [29]. In turn, protein carbonylation is an irreversible and stable oxidative modification that can lead to loss of protein function and initiation of inflammatory cell signaling [112]. This oxidative damage can occur after exposure to different reactive species or by covalent modification induced by reactive aldehydes generated by lipid peroxidation [113, 114]. The fact that lipids with polyunsaturated fatty acid chains are among the first targets of free radicals [115], and that part of protein carbonylation occurs subsequent to lipid oxidative damage [116], we reinforce the hypothesis that the antioxidant system of the salivary glands was able to contain the generation of oxidants and lipid peroxidation, which led to the maintenance of the concentration of the carbonyl protein between the experimental groups.

Conclusion

Chronic treatment for 21 days with VPA modified the salivary biochemical composition and caused disturbances in the redox state of the parotid gland in rats. The reduction in the concentration of calcium and phosphate associated with the increase in salivary amylase can be considered potential risks to oral homeostasis in view of the dysfunction of the salivary glands induced by VPA.

Financing

This study was funded by the State of São Paulo Research Foundation (FAPESP) [Process nº2021/10656-7] and partially by the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) - Financial Code 001.

Compliance with ethical standards

Interest conflicts: All authors declare no conflict of interest.

Ethical approval: The study was conducted in accordance with national (CONCEA - Conselho Nacional de Controle de Experimentação Animal: http://concea.mct.gov.br) and institutional laws and was approved by the Ethics in Animal Use

Committee (CEUA), Faculty of Dentistry, Paulista State University (Unesp), Araçatuba, São Paulo, Brazil (Authorization Protocol 0215-2021). All surgeries were performed under anesthesia with the association of ketamine hydrochloride and xylazine hydrochloride, in addition, every effort was made to minimize suffering.

Informed consent: For this type of study, formal consent is not required.

Authors' Contributions: All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were carried out by Rayara Nogueira de Freitas, Gabriela Alice Fiais, Lucas Guilherme Leite da Silva, Douglas Sadrac de Biagi Ferreira, Allice Santos Cruz Veras, Giovana Rampazzo Teixiera, Sandra Helena Penha de Oliveira, Rita Cássia Menegati Dornelles, Ana Cláudia de Melo Stevanato Nakamune, Antonio Hernandes Chaves-Neto. The first version of the manuscript was written by Rayara Nogueira de Freitas, Gabriela Alice Fiais, Rita Cássia Menegati Dornelles, Ana Cláudia de Melo Stevanato Nakamune, Antonio Hernandes Chaves-Neto and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J, Jr., Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshe SL, Perucca E, Scheffer IE, Tomson T, Watanabe M and Wiebe S (2014) ILAE official report: a practical clinical definition of epilepsy. Epilepsia 55:475-82. doi: 10.1111/epi.12550

2. Johnson EL (2019) Seizures and Epilepsy. Med Clin North Am 103:309-324. doi: 10.1016/j.mcna.2018.10.002

3. WHO (2022) Epilepsy. https://www.who.int/news-room/fact-sheets/detail/epilepsy. Accessed Acces Date 2022

4. Beghi E (2020) The Epidemiology of Epilepsy. Neuroepidemiology 54:185-191. doi: 10.1159/000503831

5. Aaberg KM, Gunnes N, Bakken IJ, Lund Soraas C, Berntsen A, Magnus P, Lossius MI, Stoltenberg C, Chin R and Suren P (2017) Incidence and Prevalence of Childhood Epilepsy: A Nationwide Cohort Study. Pediatrics 139. doi: 10.1542/peds.2016-3908

6. Camfield P and Camfield C (2015) Incidence, prevalence and aetiology of seizures and epilepsy in children. Epileptic Disord 17:117-23. doi: 10.1684/epd.2015.0736

7. Sampaio LP, Caboclo LO, Kuramoto K, Reche A, Yacubian EM and Manreza ML (2010) Prevalence of epilepsy in children from a Brazilian area of high deprivation. Pediatr Neurol 42:111-7. doi: 10.1016/j.pediatrneurol.2009.09.002

8. Moosa ANV (2019) Antiepileptic Drug Treatment of Epilepsy in Children. Continuum (Minneap Minn) 25:381-407. doi: 10.1212/CON.0000000000000712

9. Katyayan A and Diaz-Medina G (2021) Epilepsy: Epileptic Syndromes and Treatment. Neurol Clin 39:779-795. doi: 10.1016/j.ncl.2021.04.002

10. Romoli M, Mazzocchetti P, D'Alonzo R, Siliquini S, Rinaldi VE, Verrotti A, Calabresi P and Costa C (2019) Valproic Acid and Epilepsy: From Molecular Mechanisms to Clinical Evidences. Curr Neuropharmacol 17:926-946. doi: 10.2174/1570159X17666181227165722

11. Rahman M and Nguyen H (2022) Valproic Acid. Book title., Treasure Island (FL)

12. Mishra MK, Kukal S, Paul PR, Bora S, Singh A, Kukreti S, Saso L, Muthusamy K, Hasija Y and Kukreti R (2021) Insights into Structural Modifications of Valproic Acid and Their Pharmacological Profile. Molecules 27. doi: 10.3390/molecules27010104

13. Zhang XZ, Li XJ and Zhang HY (2010) Valproic acid as a promising agent to combat Alzheimer's disease. Brain Res Bull 81:3-6. doi: 10.1016/j.brainresbull.2009.09.003

14. Brown BL, Craycraft LK and Justice SB (2020) Valproic Acid in the Treatment of Migraines. Adv Emerg Nurs J 42:243-253. doi: 10.1097/TME.0000000000000319

15. Yee CS, Vazquez GH, Hawken ER, Biorac A, Tondo L and Baldessarini RJ (2021) Long-Term Treatment of Bipolar Disorder with Valproate: Updated Systematic Review and Meta-analyses. Harv Rev Psychiatry 29:188-195. doi: 10.1097/HRP.0000000000000292

16. Chen YB, Liang CS, Wang LJ, Hung KC, Carvalho AF, Solmi M, Vieta E, Tseng PT, Lin PY, Tu YK, Hsu CW and Lai EC (2022) Comparative effectiveness of valproic acid in different serum concentrations for maintenance treatment of bipolar disorder: A retrospective cohort study using target trial emulation framework. EClinicalMedicine 54:101678. doi: 10.1016/j.eclinm.2022.101678

17. Meseguer ES, Elizalde MU, Borobia AM and Ramirez E (2021) Valproic Acid-Induced Liver Injury: A Case-Control Study from a Prospective Pharmacovigilance Program in a Tertiary Hospital. J Clin Med 10. doi: 10.3390/jcm10061153

18. Norgaard M, Jacobsen J, Ratanajamit C, Jepsen P, McLaughlin JK, Pedersen L and Sorensen HT (2006) Valproic acid and risk of acute pancreatitis: a population-based case-control study. Am J Ther 13:113-7. doi: 10.1097/00045391-200603000-00005

19. Jentink J, Loane MA, Dolk H, Barisic I, Garne E, Morris JK, de Jong-van den Berg LT and Group EASW (2010) Valproic acid monotherapy in pregnancy and major congenital malformations. N Engl J Med 362:2185-93. doi: 10.1056/NEJMoa0907328

20. Goyal A, Bhadravathi MC, Kumar A, Narang R, Gupta A and Singh H (2016) Comparison of Dental Caries Experience in Children Suffering From Epilepsy with and without Administration of Long Term Liquid Oral Medication. J Clin Diagn Res 10:ZC78-82. doi: 10.7860/JCDR/2016/18211.8024

21. Tiwari S, Verma RK, Dhull KS and Suma G (2021) Assessment of Oral Health Status in Epileptic Children and Healthy Children in Bengaluru City: A Comparative Study. Int J Clin Pediatr Dent 14:768-773. doi: 10.5005/jp-journals-10005-2081

22. Suneja B, Chopra S, Thomas AM and Pandian J (2016) A Clinical Evaluation of Gingival Overgrowth in Children on Antiepileptic Drug Therapy. J Clin Diagn Res 10:ZC32-6. doi: 10.7860/JCDR/2016/16443.7069

23. Joshi NH, Deshpande AN, Deshpande NC and Rathore AS (2017) Comparative evaluation of oral hygiene status and gingival enlargement among epileptic and healthy children as related to various antiepileptic drugs. J Indian Soc Periodontol 21:125-129. doi: 10.4103/jisp.jisp_48_17

24. Alhajj M and Babos M (2022) Physiology, Salivation. Book title., Treasure Island (FL)

25. de Almeida Pdel V, Gregio AM, Machado MA, de Lima AA and Azevedo LR (2008) Saliva composition and functions: a comprehensive review. J Contemp Dent Pract 9:72-80.

26. Brazen B and Dyer J (2022) Histology, Salivary Glands. Book title., Treasure Island (FL)

27. Maciejczyk M, Skutnik-Radziszewska A, Zieniewska I, Matczuk J, Domel E, Waszkiel D, Zendzian-Piotrowska M, Szarmach I and Zalewska A (2019) Antioxidant Defense, Oxidative Modification, and Salivary Gland Function in an Early Phase of Cerulein Pancreatitis. Oxid Med Cell Longev 2019:8403578. doi: 10.1155/2019/8403578

28. Zukowski P, Maciejczyk M, Matczuk J, Kurek K, Waszkiel D, Zendzian-Piotrowska M and Zalewska A (2018) Effect of N-Acetylcysteine on Antioxidant Defense, Oxidative Modification, and Salivary Gland Function in a Rat Model of Insulin Resistance. Oxid Med Cell Longev 2018:6581970. doi: 10.1155/2018/6581970

29. Gomes MA, Manzano C, Alves TM, Fiais GA, Freitas RN, Coutinho Mattera MSL, Dornelles RCM, Matsushita DH, Stevanato Nakamune ACM and Chaves-Neto AH (2022) Assessment of redox state and biochemical parameters of salivary glands in streptozotocin-induced diabetic male rats treated with mate tea (Ilex paraguariensis). Arch Oral Biol 143:105551. doi: 10.1016/j.archoralbio.2022.105551

30. Ye ZW, Zhang J, Townsend DM and Tew KD (2015) Oxidative stress, redox regulation and diseases of cellular differentiation. Biochim Biophys Acta 1850:1607-21. doi: 10.1016/j.bbagen.2014.11.010

31. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D and Bitto A (2017) Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev 2017:8416763. doi: 10.1155/2017/8416763

32. Bomfin LE, Braga CM, Oliveira TA, Martins CS, Foschetti DA, Santos A, Costa DVS, Leitao RFC and Brito GAC (2017) 5-Fluorouracil induces inflammation and oxidative stress in the major salivary glands affecting salivary flow and saliva composition. Biochem Pharmacol 145:34-45. doi: 10.1016/j.bcp.2017.08.024

33. Dos Santos DR, Fiais GA, Oliveira HA, Ribas TB, Souza RO, Tsosura TVS, Matsushita DH, Ervolino E, Dornelles RCM, Nakamune A and Chaves-Neto AH (2022) Assessment of redox state and biochemical parameters of salivary glands in rats treated with anti-obesity drug sibutramine hydrochloride. Clin Oral Investig 26:5833-5846. doi: 10.1007/s00784-022-04539-1

34. El-Shenawy NS and Hamza RZ (2016) Nephrotoxicity of sodium valproate and protective role of L-cysteine in rats at biochemical and histological levels. J Basic Clin Physiol Pharmacol 27:497-504. doi: 10.1515/jbcpp-2015-0106

35. Chaudhary S and Parvez S (2012) An in vitro approach to assess the neurotoxicity of valproic acid-induced oxidative stress in cerebellum and cerebral cortex of young rats. Neuroscience 225:258-68. doi: 10.1016/j.neuroscience.2012.08.060

36. Abdelkader NF, Elyamany M, Gad AM, Assaf N, Fawzy HM and Elesawy WH (2020) Ellagic acid attenuates liver toxicity induced by valproic acid in rats. J Pharmacol Sci 143:23-29. doi: 10.1016/j.jphs.2020.01.007

37. Chang TK and Abbott FS (2006) Oxidative stress as a mechanism of valproic acid-associated hepatotoxicity. Drug Metab Rev 38:627-39. doi: 10.1080/03602530600959433

38. Suzuki Y, Uematsu T, Mizuno A, Ninchoji T, Fujii K and Nakashima M (1994) Analysis of the transport of valproic acid into saliva from serum. Biol Pharm Bull 17:340-4. doi: 10.1248/bpb.17.340

39. Dwivedi R, Gupta YK, Singh M, Joshi R, Tiwari P, Kaleekal T and Tripathi M (2015) Correlation of saliva and serum free valproic acid concentrations in persons with epilepsy. Seizure 25:187-90. doi: 10.1016/j.seizure.2014.10.010

40. Reagan-Shaw S, Nihal M and Ahmad N (2008) Dose translation from animal to human studies revisited. FASEB J 22:659-61. doi: 10.1096/fj.07-9574LSF

41. DEPAKENE (2021) Bulla. :< https://dam.abbott.com/pt-br/documents/pdfs/nossas-bulas/D/BU- 18-Depakene-Bula-Profissional-Xpe-final.pdf>. Accessed Acces Date

42. Khan S, Ahmad T, Parekh CV, Trivedi PP, Kushwaha S and Jena G (2011) Investigation on sodium valproate induced germ cell damage, oxidative stress and genotoxicity in male Swiss mice. Reprod Toxicol 32:385-94. doi: 10.1016/j.reprotox.2011.09.007

43. Ourique GM, Saccol EM, Pes TS, Glanzner WG, Schiefelbein SH, Woehl VM, Baldisserotto B, Pavanato MA, Goncalves PB and Barreto KP (2016) Protective effect of vitamin E on sperm motility and oxidative stress in valproic acid treated rats. Food Chem Toxicol 95:159-67. doi: 10.1016/j.fct.2016.07.011

44. Raza M, Dhariwal MA, Ageel AM and Qureshi S (1996) Evaluation of the antiinflammatory activity of sodium valproate in rats and mice. Gen Pharmacol 27:1395-400. doi: 10.1016/s0306-3623(96)00073-0

45. Sveberg Roste L, Tauboll E, Isojarvi JI, Pakarinen AJ, Huhtaniemi IT, Knip M and Gjerstad L (2002) Effects of chronic valproate treatment on reproductive endocrine hormones in female and male Wistar rats. Reprod Toxicol 16:767-73. doi: 10.1016/s0890-6238(02)00054-0

46. Akindele AJ, Otuguor E, Singh D, Ota D and Benebo AS (2015) Hypoglycemic, antilipidemic and antioxidant effects of valproic acid in alloxan-induced diabetic rats. Eur J Pharmacol 762:174-83. doi: 10.1016/j.ejphar.2015.05.044

47. Ezz HS, Khadrawy YA and Noor NA (2011) The neuroprotective effect of curcumin and Nigella sativa oil against oxidative stress in the pilocarpine model of epilepsy: a comparison with valproate. Neurochem Res 36:2195-204. doi: 10.1007/s11064-011-0544-9

48. Noor NA, Aboul Ezz HS, Faraag AR and Khadrawy YA (2012) Evaluation of the antiepileptic effect of curcumin and Nigella sativa oil in the pilocarpine model of epilepsy in comparison with valproate. Epilepsy Behav 24:199-206. doi: 10.1016/j.yebeh.2012.03.026

49. Cypriano ML, Dos Santos Ramos GHA, de Oliveira ACF, Dos Santos DR, Fiais GA, de Oliveira AP, Antoniali C, Dornelles RCM, de Melo Stevanato Nakamune AC and Chaves-Neto AH (2021) Effect of testosterone replacement therapy and mate tea (Ilex paraguariensis) on biochemical, functional and redox parameters of saliva in orchiectomized rats. Arch Oral Biol 132:105289. doi: 10.1016/j.archoralbio.2021.105289

50. Dos Santos DR, Fiais GA, de Oliveira Passos A, Dos Santos LFG, Kayahara GM, Crivelini MM, Matsushita DH, Antoniali C, Nakamune A, Dornelles RCM and Chaves-Neto AH (2022) Effects of orchiectomy and testosterone replacement therapy on redox balance and salivary gland function in Wistar rats. J Steroid Biochem Mol Biol 218:106048. doi: 10.1016/j.jsbmb.2021.106048

51. Hartree EF (1972) Determination of protein: A modification of the lowry method that gives a linear photometric response. Analytical Biochemistry 48:422-427. doi: 10.1016/0003-2697(72)90094-2

52. Nagy A, Barta A, Varga G and Zelles T (2001) Changes of salivary amylase in serum and parotid gland during pharmacological and physiological stimulation. J Physiol Paris 95:141-5. doi: 10.1016/s0928-4257(01)00018-3

53. Correia RR, Batista VRG, Veras ASC, Tavares MEA, Souza FLA, Pacagnelli FL, Campos DHS, Giometti IC and Teixeira GR (2022) High-intensity interval training attenuates the effects caused by arterial hypertension in the ventral prostate. Prostate 82:373-387. doi: 10.1002/pros.24285

54. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103-11. doi: 10.1016/j.clinbiochem.2005.08.008

55. Buege JA and Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302-10. doi: 10.1016/s0076-6879(78)52032-6

56. Mesquita CS, Oliveira R, Bento F, Geraldo D, Rodrigues JV and Marcos JC (2014) Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Anal Biochem 458:69-71. doi: 10.1016/j.ab.2014.04.034

57. Benzie IF and Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70-6. doi: 10.1006/abio.1996.0292

58. Beutler E, Duron O and Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882-8.

59. Wendel A (1981) Glutathione peroxidase. Methods Enzymol 77:325-33. doi: 10.1016/s0076-6879(81)77046-0

60. Korkmazer N, Vurucu S, Demirkaya E, Unay B, Kul M, Akin R and Gokcay E (2006) Serum and liver tissue biotinidase enzyme activity in rats which were administrated to valproic acid. Brain Dev 28:515-20. doi: 10.1016/j.braindev.2006.03.003

61. Oztopuz O, Turkon H, Buyuk B, Coskun O, Sehitoglu MH, Ovali MA and Uzun M (2020) Melatonin ameliorates sodium valproate-induced hepatotoxicity in rats. Mol Biol Rep 47:317-325. doi: 10.1007/s11033-019-05134-6

62. Alqarni F, Eweis HS, Ali A, Alrafiah A, Alsieni M, Karim S and Alkathyri MA (2022) The Effect of Coenzyme Q10 on Liver Injury Induced by Valproic Acid and Its Antiepileptic Activity in Rats. Biomedicines 10. doi: 10.3390/biomedicines10010168

63. Nazmy EA, El-Khouly OA, Atef H and Said E (2017) Sulforaphane protects against sodium valproate-induced acute liver injury. Can J Physiol Pharmacol 95:420-426. doi: 10.1139/cjpp-2016-0447

64. Shakya R, Hoque MK, Sapkota AS and Gupta PK (2018) Differential Hepatotoxic Effects of Sodium Valproate at Different Doses in Albino Rats. Kathmandu Univ Med J (KUMJ) 16:78-82.

65. Koroglu OF, Gunata M, Vardi N, Yildiz A, Ates B, Colak C, Tanriverdi LH and Parlakpinar H (2021) Protective effects of naringin on valproic acid-induced hepatotoxicity in rats. Tissue Cell 72:101526. doi: 10.1016/j.tice.2021.101526

66. Haznedar P, Dogan O, Albayrak P, Oz Tuncer G, Teber S, Deda G and Eminoglu FT (2019) Effects of levetiracetam and valproic acid treatment on liver function tests, plasma free carnitine and lipid peroxidation in childhood epilepsies. Epilepsy Res 153:7-13. doi: 10.1016/j.eplepsyres.2019.03.009

67. Ahmed SN and Siddiqi ZA (2006) Antiepileptic drugs and liver disease. Seizure 15:156-64. doi: 10.1016/j.seizure.2005.12.009

68. Khan S, Kumar S and Jena G (2016) Valproic acid reduces insulin-resistance, fat deposition and FOXO1-mediated gluconeogenesis in type-2 diabetic rat. Biochimie 125:42-52. doi: 10.1016/j.biochi.2016.02.014

69. Zhang H, Lu P, Tang HL, Yan HJ, Jiang W, Shi H, Chen SY, Gao MM, Zeng XD and Long YS (2021) Valproate-Induced Epigenetic Upregulation of Hypothalamic Fto Expression Potentially Linked with Weight Gain. Cell Mol Neurobiol 41:1257-1269. doi: 10.1007/s10571-020-00895-2

70. Wolden-Hanson T, Gidal BE and Atkinson RL (1998) Evaluation of a rat model of valproate-induced obesity. Pharmacotherapy 18:1075-81.

71. Shibata K, Kondo R, Sano M and Fukuwatari T (2013) Increased conversion of tryptophan to nicotinamide in rats by dietary valproate. Biosci Biotechnol Biochem 77:295-300. doi: 10.1271/bbb.120716

72. Mazzeo MA, Linares JA, Lopez MM, Gallara RV, Bachmeier E, Wietz FM and Finkelberg AB (2012) Functional impairment in submandibular gland of rats induced by 5-fluorouracil and calcium leucovorin. Acta Odontol Latinoam 25:262-8.

73. Stewart CR, Obi N, Epane EC, Akbari AA, Halpern L, Southerland JH and Gangula PR (2016) Effects of Diabetes on Salivary Gland Protein Expression of Tetrahydrobiopterin and Nitric Oxide Synthesis and Function. J Periodontol 87:735-41. doi: 10.1902/jop.2016.150639

74. Kimura I, Miyamoto H, Chen FJ and Kimura M (1996) The streptozocin-diabetic state depresses saliva secretion stimulated by pilocarpine and noradrenaline in mice. Biol Pharm Bull 19:384-7. doi: 10.1248/bpb.19.384

75. de Jesus VC, Beanes G, Paraguassu GM, Ramalho LM, Pinheiro AL, Ramalho MJ and Rodriguez TT (2015) Influence of laser photobiomodulation (GaAlAs) on salivary flow rate and histomorphometry of the submandibular glands of hypothyroid rats. Lasers Med Sci 30:1275-80. doi: 10.1007/s10103-015-1725-6

76. Lee HJ, Lee YJ, Kwon HC, Bae S, Kim SH, Min JJ, Cho CK and Lee YS (2006) Radioprotective effect of heat shock protein 25 on submandibular glands of rats. Am J Pathol 169:1601-11. doi: 10.2353/ajpath.2006.060327

77. Lasisi TJ and Alada AR (2015) Effects of induced-kwashiorkor on salivary parameters in Wistar rats. Afr J Med Med Sci 44:125-32.

78. Nishimura T, Sakai M and Yonezawa H (2000) Effects of valproic acid on fertility and reproductive organs in male rats. J Toxicol Sci 25:85-93. doi: 10.2131/jts.25.85

79. Mauz PS, Morike K, Kaiserling E and Brosch S (2005) Valproic acid-associated sialadenosis of the parotid and submandibular glands: diagnostic and therapeutic aspects. Acta Otolaryngol 125:386-91. doi: 10.1080/00016480410024460

80. Derin H, Derin S, Oltulu P, Ozbek O and Caksen H (2017) Pediatric Sialadenosis Due to Valproic Acid. J Craniofac Surg 28:e127-e129. doi: 10.1097/SCS.0000000000003303

81. Reisser C and Maier H (1991) [Sialadenosis caused by long-term valproate medication]. Laryngorhinootologie 70:384-6. doi: 10.1055/s-2007-998058

82. Narita T, Qi B, Murakami M and Sugiya H (2019) Pilocarpine induces the residual secretion of salivary fluid in perfused submandibular glands of rats. PLoS One 14:e0221832. doi: 10.1371/journal.pone.0221832

83. Bureau M, Laschet J, Minier F and Chauvel P (1997) [Intervention of GABAergic neurotransmission in partial epilepsies]. Rev Neurol (Paris) 153 Suppl 1:S46-54.

84. Fritschy JM and Mohler H (1995) GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359:154-94. doi: 10.1002/cne.903590111

85. Sawaki K, Ouchi K, Sato T and Kawaguchi M (1995) Existence of gamma-aminobutyric acid and its biosynthetic and metabolic enzymes in rat salivary glands. Jpn J Pharmacol 67:359-63. doi: 10.1254/jjp.67.359

86. Kosuge Y, Kawaguchi M, Sawaki K, Okubo M, Shinomiya T and Sakai T (2009) Immunohistochemical study on GABAergic system in salivary glands. Eur J Pharmacol 610:18-22. doi: 10.1016/j.ejphar.2009.02.043

87. Shida T, Kondo E, Ueda Y, Takai N, Yoshida Y, Araki T, Kiyama H and Tohyama M (1995) Role of amino acids in salivation and the localization of their receptors in the rat salivary gland. Brain Res Mol Brain Res 33:261-8. doi: 10.1016/0169-328x(95)00138-i

88. Proctor GB (2016) The physiology of salivary secretion. Periodontol 2000 70:11-25. doi: 10.1111/prd.12116

89. Jakutiene E, Grikiniene J, Vaitkevicius A, Tschaika M, Didziapetriene J and Stakisaitis D (2007) Sodium valproate stimulates potassium and chloride urinary excretion in rats: gender differences. BMC Pharmacol 7:9. doi: 10.1186/1471-2210-7-9

90. Grikiniene J, Stakisaitis D and Tschaika M (2005) Influence of sodium valproate on sodium and chloride urinary excretion in rats, gender differences. Pharmacology 75:111-5. doi: 10.1159/000087505

91. Homann V, Kinne-Saffran E, Arnold WH, Gaengler P and Kinne RK (2006) Calcium transport in human salivary glands: a proposed model of calcium secretion into saliva. Histochem Cell Biol 125:583-91. doi: 10.1007/s00418-005-0100-2

92. Yu JH and Schneyer CA (1983) Nerve-stimulated secretion of calcium by rat submandibular gland. Proc Soc Exp Biol Med 173:386-9. doi: 10.3181/00379727-173-41661

93. Schneyer CA, Sucanthapree C, Schneyer LH and Jirakulsomchok D (1978) Total salivary calcium and amylase output of rat parotid with electrical stimulation of autonomic innervation. Proc Soc Exp Biol Med 159:478-83. doi: 10.3181/00379727-159-40374

94. Schneyer CA (1986) Calcium levels in rat submandibular gland and saliva after sympathetic nerve stimulation in the presence of alpha, beta 1 and beta 2-adrenergic antagonists. Arch Oral Biol 31:383-6. doi: 10.1016/0003-9969(86)90161-5

95. Moser SO, Haykir B, Kung CJ, Bettoni C, Hernando N and Wagner CA (2023) Expression of phosphate and calcium transporters and their regulators in parotid glands of mice. Pflugers Arch 475:203-216. doi: 10.1007/s00424-022-02764-x

96. Herguner MO, Altunbasak S, Dogan A, Yildizdas D, Incecik F, Erdogan S, Gonlusen G, Daglioglu K, Dikmen N and Burgut R (2006) Effects of sodium valproate on renal functions in rats. Ren Fail 28:593-7. doi: 10.1080/08860220600843821

97. Kuijper-Lenstra AH and Kramer MF (1975) Rate of protein synthesis in rat salivary gland cells after pilocarpine or feeding. I. Rate of (glyco) protein secretion from cells of mixed salivary glands. Cell Tissue Res 164:435-46. doi: 10.1007/BF00219935

98. Ikeno T, Nasu J and Kuzuya H (1983) Quantitative changes in amylase activity in the salivary glands, pancreas, saliva, and serum after administration of isoproterenol, pilocarpine, and acetylcholine. J Dent Res 62:56-7. doi: 10.1177/00220345830620011201

99. Busch L, Sterin-Borda L and Borda E (2002) Differences in the regulatory mechanism of amylase release by rat parotid and submandibular glands. Arch Oral Biol 47:717-22. doi: 10.1016/s0003-9969(02)00057-2

100. Voudris K, Attilakos A, Katsarou E, Mastroyianni S, Dimou S, Skardoutsou A, Prassouli A and Garoufi A (2006) Serum total amylase, pancreatic amylase and lipase activities in epileptic children treated with sodium valproate monotherapy. Brain Dev 28:572-5. doi: 10.1016/j.braindev.2006.04.002

101. Jimenez-Rodriguezvila M, Caro-Paton A, Conde M, Duenas-Laita A, Martin-Lorente JL, Fernandez Perez de Villalain MJ, Gonzalez Martinez de Zarate JL and Maranon A (1986) Side-effects of sodium valproate, mainly related to its hepatic and pancreatic toxicity. Int J Clin Pharmacol Res 6:217-24.

102. Henskens YM, Strooker H, van den Keijbus PA, Veerman EC and Nieuw Amerongen AV (1996) Salivary protein composition in epileptic patients on different medications. J Oral Pathol Med 25:360-6. doi: 10.1111/j.1600-0714.1996.tb00279.x

103. Knas M, Maciejczyk M, Daniszewska I, Klimiuk A, Matczuk J, Kolodziej U, Waszkiel D, Ladny JR, Zendzian-Piotrowska M and Zalewska A (2016) Oxidative Damage to the Salivary Glands of Rats with Streptozotocin-Induced Diabetes-Temporal Study: Oxidative Stress and Diabetic Salivary Glands. J Diabetes Res 2016:4583742. doi: 10.1155/2016/4583742

104. Kolodziej U, Maciejczyk M, Niklinska W, Waszkiel D, Zendzian-Piotrowska M, Zukowski P and Zalewska A (2017) Chronic high-protein diet induces oxidative stress and alters the salivary gland function in rats. Arch Oral Biol 84:6-12. doi: 10.1016/j.archoralbio.2017.09.006

105. Campos SC, Moreira DA, Nunes TD, Colepicolo P and Brigagao MR (2005) Oxidative stress in alcohol-induced rat parotid sialadenosis. Arch Oral Biol 50:661-8. doi: 10.1016/j.archoralbio.2004.11.013

106. Raza M, Al-Bekairi AM, Ageel AM and Qureshi S (1997) Biochemical basis of sodium valproate hepatotoxicity and renal tubular disorder: time dependence of peroxidative injury. Pharmacol Res 35:153-7. doi: 10.1006/phrs.1997.0134

107. Yildiz A, Vardi N, Parlakpinar H, Ates B and Colakoglu N (2022) Effects of Low- and High-Dose Valproic Acid and Lamotrigine on the Heart in Female Rats. Cardiovasc Toxicol 22:326-340. doi: 10.1007/s12012-021-09714-6

108. Naderi M, Ahangar N, Badakhshan F, Ghasemi M and Shaki F (2021) Zinc and selenium supplement mitigated valproic acid-induced testis toxicity by modulating the oxidative redox balance in male rats. Anat Cell Biol 54:387-394. doi: 10.5115/acb.20.280

109. Hussein AM, Awadalla A, Abbas KM, Sakr HF, Elghaba R, Othman G, Mokhtar N and Helal GM (2021) Chronic valproic acid administration enhances oxidative stress, upregulates IL6 and downregulates Nrf2, Glut1 and Glut4 in rat's liver and brain. Neuroreport 32:840-850. doi: 10.1097/WNR.0000000000001663

110. Tong V, Teng XW, Chang TK and Abbott FS (2005) Valproic acid I: time course of lipid peroxidation biomarkers, liver toxicity, and valproic acid metabolite levels in rats. Toxicol Sci 86:427-35. doi: 10.1093/toxsci/kfi184

111. Zalewska A, Knas M, Maciejczyk M, Waszkiewicz N, Klimiuk A, Choromanska M, Matczuk J, Waszkiel D and Car H (2015) Antioxidant profile, carbonyl and lipid oxidation markers in the parotid and submandibular glands of rats in different periods of streptozotocin induced diabetes. Arch Oral Biol 60:1375-86. doi: 10.1016/j.archoralbio.2015.06.012

112. Griesser E, Vemula V, Raulien N, Wagner U, Reeg S, Grune T and Fedorova M (2017) Cross-talk between lipid and protein carbonylation in a dynamic cardiomyocyte model of mild nitroxidative stress. Redox Biol 11:438-455. doi: 10.1016/j.redox.2016.12.028

113. Gonos ES, Kapetanou M, Sereikaite J, Bartosz G, Naparlo K, Grzesik M and Sadowska-Bartosz I (2018) Origin and pathophysiology of protein carbonylation, nitration and chlorination in age-related brain diseases and aging. Aging (Albany NY) 10:868-901. doi: 10.18632/aging.101450

114. Levine RL, Williams JA, Stadtman ER and Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346-57. doi: 10.1016/s0076-6879(94)33040-9

115. Ayala A, Munoz MF and Arguelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014:360438. doi: 10.1155/2014/360438

116. Akagawa M (2021) Protein carbonylation: molecular mechanisms, biological implications, and analytical approaches. Free Radic Res 55:307-320. doi: 10.1080/10715762.2020.1851027

Figures

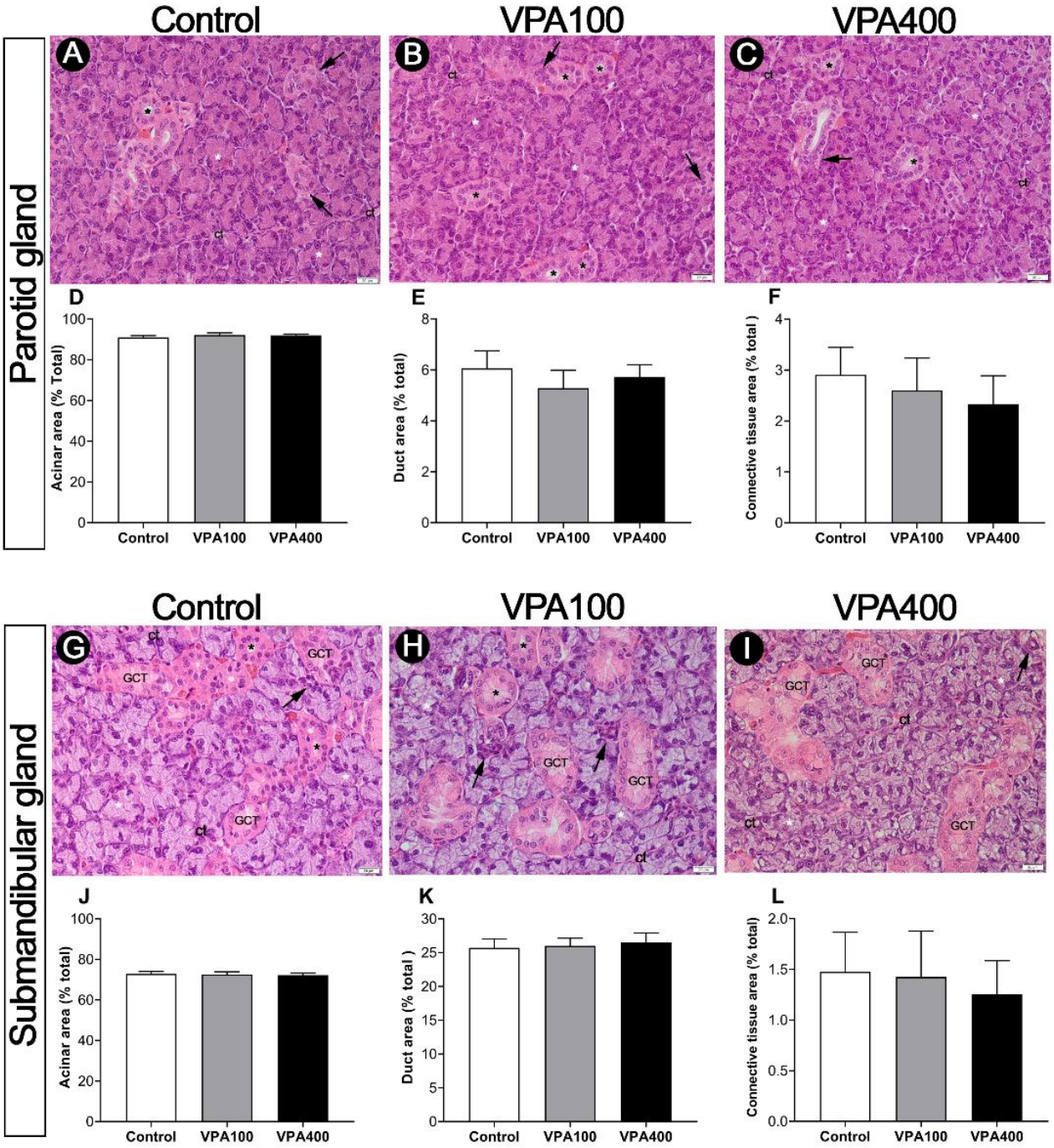

Fig. 1 Effects of VPA on histomorphometric parameters in rat salivary glands. Staining: hematoxylin and eosin. 400x magnification. Photomicrographs of the parotid gland (A, B, and C) and submandibular gland (G, H, and I) and graphical representation of the percentage of the acinar area (D and J), duct area (E and K), and connective tissue (F and L). Abbreviations and symbols: (white asterisks) acinar secretory units; (black asterisks) striated ducts; (arrows) intercalated ducts; (GCT) granular convoluted tubules; (ct) connective tissue. Results were obtained by analysis of variance (One-Way ANOVA) followed by Tukey's *post hoc* test. For all analyses, $p < 0.05$ probability levels were considered statistically significant.

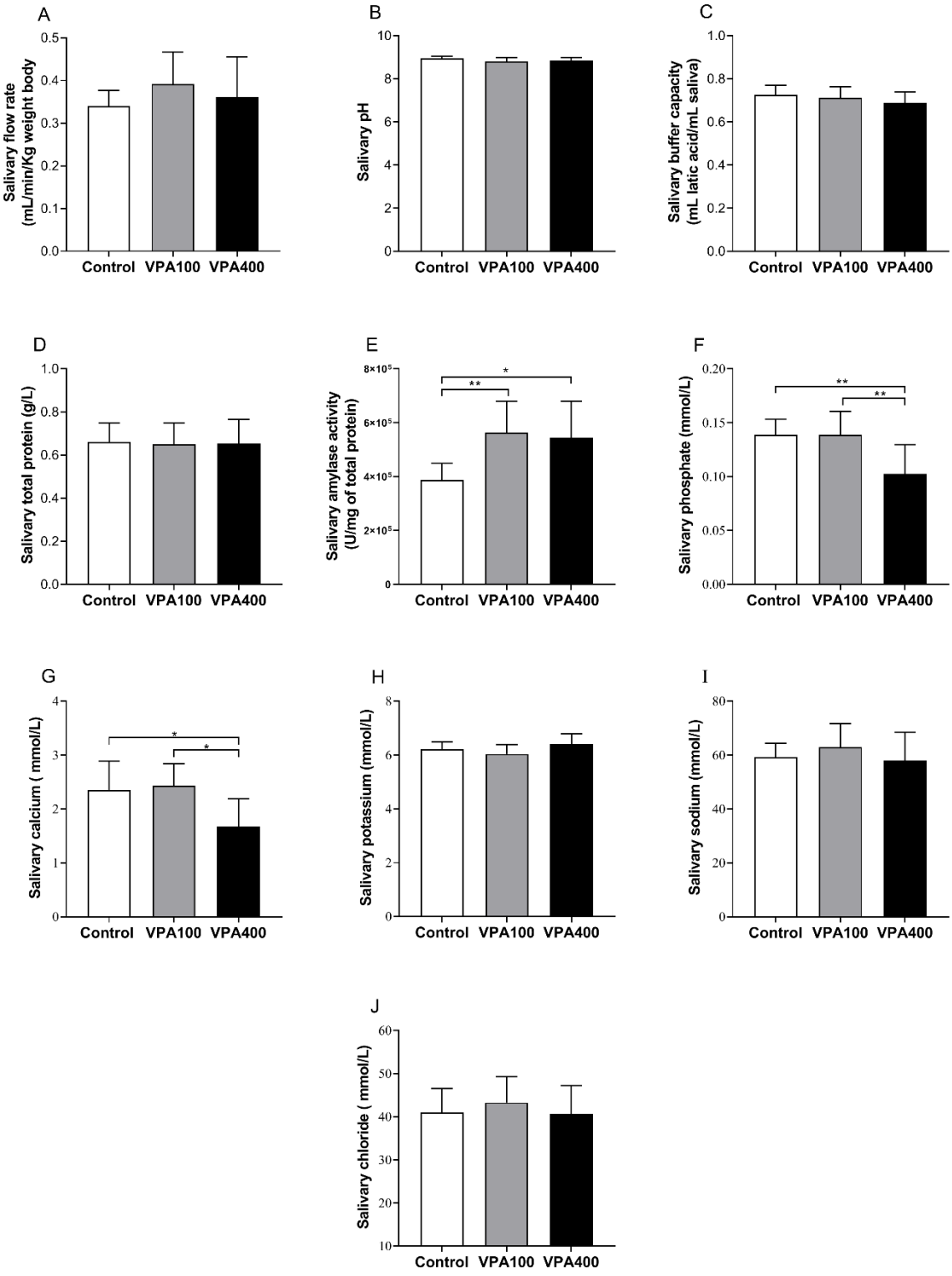

Fig. 2 Effects of VPA on salivary flow rate (A), salivary pH (B), salivary buffering capacity (C), salivary total protein (D), amylase (E), phosphate (F), calcium (G), potassium (H), sodium (I), and chloride (J) from Wistar rats. Data are expressed as mean ± standard deviation. Results were obtained by analysis of variance (One-Way ANOVA) followed by Tukey's *post hoc* test (*$p < 0.05$; **$p < 0.01$). For all analyses, $p < 0.05$ probability levels were considered statistically significant.

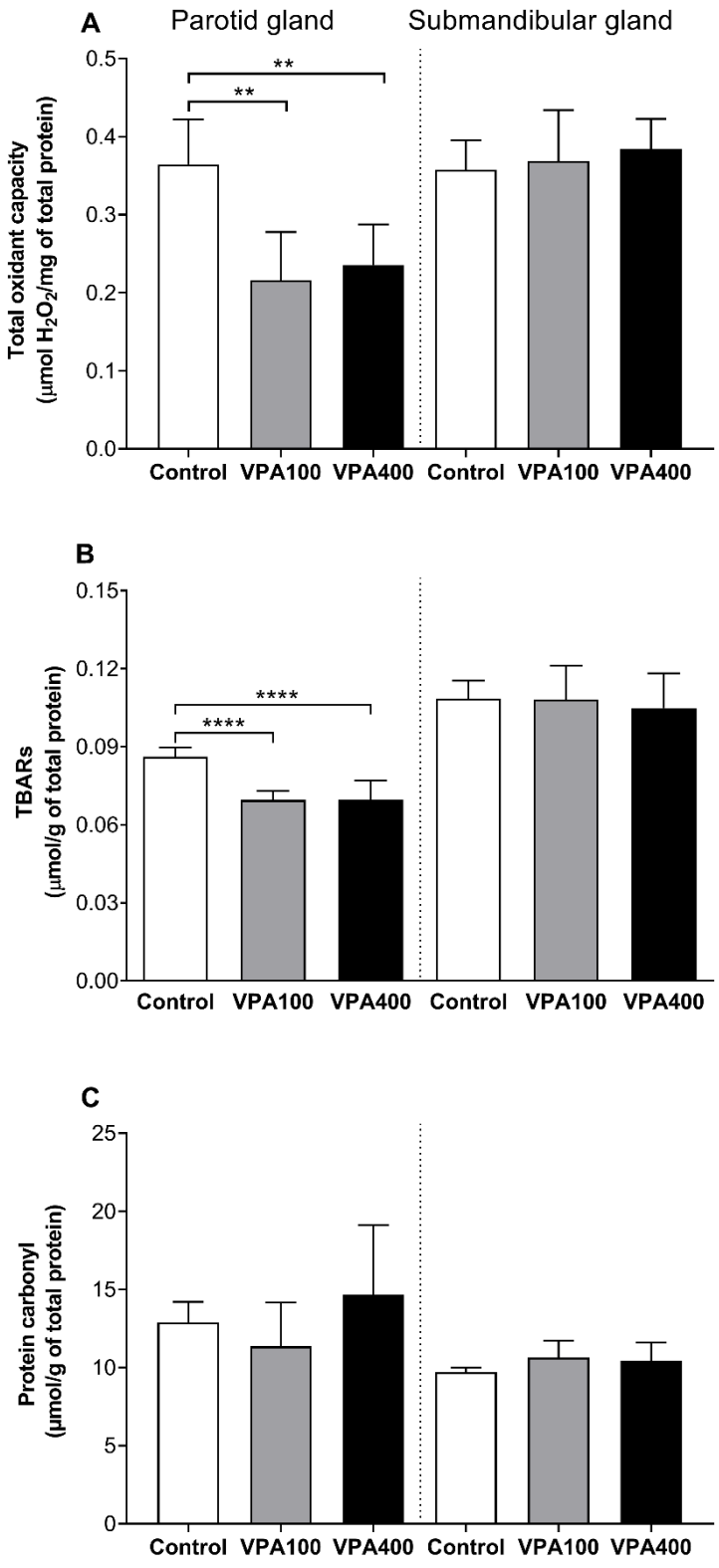

Fig. 3 Effects of VPA on total oxidant capacity (A), lipid oxidative damage - TBARs (B), and carbonyl protein (C) in parotid and submandibular glands of Wistar rats. Data are expressed as mean ± standard deviation. The results were obtained by analysis of variance (One-Way ANOVA) followed by Tukey's *post hoc* test (**$p < 0.01$; ****$p < 0.0001$). For all analyses, $p < 0.05$ probability levels were considered statistically significant.

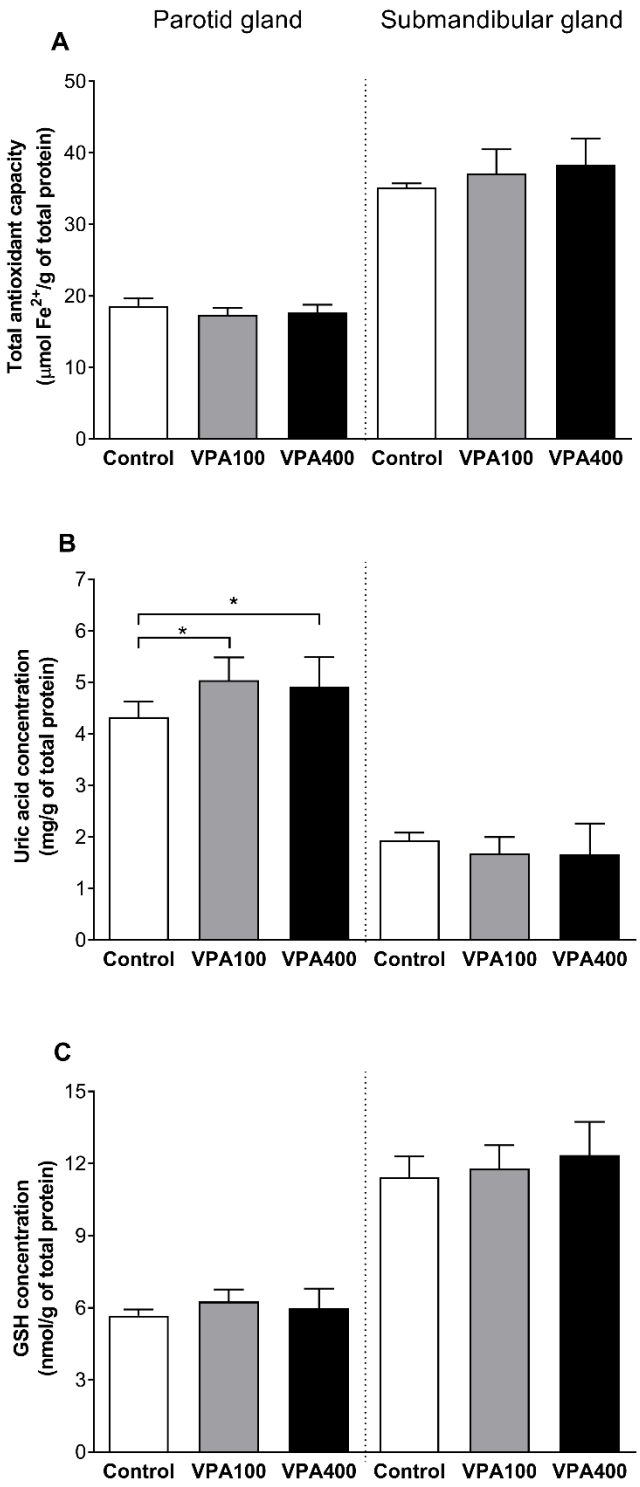

Fig. 4 Effects of VPA on total antioxidant capacity (A), uric acid (B), and GSH (C) of parotid and submandibular glands in Wistar rats. Data are expressed as mean ± standard deviation. Results were obtained by analysis of variance (One-Way ANOVA) followed by Tukey's *post hoc* test. (*$p < 0.05$). For all analyses, $p < 0.05$ probability levels were considered statistically significant.

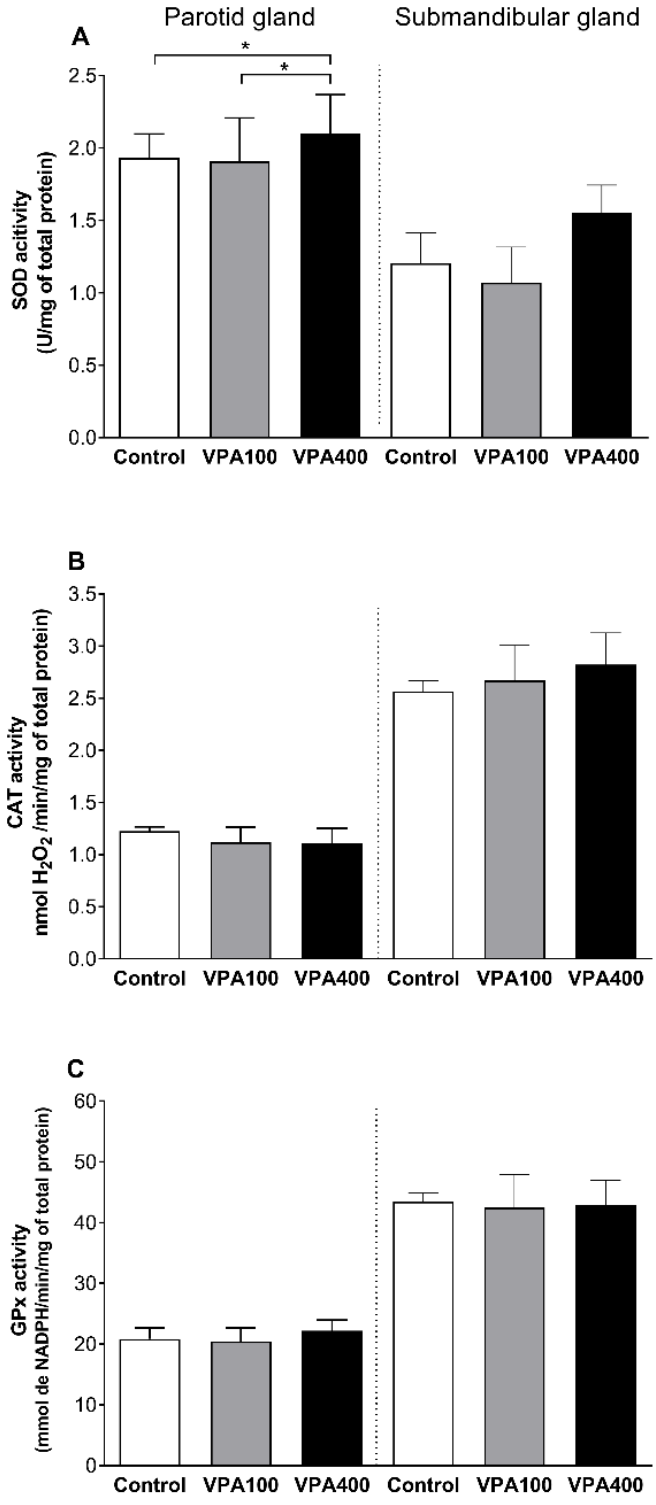

Fig. 5 Effects of VPA on SOD (A), CAT (B), and GPx (C) activities in the parotid and submandibular glands of Wistar rats. Data are expressed as mean ± standard deviation. Results were obtained by analysis of variance (One-Way ANOVA) followed by Tukey's *post hoc* test. (*$p < 0.05$). For all analyses, $p < 0.05$ probability levels were considered statistically significant.

Tables

Table 1 Effects of VPA on ALT and AST activities, body mass, feed consumption and water intake, salivary gland mass and index, and salivary gland total protein concentrations in rats.

Parameters	Control	VPA100	VPA400
ALT activity (U/L)	76.61± 6.42	74.72 ± 7.50	68.97 ± 6.73
AST activity (U/L)	156.9 ± 10.93	145.2 ± 22.37	135.5 ± 15.76
Initial body weigth (g)	207.5 ± 9.06	211.0 ± 19.15	208.3 ± 12.99
Final body weight (g)	366.3 ± 8.37	370.8 ± 19.18	350.3 ± 25.04
Chow intake (kg/ week /group)	781.5 ± 47.38	741.0 ± 164.0	613.0 ± 39.60
Water intake (L/week/group)	1.60 ± 0.04	1.68 ± 0.31	1.38 ± 0.66
Parotid gland weight (mg)	300.0 ± 21.06	314.5 ± 37.34	295.5 ± 10.95
Submandibular gland weight (mg)	239.7 ± 9.88	254.3 ± 20,96	236.9 ± 25.75
Parotid gland index	0.834 ± 0.06	0.853 ± 0.08	0.830 ± 0.07
Submandibular gland index	0.658 ± 0.02	0,697 ± 0,06	0.636 ± 0.06
Parotid gland total protein (mg/g of tissue)	18.78 ± 0.76	19.43 ± 1.34	18.72 ± 1.55
Submandibular gland total protein (mg/g of tissue)	18.77 ± 0.52	17.77 ± 1.94	17.36 ± 1.38

Data are expressed as mean ± standard deviation. Results were obtained by analysis of variance (One-Way ANOVA) followed by Tukey's *post hoc* test. For all analyses, $p < 0.05$ probability levels were considered statistically significant.

ANEXO: Aprovação da comissão de ética no uso de animais

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

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

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado **"Efeitos do anticonvulsivante ácido valproico no stresse oxidativo e função secretora das glândulas salivares de ratos Wistar"**, Processo FOA nº 0215-2021, sob responsabilidade de Antonio Hernandes Chaves Neto apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 27 de Maio de 2021.

VALIDADE DESTE CERTIFICADO: 30 de Novembro de 2023.
DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 30 de Dezembro de 2023.

CERTIFICATE

We certify that the study entitled **"Effects of the anticonvulsant valproic acid on oxidative stress and secretory function of the salivary glands of Wistar rats"**, Protocol FOA nº 0215-2021, under the supervision of Antonio Hernandes Chaves Neto presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on May 27, 2021.

VALIDITY OF THIS CERTIFICATE: November 30, 2023.
DATE OF SUBMISSION OF THE FINAL REPORT: December 30, 2023.

Prof. Associado João Carlos Callera
Coordenador da CEUA
CEUA Coordinator

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