Research, Society and Development, v. 11, n. 7, e13211729619, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i7.29619

Effect of chronic alcoholism on morphology and cell proliferation in salivary glands of Wistar rats

Efeito do alcoolismo crônico sobre a morfologia e a proliferação celular em glândulas salivares de ratos Wistar

Efecto del alcoholismo crónico sobre la morfología y proliferación celular en glándulas salivales de ratas Wistar

Received: 04/26/2022 | Reviewed: 05/05/2022 | Accept: 05/13/2022 | Published: 05/18/2022

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Abstract
Several disorders which affect the oral cavity, salivary composition and, flow have been described in alcoholics chronic, thus this study aimed to evaluate the effects of chronic alcoholism on the salivary glands of Wistar rats. It was used 18 animals randomly subdivided into two groups: control (C) and treated with ethanol (T). C group (n=9) received water ad libitum and T group (n=9) received alcoholic solution ad libitum: 5% ethanol (1st week), 10% ethanol (2nd week) and 20% ethanol (for 9 weeks). After the treatment period, the animals were anesthetized and

euthanized by decapitation. The parotid, submandibular and sublingual salivary glands of both groups were dissected, fixed and processed for paraffin. Histological sections were stained with hematoxylin/eosin and Picro-Sirius red stained, and the rate of cell proliferation was determined by immunohistochemistry with the marker KI-67. The salivary glands of group C showed preserved parenchyma, while the parotid and submandibular glands of group T showed hydropic degeneration, atypical nuclei, non-evident nucleolus and condensed chromatin in serous acini. The intercalated, striated and excretory ducts were seen with punctual areas of degeneration and nuclear atypia. In the submandibular glands there was a 73% increase in proliferative cells in T, when compared to C ($p<0.05$). However, in the parotid glands, there was no significant difference in the rate of cell proliferation. The results suggest that there is an increase in the rate of cell proliferation in submandibular glands of Wistar rats subjected to chronic alcohol consumption, and also show that chronic alcohol consumption promotes morphological changes in the salivary glands of these rats, causing a deleterious effect on cellular structures.
Keywords: Alcoholism; Parotid gland; Salivary gland; Sublingual gland; Submandibular gland.

Resumo

Em alcoólatras crônicos são descritos vários distúrbios que afetam a cavidade oral e a composição e o fluxo salivar. Desta forma, este estudo visou avaliar os efeitos do etilismo crônico sobre as glândulas salivares de ratos Wistar. Foram utilizados 18 animais subdivididos aleatoriamente em dois grupos: controle (C) e tratados com etanol (T). O grupo C (n=9) recebeu água ad libitum e o T (n=9) recebeu solução alcoólica ad libitum: etanol 5% (1ª semana), etanol 10% (2a semana) e etanol 20% (por nove semanas). Após o período de tratamento, os animais foram anestesiados e sacrificados por decapitação. As glândulas salivares parótidas, submandibulares e sublinguais de ambos os grupos foram dissecadas, fixadas e processadas para parafina. Os cortes histológicos foram corados com hematoxilina/eosina e picro-sirius, e a taxa de proliferação celular foi determinada por imuno-histoquímica com o marcador KI-67. As glândulas salivares do grupo C apresentaram o parênquima preservado, ao passo que as glândulas parótidas e submandibulares do grupo T apresentaram degeneração hidrópica, núcleos atípicos, nucléolo não evidente e cromatina condensada nos ácinos serosos. Os ductos intercalares, estriados e excretores foram vistos com áreas pontuais de degeneração e atipia nuclear. Nas glândulas submandibulares houve um aumento de 73% de células proliferativas em T, quando comparado com C ($p<0.05$). Contudo, nas glândulas parótidas, não houve diferença significativa na taxa de proliferação celular. Os resultados sugerem que ocorre aumento da taxa de proliferação celular em glândulas submandibulares de ratos Wistar submetidos ao consumo crônico de álcool, e também mostram que o etilismo crônico promove alterações morfológicas nas glândulas salivares destes ratos, causando um efeito deletério nas estruturas celulares.
Palavras-chave: Glândulas salivares; Alcoolismo; Glândula parótida; Glândula sublingual; Glândula submandibular.

Resumen

En los alcohólicos crónicos se describen varios trastornos que afectan la cavidad oral y la composición y flujo salival. Así, este estudio tuvo como objetivo evaluar los efectos del consumo de alcohol enfermedad crónica en las glándulas salivales de ratas Wistar. Dieciocho animales se dividieron aleatoriamente en dos grupos: control (C) y tratados con etanol (T). El grupo C (n=9) recibió agua ad libitum y el grupo T (n=9) recibió solución alcohólica ad libitum: etanol al 5% (1ª semana), etanol al 10% (2ª semana) y etanol al 20% (durante nueve semanas). Después del período de tratamiento, los animales fueron anestesiados y sacrificados por decapitación. las glándulas Se disecaron glándulas salivales parótidas, submaxilares y sublinguales de ambos grupos, fijado y transformado en parafina. Las secciones histológicas se tiñeron con hematoxilina/eosina y picrosirius, y la tasa de proliferación celular se determinó mediante inmunohistoquímica con el marcador KI-67. Las glándulas salivales del grupo C presentaban parénquima preservado, mientras que las glándulas parótidas y submandibulares del grupo T presentaban degeneración hidrópica, núcleos atípicos, nucléolo no evidente y cromatina condensada en acinos serosos. Los conductos intercalados, estriados y excretores se observaron con áreas puntuales de degeneración y atipia nuclear. En las glándulas submandibulares hubo un aumento del 73% de células proliferativas en T, en comparación con C ($p<0,05$). Sin embargo, en las glándulas parótidas no hubo diferencia significativa en la tasa de proliferación celular. Los resultados sugieren que existe un aumento en la tasa de proliferación celular en las glándulas submandibulares de ratas Wistar sometidas al consumo crónico de alcohol, y también muestran que el consumo crónico de alcohol promueve cambios morfológicos en las glándulas salivales de estas ratas, provocando un efecto deletéreo en la función celular estructuras.
Palabras clave: Glándulas salivales; Alcoholismo; Glándula parótida; Glándula sublingual; Glándula submandibular.

1. Introduction

Saliva is a composite fluid that performs important functions such as food bolus formation, lubrication, digestion, protection against infectious agents, tissue repair, and maintenance of mouth moisture and teeth integrity (Katchburian & Arana, 2012; Nanci, 2013; Saito, 2021; Chibly et al., 2022). The major salivary glands are the main producers of saliva, and the parotid gland is responsible for the initial digestion of carbohydrates due to the high activity of amylase. In turn, the

submandibular glands have the function of hydrolyzing some bacteria, as they secrete lysozymes and lactoferrin (Katchburian & Arana, 2012; Nanci, 2013). And, the duct system originates from the glandular parenchyma and acts as a means of transporting fluid from the internal environment to the external environment, in addition to altering the composition of saliva (Nanci, 2013).

The oral health depends on salivary flow due its regulatory functions (Cotroneo et al., 2008), since reduced flow of this substance can occur in pathological situations, like xerostomia, or in non-pathological, like during sleep, or drugs or medicine uses (Katchburian & Arana, 2012). Another saliva feature is that can be used for substance detection such as amphetamines, benzodiazepines, barbiturates, cocaine, tobacco, opioids and alcohol (Guo et al., 2007).

Ethyl alcohol is a widely consumed beverage which causes mood swings and social disorders, besides clinical and physiological problems. This drink has a rapid diffusion in oral cavity but it also occurs in gastric and duodenal regions, reaching bloodstream and tissues for consequence (Waszkiewicz et al., 2013). Its chronic consumption leads to pathological disorders like cirrhosis, bleeding episodes, ascites, neurological disorders, vitamin deficiency, among others (Banderas et al., 1991).

Alcohol and cigarettes consumption cause oral cavity changes in salivary flow and composition, injuries, atrophy, hyperproliferation, and even cancer (Chang et al., 2012; Waszkiewicz et al., 2013). Furthermore, ethanol removes the mucosal lipid layer, leaving it more permeable and vulnerable by carcinogenic agents (Ogden & Wight, 1998; Wight & Ogden, 1998).

Chronic alcoholism causes mucosal atrophy, dysplasia, hyper regeneration, fat deposition on salivary glands, increase of acinar cells, reduction of salivary flow, reduction in electrolytic and protein concentration on saliva, and diffusion of inflammatory cells (Riedel et al., 2003; Waszkiewicz et al., 2013). In such context, this study aimed to evaluate the alcoholism chronic effects of morphology and cellular proliferation profile of salivary glands of adult Wistar rats.

2. Methodology

This is a prospective, experimental, quantitative and analytical study (Pereira et al, 2018). The research protocol complies with the Ethical Principles of Animal Experimentation, adopted by the Brazilian College of Animal Ethics (COBEA) and was approved (Process 323) by the Committee on Ethics in the Use of Animals (CEUA) of the Federal University of Triângulo Mineiro (UFTM).

Animals

18 Wistar rats with eight weeks of life, were maintained in a vivarium under controlled conditions: temperature between 22ºC and 24ºC and light/dark cycle of twelve hours.

The animals were randomly divided into two groups: control (C) and treated with ethanol (T). Group C (n=9) received water ad libitum. And, the T group (n=9) received alcoholic solution ad libitum: 5% ethanol in the first week, 10% ethanol in the second week, and 20% ethanol for a period of nine weeks (Tirapelli et al., 2008). All rats were fed (NUVILAB CR-1 rodent chow) *ad libitum*.

After the experimental period, animals were anesthetized with Thiopental (Cristália, Itapira, SP) at a dose of 60mg/Kg by body weight, intraperitoneally. Once the anesthetic state was confirmed, they were euthanized by decapitation, and the parotid, submandibular and sublingual salivary glands (right and left) of groups C and E were dissected and collected for histological processing.

Histological Processing of the Salivary Glands

After removal of the salivary glands (parotid, submandibular and sublingual), these were fixed in 10% formaldehyde solution, with 0.1M phosphate buffer and pH 7.4, for 48 hours. Then, the glands were dehydrated in ethyl alcohol (alcohol 70%, 80%, 90%, absolute I, absolute II for two hours each), diaphanized in Xylol I and II for fifteen minutes each, and embedded in paraffin.

Histological sections, 5 µm thick, were obtained using a microtome (Leica®, São Paulo, SP) and placed centrally on slides, covered with Poly-y-lisine® (Sigma-Aldrich, St. Louis, MO).

Histological sections were stained with hematoxylin and eosin (HE) by conventional methods. Samples were kept in xylene overnight for deparaffinization. Subsequently, hydration was performed (absolute alcohol I, absolute alcohol II and 70% alcohol) and washing with running water. The sections were bathed in Hematoxylin for nine minutes and kept in water for differentiation. Then, the material was stained with Eosin for 5 minutes and then the excess dye was removed with water. After staining, the sections were dehydrated (90% alcohol, absolute alcohol I, and absolute alcohol II) and cleared with xylene for 20 minutes, and the slides were mounted with Entellan® (Merk KGaA, 64271 Dannstadt, Germany).

The histological technique of staining with picro-sirius was also performed, which evidences the collagen fibers. For this technique, the samples were kept overnight in xylene for deparaffinization, followed by hydration (absolute alcohol I, absolute alcohol II and 70% alcohol) and washing with running water. The sections were stained for 30 minutes with Sirius Red solution, then counterstained with Hematoxylin for three minutes and then placed in water for differentiation. After staining, the sections were dehydrated (90% alcohol, absolute alcohol I, and absolute alcohol II) and cleared with xylene for 20 minutes, and the slides were mounted with Entellan® (Merk KGaA, 64271 Dannstadt, Germany).

Morphological and immunohistochemical evaluation

Qualitative morphological evaluation of samples from groups C and T was performed under ordinary light microscopy by two independent evaluators. The secretory portions, the excretory duct system and the stroma of the samples from groups C and T were histologically analyzed in the entire histological section of the salivary glands (right and left). In sections stained with HE, the observation of the parenchyma was performed, describing the normal parameters and the parameters of pathological alterations, such as: presence of enlarged cells, pyknotic nuclei, cell death, and degeneration. Using the histological technique of staining with Picro-Sirius, the stroma of the salivary glands was evaluated to verify normal collagen fibers and possible presence of fibrosis. The images were obtained using an Axio Vert.A1® microscope (Carl Zeiss Microscopy GmbH 37081 Gottingen, Germany) and processed with the Photoshop® program.

In the immunohistochemical reactions with cell proliferation marker KI-67, initially deparaffinization was performed in xylene. For antigen recovery, slides were placed in Tris/EDTA buffer solution and incubated in a Decloaking Chamber. After cooling, the slides were washed with PBS (Phosphate Buffer Saline) and endogenous peroxidase was blocked with H_2O_2. Protein blocking solution was applied and incubated with anti-KI-67 primary antibody (MIB-1, sc-101861, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight. The slides were incubated with complement reagent and the development was done with DAB chromogen solution (diaminobenzidine), REVEAL biotin-free detection system and DAB substrate system (BIOGEN®, Pleasanton, CA), a. For counterstaining, Harris' hematoxylin was used. Negative controls consisted of omission of the primary antibody and palatine tonsils were used as positive controls. Cells that had brown staining of the nucleus were considered positive for KI-67.

Morphometric evaluation was performed (using an optical microscope with a 100x objective) quantifying the number of KI-67 positive cells in relation to the total number of cells in the intercalated ducts, in 10 random fields, with the final value

corresponding to the mean of the values obtained in the evaluated fields. The results were analyzed with the SPSS 16.0® program and the graphs made with the Graphpad Prism® program. The Kolmogorov-Smirnov and Mann Whitney tests were applied, with $p<0.05$ being considered significant.

3. Results

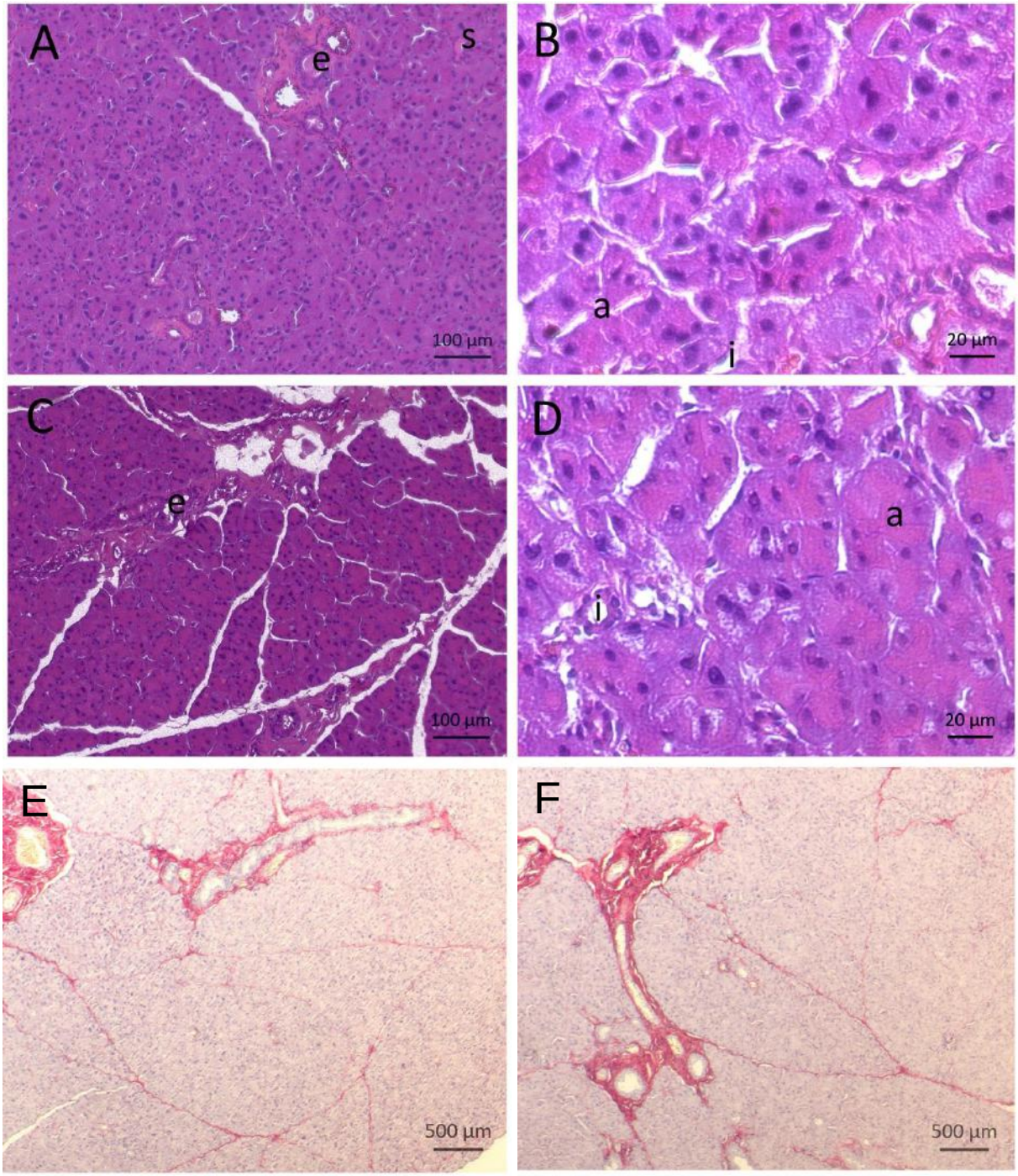
Morphological Evaluation of the Parotid Glands

In the control group, the parotid glands presented serous acini consisting of pyramidal cells, which have rounded basophilic nuclei with loose chromatin and evident nucleoli, located in the basal portion of the cell. The cytoplasm appeared basophilic due to the presence of zymogenic granules, which contain enzymes. Serous acini were characterized by the basophilic appearance with a small central lumen, lumen. In the excretory portion, the intercalated ducts presented simple cubic epithelium. From the intercalated ducts, the striated ducts originate, which were identified by the presence of tall columnar cells, simple columnar epithelium, with large and ovoid nuclei, located at the apex of the cell; and the cytoplasm of the cells of this duct was characterized by the presence of striations, which originate from the interdigitations and cytoplasmic extensions of adjacent cells (Figure 1 A and B).

In the alcoholic group, the parotid glands presented, in the serous acini, hydropic degeneration, atypical nuclei, non-evident nucleolus and condensed chromatin. In some acini, a basophilic staining was observed at the ends and acidophilic in the center. The striated ducts had a degraded and atypical shape, with a decrease in the constitution of the cytoplasm and atypical nuclei stained in purple. In the histological section, intercalated and excretory ducts were visualized with punctual areas of degeneration and nuclear atypia (Figure 1 C and D).

The morphological aspect of the stroma of the parotid glands from the control group to the alcoholic group was similar, analyzed by Picro-Sirius staining (Figure 1 E and F). There was the presence of a capsule of dense non-modeled connective tissue surrounding the gland, which is composed of collagen fibers (stained in red). Between the lobules and between the acini of the glands, collagen fibers were observed, arranged around the cells. And, long collagen bundles were seen around the striated and excretory ducts. It was also observed the presence of collagen fibers involving the blood vessels.

Figure 1. Morphology and stroma of Wistar rats Parotid Glands from the control and ethanol-treated groups: (A and B) parotid gland from a control animal, stained with Hematoxylin and Eosin (HE); (C and D) parotid gland from an animal from the ethanol-treated group, stained with HE; (E and F) parotid glands of rats from the control and ethanol-treated groups, respectively, stained with Picro Sirius and Hematoxylin, showing collagen fibers in red and nuclei in blue. Legends referenced in the figures: “e” excretory duct; “s” striated duct; "a" acini; “i” intercalary duct.



Source: Authors.

Morphological Evaluation of the Submandibular Glands

In the control group, serous and mainly mucous acini were seen in the secretory portion. Mucous acini had clear cell boundaries stained in purple, with weakly stained cytoplasm, flattened nuclei located in the basal part of the cell, and

mucinogen granules. In serous acini, it was not possible to observe the cell limit, their nuclei were rounded and their cytoplasm basophilic. Next to the acini, intercalated ducts lined by simple pavement epithelium and a small lumen were observed. At higher and lower magnification, the striated ducts were observed, which are larger than the intercalated ducts and lined by a columnar epithelium with tall and short cells. In the center of the duct, there was the presence of a lumen, and, in the cytoplasm, basal striations and basophilic nuclei, located towards the apex of the cell, were evidenced. Intralobular excretory ducts and extralobular excretory ducts were observed, which are lined by cylindrical stratified epithelium. These two ducts have a wider lumen than the others, and cells with rounded basophilic nuclei located in the central region. The presence of granular ducts was verified only in the salivary glands of male rats. Such ducts are distinguished by their relatively larger size, compared to the other ducts, and by their eosinophilic staining (Figure 2 A and B).

Morphologically, in the alcoholic group, serous and mucous acini with hydropic degeneration, pyknotic and atypical nuclei, condensed chromatin and no evident nucleolus were observed. Histologically, the striated and excretory ducts of the submandibular glands are disfigured due to the presence of cellular degeneration (Figure 2 C and D).

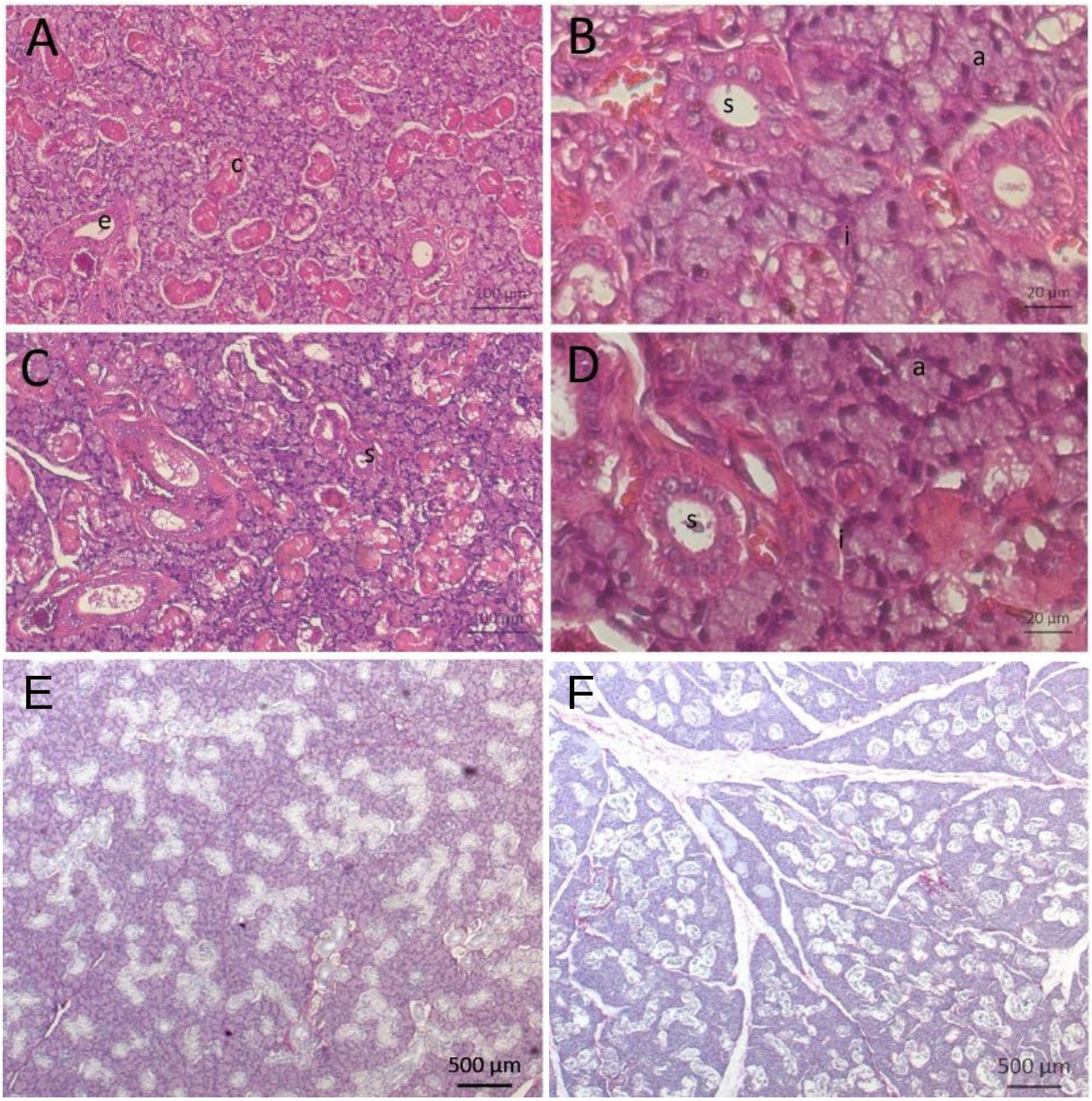
The submandibular glands appeared surrounded by a capsule of dense non-modeled connective tissue, which appeared stained in red due to the evidencing of collagen fibers. The stroma between the lobules and acini of the glands was observed with a reddish color due to collagen fibers. Around the vessels and the striated and excretory ducts, long collagen bundles were seen, highlighted in red (Figure 2 E). When comparing the alcoholic group with the control, it was observed that there were no changes in the collagen fibers and there was no presence of fibrosis (Figure 2 F).

Morphological Evaluation of the Sublingual Glands

In the control group, the presence of mucous tubules was observed, identified through the lighter color and flattened nuclei in the basal region of the cell. In the excretory portion, the intercalated and striated ducts were observed with simple cubic and simple columnar epithelium, respectively, and with basophilic rounded nuclei. The excretory ducts were seen lined by cubic stratified epithelium and ovoid basophilic nuclei (Figure 3 A and B). The morphological analysis of the sublingual glands of the alcoholic group showed no differences in relation to the control group, that is, there was no histological change in the sublingual glands (Figure 3 C and D).

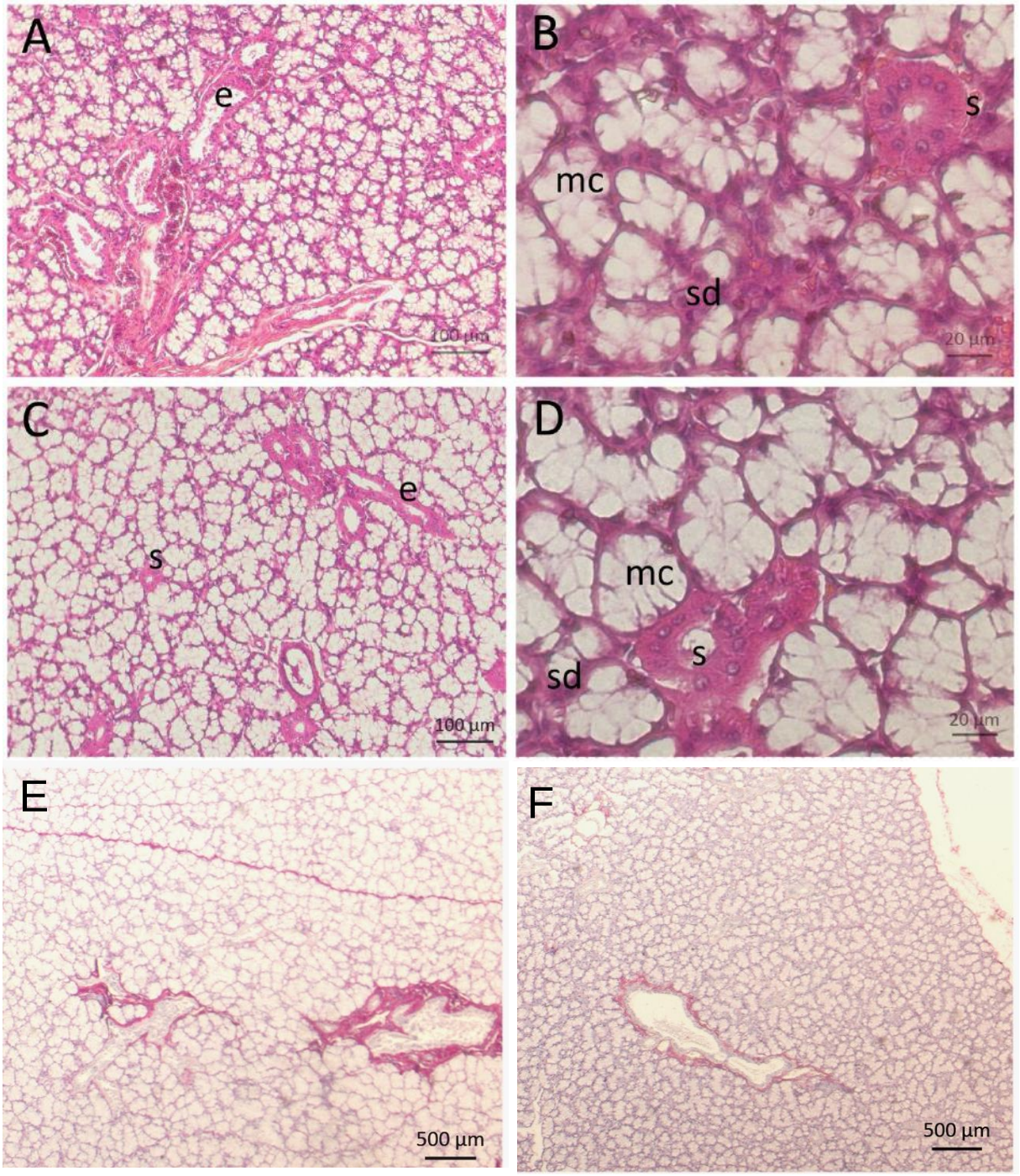
In the control group, collagen fibers were observed, stained in red, between the lobes and between the acini. Around the sublingual, a capsule was seen, consisting of dense non-modeled connective tissue, stained by Picro-Sirius. There was also a reddish color around the ducts and blood vessels (Figure 3 E). Morphologically, no changes were observed between the control and alcoholic groups in the sublingual glands by Picro-Sirius staining. That is, there were no changes in the stroma of the glands (Figure 3 F).

Figure 2. Morphology and stroma of Wistar rats Submandibular Glands from the control and ethanol-treated groups: (A and B) submandibular gland of an animal from the control group, stained with Hematoxylin and Eosin (HE); (C and D) gland from animal from the ethanol-treated group, stained with HE; (E and F) glands of rats from the control and ethanol- treated groups, respectively, stained with Picro Sirius and Hematoxylin, showing collagen fibers in red and nuclei in blue. Legends referenced in the figures: “e” excretory duct; “s” striated duct; "a" acini; “i” intercalary duct.



Source: Authors.

Figure 3. Morphology and stroma of Wistar rats sublingual glands from the control and ethanol-treated groups: (A and B) sublingual gland of an animal from the control group, stained with Hematoxylin and Eosin (HE); (C and D) gland from animal from the ethanol-treated group, stained with HE; (E and F) glands of rats from the control and ethanol-treated groups, respectively, stained with Picro Sirius and Hematoxylin, showing collagen fibers in red and nuclei in blue. Legends referenced



in the figures: “e” excretory duct; “s” striated duct; "a" acini; “i” intercalary duct.

Source: Authors.

The proliferation rate of intercalated duct cells

In the parotid glands, there was no significant difference in the rate of cell proliferation of the intercalated ducts (U =

40.5; p>0.05), with the number of proliferating cells in group C (0.10± 0.10 cells) similar to the T group (0.10±0.08 cells) (Figure 4). However, in the submandibular glands there was a significant increase (U= 11.0; p<0.05) of KI-67 positive intercalated duct cells biological process that guarantees the renewal of secretory cells) in the T group (0.40 ± 0.12 cells) when compared with group C (0.23±0.08 cells), corresponding to a 73% increase in proliferative cells (Figure 5). No KI-67 positive cells were seen in the sublingual glands of both groups.

Figure 4. Effect of chronic alcohol consumption on cell proliferation (quantity of KI-67 positive cells) in the intercalated ducts in parotid glands of Wistar rats in the control (C) and ethanol-treated (T) groups: Mann Whitney Test - U, p>0.05.

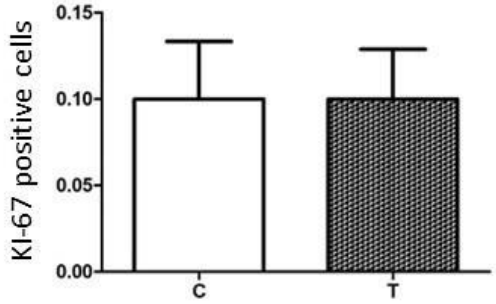


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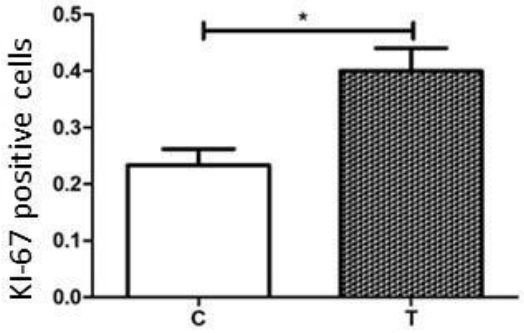
Figure 5. Effect of chronic alcohol consumption on cell proliferation (quantity of KI-67 positive cells) in the intercalated ducts in submandibular glands of Wistar rats from the control (C) and ethanol-treated (T) groups: Mann Whitney Test - U, *p<0.05.



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4. Discussion

In this study, salivary glands of adult Wistar rats were evaluated in order to observe possible histopathological changes caused by chronic consumption of ethanol. Another study with Wistar rats evaluated the effect of chronic alcohol intake on the regeneration of the submandibular glands, through histological analysis of the extraction of part of these glands. The authors observed that animals that received ethanol showed changes in the pattern of glandular regeneration, with changes in basal laminin expression and glycoprotein production, causing advanced morphogenesis and delayed cytodifferentiation during salivary gland regeneration, probably due to the effects of alcohol (Nor et al., 2013).

Toxic metabolites derived from chronic alcoholism promote oxidative stress, and, consequently, cell necrosis and

secretion of chemical mediators that cause recruitment and activation of inflammatory cells (Szabo et al., 2007). Other morphometric studies of rat salivary glands showed: the serous and mucous acini of the submandibular and sublingual glands were increased in size in chronic alcoholics (Carranza et al., 2005); and that there was a significant difference between the size of the acini of the parotid gland in the control group and in the alcoholic group, which varied between 70 and 100 microns (Mandel & Hamele-Bena, 1997). In this study, morphologically, a possible increase in the size of acini was observed in alcoholic animals in relation to non-alcoholic animals. In another study, it was found that the normal sizes of acini were 40 microns and that in alcoholic rats the parotid and submandibular glands show larger acini (Donat, 1975). In contrast to other authors, normal acini were reported to have a size of 54 microns, while serous acini of alcoholics had 74 microns (Gupta & Sodahani, 1998).

Another study also showed, in alcoholic rats, the area occupied by acini was larger due to a greater number of cells, and that the lumen was increased, suggesting that these characteristics could be associated with hypertrophy and hyperplasia of the salivary gland (Carranza et al., 2005). In addition, some authors suggested that in the parotid glands of alcoholics, the acini had increased function and suffered hypertrophy (Abelson et al., 1976; Mandel & Hamele-Bena, 1997).

In the present study, it was possible to observe changes in the duct system of the parotid and submandibular glands, with the cytoplasm of the ductal cells showing a reduced constitution, the nuclei were atypical and there was the presence of cellular degeneration. Alcohol consumption affects the duct system in salivary glands (Smith and Esguep, 1995) and, in autopsies and salivary gland biopsy of chronic alcoholics, an increase in the size of the lumen of the ducts that constitute the salivary glands was observed (Ferraris et al., 1999; Carda et al., 2004).

Marked structural alterations, such as atrophy with interstitial fibrosis and fatty infiltration, have already been reported in the salivary glands of adult rats submitted to chronic ethanol consumption (Carvalho et al., 1993). The appearance of lipid inclusions, or hydropic degeneration, which were present in the cytoplasm of the acini and in the constitution of the ducts, in most structures of the parotid and submandibular glands were observed in this study. It can be suggested that these inclusions originate from ethanol metabolites, and that alcohol and its metabolites cause functional changes in the salivary glands, promoting the formation of lipid inclusions in acini and ducts (Carda et al., 2004).

In necropsies of parotid and submandibular glands of alcoholics, a minority of cases were affected by alcohol, and in the presence of cirrhosis of the liver, the parotid and submandibular glands had reduced volume of acini and the presence of adipose tissue, and the submandibular glands had minor changes (Scott et al., 1988). In agreement with these results, the present study showed that, among the glands studied, the parotid glands were the most morphologically affected by the chronic consumption of ethanol. This increase in adipose tissue in the glands suggests a disorder in the metabolism of fat in the liver resulting from liver cirrhosis (Scott, 1977; Scott et al., 1987). In chronic alcoholics, there are changes in the structure of the parotid glands, with the presence of accumulation of adipocytes in the cytoplasm (Ferraris et al., 1995; Ferraris et al., 1999), however, lipid accumulation occurs only at the beginning of alcoholism due to alteration in the cell membrane (Gorlin & Goldman, 1973).

In this work, it was possible to verify an accumulation of lipid inclusions in rats after 90 days of treatment with ethanol. There are reports that lipids inside the cytoplasm can affect the duct system and the innervation of the salivary glands (Kim et al., 1998). However, another author observed only dilatation in the excretory ducts, but without significant alterations in the ducts of the parotid glands in alcoholic patients (Gomez, 1973).

In liver cirrhosis, some of the main clinical alterations are the enlargement of the parotid glands, which is painless, and sialosis (Mandel & Baurmash, 1971; Abelson et al., 1976). However, in chronic alcoholism there is no enlargement of the gland, nor the occurrence of sialosis (Vicary et al., 1988), however, in this study we observed an enlargement of parotid and

submandibular glands.

In addition to the increase in adipose tissue, in the salivary glands in chronic alcoholism, there is no presence of any other structural alteration, therefore, there is no correlation with high salivary flow rates (Durr et al., 1975; Abelson et al., 1976; Vicary et al., 1988). Thus, it is suggested that the modification of salivary flow is due to physiological and hemodynamic changes caused by alcoholism (Abelson et al., 1976). However, there is evidence that alterations occur in the vascular tissue of the salivary glands in chronic alcoholics, that is, the presence of lower concentrations of vascular tissue was reported when compared to the control group (Scott et al., 1988).

There are reports which suggest that ethanol induces histological and morphometric changes in the salivary glands of rats intoxicated with ethanol during adolescence (Fernandes et al., 2015), and, recently, the effect of excessive alcoholism during pregnancy in Wistar rats has been evaluated. oxidative stress and morphometric alterations were detected in the pups' salivary glands, in addition to a functional reduction of the salivary amylase enzyme (Ferreira et al., 2021).

Considering that chronic alcohol consumption can lead to important changes in the salivary glands, this study was important to elucidate the effect of alcohol consumption on cell proliferation in submandibular glands in Wistar rats. Although some studies suggest that alcohol consumption may be associated with a decrease in the epithelial thickness of the oral mucosa, there are still contradictory results that showed an increase in proliferative activity (Maier et al., 1994; Maito et al., 2003).

In submandibular glands, there were a significant increase in KI-67 positive cells in the intercalated ducts of group T, compared to group C, corresponding to a 73% increase in proliferative cells. These results demonstrate that alcohol consumption stimulates cell proliferation in the submandibular glands, corroborating other studies (Maier et al., 1994; Homann et al., 1997; Maito et al., 2003; Carrard et al., 2004). Furthermore, alcohol ingestion induces 40% increase in the proliferation of epithelial cells in the rat tongue mucosa (Carrard et al., 2004).

Although continuous alcohol intake is associated with increased cell proliferation, and topical and intermittent exposure to alcohol causes only an increase in cell desquamation (Carrard et al., 2004), in this study, on parotid glands, there were no significant difference in the rate of cell proliferation on intercalated ducts, with, on average, the number of proliferating cells in Control group being similar to Treated group.

5. Conclusion

In summary, the study shows that alcohol plays a direct role in altering the morphology of the salivary glands, and therefore can lead to pathological disorders. The results also suggest that there is an increase in the rate of cell proliferation in submandibular glands of Wistar rats subjected to chronic alcohol consumption at the concentrations studied.

Future studies will be able to compare the amount and composition of saliva produced by control animals and those submitted to chronic alcohol consumption.

Acknowledgments

We are grateful for financial support of Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG [Scholarship] and - Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq [Scholarship].

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