Original Article

Extra Hepatic Effects of Sofosbuvir on A Serous Model of Major and Minor Salivary Glands in Albino Rats

Fatma Adel Saad[1], Aboushady I.M[2], Samah Mehanni[3]

[1]Oral Biology Department, Faculty of Dentistry, Future University[1], Cairo University[2], Egypt

ABSTRACT

Background: Extra hepatic adverse effects associated with the therapy of chronic HCV infection with sofosbuvir treatment regimens have recently arisen.

Objective: This study aimed to assess the inflammatory effect of sofosbuvir and its influence on cellular proliferation, functionality and differentiation of both submandibular (SMGs) and Von Ebner's salivary glands (EGs).

Methodology: 21 adult male albino rats were divided into three equal groups: GroupI (control) received orally distilled water; GroupII received orally sofosbuvir (40 mg/kg/day) dissolved in distilled water for one month and GroupIII received sofosbuvir for 2 months. SMGs and EGs sections were processed for H&E, immunohistochemical (using anti-COX-2 and anti-PCNA antibodies) and immunofluorescence (using anti α-amylase antibody) examination.

Results: Compared to control group, groupII displayed atrophic changes in SMGs and EGs which were accentuated in groupIII; shrunken acini, glandular cell vacuolization, nuclear degenerative signs, wide degenerative stromal areas and flattening of excretory ductal lining with stagnant secretion as well as the transformation of few serous glandular cells into mucous-like cells particularly in SMGs of groupIII. Likewise, both glands of groupII showed significantly increased immunoreactivity to COX-2 in acini and some ductal cells but with a significant decrease in those of groupIII. Regarding PCNA immunoreactivity and α-amylase immunofluorescence, significantly diminished positivity in the glandular cells of both glands in groupII was detected compared to control group whilst insignificant improvement was elucidated in those of groupIII comparing to groupII except for the significant reactivity to α-amylase in EGs of groupIII.

Conclusions: It was concluded that the oxidative stress associated degenerative changes caused by sofosbuvir in salivary glands after one month of administration seemed to be diminished after two months of administration due to the body acquired drug tolerance to restore the disturbed physiological processes. Hence, the use of anti-oxidants as an adjuvant treatment could be beneficial.

Received: 17 October 2019, **Accepted:** 25 October 2019

Key Words: α-amylase, COX-2, PCNA, salivary gland, sofosbuvir.

Corresponding Author: Fatma Adel Saad, PhD, Oral Biology Department, Faculty of Dentistry, Future University, Egypt, **Tel.**: +20 1223739140, **E-mail:** femy76@hotmail.com

ISSN: 1110-0559, Vol. 43, No. 1

INTRODUCTION

Hepatitis C virus (HCV) is a blood borne infectious pathogen with high spread rates and belongs to family Flaviviridae[1]. There are eleven major genotypes, HCV genotype-4 accounts for more than 90% of HCV infection in Egypt. HCV-4 is a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and transplantation in Egypt. The world health organization (WHO) reported, in 2014, that Egypt has the highest prevalence rate (14.7%) of HCV infection worldwide so that it became a national catastrophe[2].

HCV infection is associated with some extra hepatic manifestations including a number of organ systems[3]. Because of HCV sialotropism, conditions related to salivary glands such as hyposalivation and Sjögren-like sialoadenitis were manifested. Many therapeutic strategies have been assumed starting from interferon (IFN) in 1991 till Sofosbuvir (Sovaldi®) in 2014[2]. Sofosbuvir (Sovaldi®) was approved by the United States Food and Drug Administration (FDA) in 2013 and the European regulators in 2014. Sofosbuvir is a potent nucleotide analog, administered orally as a single daily dose and it has a potent antiviral activity. It is used to treat chronic hepatitis C in adults with HCV genotypes (1-6) in combination treatment regimens. It can cause serious side effects including: bradycardia, tiredness, headache, nausea, difficulty in sleeping and low red blood cell count. Moreover, sofosbuvir, in combination with other direct antiviral drugs, may cause pneumonia, intestinal inflammation, abdominal pain, discomfort, vomiting, mouth rashes or ulcers and heart diseases[4,5]. Some histopathological changes of salivary glands following sofosbuvir administration were also reported[6].

Cyclooxygenase (COX) is a key regulatory enzyme in prostaglandin (PG) biosynthesis. COX exists in at least two

Personal non-commercial use only. EJH copyright © 2020. All rights served *DOI: 10.21608/ejh.2019.18330.1187*

isoforms, termed COX- 1 and COX-2 encoded by different genes. COX-1 is constitutively expressed in many tissues and cell types and is involved in regulating normal physiological functions. COX-2 is induced in response to stimuli such as mitogens, oncogenes, endotoxins, inflammatory molecules, cytokines and growth factors[7]. For more than 50 years, several studies demonstrated that many medications could induce mitochondrial damage and toxicity through insulting the mitochondrial metabolic pathways[8,9].

Proliferating cell nuclear antigen (PCNA), known as cyclin1, is synthesized by proliferating cells. The existence of functional PCNA is important for cell survival because it is essential for DNA replication, repair and DNA recombination processes as well as regulation of the cell cycle and chromatin remodeling. So, it plays a key role in the initiation of cell proliferation, studying of cell kinetics and acts as a marker for cell proliferation[10-12].

Alpha-amylases (α-amylases), multigene family of glycoside hydrolases, are variably expressed in salivary glands, pancreas, liver, intestine, stomach, testis and skeletal muscles. Two different genes encoding both salivary (type1) and pancreatic (type2) amylases were detected. Salivary α-amylase is involved in starch metabolism and hydrolysis into glucose and maltose in human and rat's tissues[13,14]. It is also considered an indicator for proper serous salivary glands function and differentiation as it is formed in the acinar and/or ductal cells of the salivary glands and accumulated in their secretory granules to be released[15-17].

Hence, the current study was attempted to investigate the possible extra hepatic effects of sofosbuvir on major and minor serous salivary glands; submandibular (SMGs) and von Ebner (EGs) salivary glands respectively. This was achieved histopathologically and immunohistochemically using both anti-COX-2 antibody[8,9] to correlate the effect of sofosbuvir on the mitochondrial metabolic pathway as well as anti-PCNA antibody[11] to detect cellular proliferation. Finally, immunofluorescence examination using anti- α-amylase antibody, was conducted to evaluate salivary glands function and differentiation[15,16].

MATERIALS AND METHODS

2.1. Ethical statement

This work was carried out in collaboration with the research of Mehanny *et al.*[18] where we obtained our tissue samples from the same animals used in such research[18]. All experimental procedures were conducted according to the regulations of the Research Ethics Committee animal's experimentation at the Faculty of Oral and Dental Medicine, Cairo University, (Approval number: CU III F 47 17).

2.2. Animals

Twenty one adult male albino rats, weighing about 200-220 grams and aged about 5-6 months, were used in the current study. These rats were obtained from the animal house, Faculty of medicine, Cairo University, Egypt. They were housed in a 12-hours light/dark cycle under controlled temperature, humidity, standard chow pellets and tap water ad libitum throughout the experimental period.

2.3. Experimental design

One week following adaptation, the rats were randomly divided into three equal groups, seven rats each, as follows:

Group I (Control): The rats received distilled water via oral gavage throughout the experimental period.

Group II (Experimental): The rats received sofosbuvir dissolved in distilled water (8mg/ml) via oral gavage at a single daily dose of 40 mg/kg/day[19] for one month.

Group III (Experimental): The rats received sofosbuvir dissolved in distilled water (8mg/ml) via oral gavage at a single daily dose of 40 mg/kg/day[19] for two months.

According to the corresponding experimental period of each group, animals were euthanized at morning through an intracardiac anesthetic overdose (sodium thiopental 80 mg/kg)[20]. The SMGs and EGs were immediately dissected, fixed in 10% formaldehyde solution for 48 hours, then washed and dehydrated in ascending concentrations of alcohol and finally embedded in paraffin[21].

2.4. Histopathological Examination

Serial sections of 4-5μm thickness were stained with haematoxylin and eosin for histopathological examination using the light microscope[21].

2.5. Immunohistochemical Examination

Tissue sections were processed for immunohistochemical analysis of COX-2 and PCNA proteins. Paraffin sections of 4-5 μm in thickness were mounted on positively charged microscope slides and unmasking was done at 90˚C for 30 min with 10 mM sodium citrate buffer, pH 6.0. Sections were incubated for 10 min at room temperature in 0.03% hydrogen peroxide to eliminate the endogenous peroxidase activity. Then, incubation for 30 min at room temperature in the blocking serum 0.04% bovine serum albumin and 0.5% normal goat serum in PBS. Anti-COX-2 and anti- mouse monoclonal PCNA antibodies were utilized at a dilution of 1:200 and incubated overnight at 4˚C. Afterwards, sections were washed 3 times in PBS. Blocking of non-specific staining was done for 30 minutes at room temperature with 0.5% casein and 5% normal serum. At last, staining was achieved using diaminobenzidine substrate (DAB) to obtain the desired brown color, in addition to counterstaining of the sections with Mayer's hematoxylin for 30 seconds. The negative control was obtained by omitting the primary antibody from the above mentioned protocol using normal serum or PBS[10]. Immunohistochemical examination was performed using the light microscope (Olympus® BX 60, Tokyo, Japan).

2.6. Immunofluorescence Examination

The functionality of SMGs and EGs acinar cells was studied by measuring α-amylase production via immunohistochemistry. The procedures were performed at the pharmacology department, Faculty of Pharmacy for Boys, Azhar University, Cairo. Paraffin sections of SMGs and EGs were heated in oven at 60 °C for 20 minutes and deparaffinized in 100% xylene. Rehydration was then done in graded ethanol series. Antigen retrieval was achieved by the incubation of tissue sections in 0.01 M sodium citrate buffer, pH 6.0 for 20 minutes using a 500W microwave. Following one hour incubation with the blocking buffer (0.1% Triton X-100/phosphate – buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 10% horse serum), tissue sections were incubated with the rabbit polyclonal anti–alpha amylase 1 (primary antibody) that was diluted in 1% BSA/ 10% horse serum / PBS. After washing, the bound antibodies were identified by goat anti-rabbit Tex Red, Alexa Flour and goat anti-rat Tex Red, Alexa Fluor secondary antibodies. Staining of the nuclei was done with DAPI and then the slides were mounted in Fluoromount G. The slides were examined by Nikon fluorescence microscope (Model: Nikooneclipse 90i with a DS-U3 imaging system, Nikon Metrology, Inc. USA) under green, blue and red channels. Fluorometric analysis (fluorometric intensity in at least 9 microscopic fields) was assessed for each section by Image J/NHI software[16,22].

2.7. Statistical Analysis

Data obtained from the analysis of COX-2, PCNA and alpha amylase immunoexpressions in SMGs and EGs tissues were coded and entered using the statistical package SPSS version 22. Data were summarized using mean and standard deviation (SD).Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc tests when comparing more than 2 groups[6,10].

RESULTS

3.1. Histopathological Results

Group I: This group showed a normal SMG structure, consisting primarily of serous acini lined with pyramidal cells having basal nuclei. The granular convoluted tubules (GCTs) were lined with high columnar cells having apical eosinophilic secretory granules and round basal nuclei. The striated duct displayed a single layer of columnar cells with basal striations. Excretory ducts with normal histology were also observed, being lined by pseudo stratified columnar epithelium and surrounded by normal C.T. stroma (Figure 1a). Additionally, EGs displayed non capsulated lobular aggregates interposed between skeletal muscle bundles. Their serous acini were lined with pyramidal cells having variably distinct borders, eosinophilic apical granules and basal nuclei, in addition to few intra-lobular ducts (Figure 1b).

Group II: After one month of sofosbuvir administration, atrophic changes of both glands were noticed. Apparent shrinkage of serous acini and intracytoplasmic vacuolization were also detected in many acini as well as in GCTs, striated and excretory ducts. Marked decrease of eosinophilia and granular content of GCTs was noticed. Some acini exhibited degenerative nuclear signs including pleomorphism, hyperchromatism and pyknosis as well as karyolysis. Some excretory ducts appeared dilated with stagnant secretion and flattening of epithelial lining along with vacuolization in some cells. Wide degenerative areas of C.T. septa with inflammatory cells were clearly observed in between acini, intralobular ducts and surrounding the excretory ducts. Dilated and congested blood vessels were observed in the C.T. septa (Figure 1c). Likewise, EGs revealed apparently wide areas of degeneration in the C.T. stroma as well as degenerating acini while few ducts displayed stagnant secretion and thinning of their lining (Figure 1d).

Group III: Accentuated atrophic changes were detected after two months of sofosbuvir administration. Apparent increase of the acinar shrinkage and some of them lost their typical form, while others showed complete degeneration. Marked vacuolization was noticed in the serous acini as well as in different ducts. Besides, nuclear mitotic figures were also observed in scattered deeply basophilic acini. Some serous glandular cells presented histological features of mucous cells. In addition, the GCTs revealed decreased eosinophilic granular content whilst the excretory ducts showed similar changes to group II. Apparently wide degenerative areas of C.T. stroma and inflammatory cells infiltrate were also noticed around the degenerating acini (Figure 1e). Regarding the EGs, they displayed obvious acinar degeneration as well as noticeable degenerative areas of the C.T. stroma. Moreover, mitotic figures were also illustrated in few acinar cells and some ducts appeared with stagnant secretion in their lumen (Figure 1f).

3.2. Immunohistochemical and Statistical Results

3.2.1. Immunohistochemical and Statistical Results for COX-2 Immunoreactivity

Group I: SMGs of the control group showed negative cytoplasmic immunoreactivity to COX-2 with mild nuclear reaction in very few nuclei, while the ductal cells negatively reacted to COX-2 (Figure 2a). Regarding the EGs, both acini and ducts displayed a negative immunoexpression of COX-2 (Figure 2b).

Group II: Immunohistochemical examination of both SMGs and EGs exhibited statistically significant increase (P *value* < 0.001 in SMGs and P *value* $= 0.006$ in EGs) in the immunoreactivity to COX-2 compared to group I. The expression of COX-2 in SMGs was detected in the membrane, cytoplasm and nuclei of the acinar cells. Similar reaction was elucidated in EGs except for the nuclei that were negatively stained. However, cells of the duct system revealed a negative immunoreaction, but some ducts displayed a cytoplasmic reaction particularly in SMGs (Figures 2c, 2d and Figure 5).

Group III: Two months following treatment with sofosbuvir, the SMGs presented a significant increase (*P value* < 0.001) in the expression of COX-2 (membranous, cytoplasmic and nuclear reaction) compared to group I whereas the duct system reacted negatively. Comparing to group II, a statistically significant decrease in COX-2 expression was observed (*P value* 0.004) in SMGs (Figure 2e and Figure 5). For the EGs, COX-2 expression was non-significantly increased in the acinar cells (membranous and cytoplasmic expression but with negatively reacted nuclei) compared to group I (*P value* = 0.74). On the other hand, there was significant decrease in COX-2 expression compared to group II (*P value* = 0.029) (Figure 2f and Figure 5).

3.2.2. Immunohistochemical and Statistical Results for PCNA Immunoreactivity

Group I: SMGs of the control group revealed both cytoplasmic and nuclear immunoreactivity to PCNA in the gland parenchyma. Negative nuclear reaction was rarely observed (Figure 3a). Regarding the EGs, they displayed both cytoplasmic and nuclear immunopositivity in all acini and ducts (Figure 3b).

Group II: Immunohistochemical examination of PCNA expression in the SMGs cells showed cytoplasmic and nuclear immunoreactivity in addition to the increase of the negative nuclei. This expression was significantly lower than that detected in group I (*P value* = 0.003) (Figure 3c and Figure 6). In the EGs, a weak cytoplasmic and nuclear expression was detected with few negative nuclei. This expression was also significantly lower than that of group I (*P value* <0.001) (Figure 3d and Figure 6).

Group III: Two months after sofosbuvir treatment, SMGs sections presented cytoplasmic and nuclear expression to PCNA with rare negative nuclei; some mitotic figures were also noticed. This reactivity to PCNA was insignificantly decreased compared to group I (*p value* = 0.08) while in comparison with group II, the non significant increase (*p value*= 0.26) was elucidated (Figure 3e and Figure 6). Regarding EGs, the cytoplasmic expression was obvious with strong nuclear immunopositivity besides the presence of mitotic figures. However, the expression of PCNA in EGs was significantly lower than that detected in group I (*P value* <0.001) but comparing to group II, a non-significant increase (*p value* = 0.19) was observed (Figure 3f and Figure 6).

3.3. Immunofluorescence and Statistical Results

3.3.1. Immunofluorescence and Statistical Results for α-amylase Immunoreactivity

Group I: SMGs of the control group revealed marked α-amylase expression among different portions of the duct system whereas it was less observed in the serous acini (Figure 4a). Regarding the EGs, some acinar cells exhibited α-amylase immunoreactivity (Figure 4b).

Group II: Immunofluorescence examination of α-amylase expression in SMGs showed statistically significant decrease (*P value* < 0.001) in the expression of α-amylase compared to group I. This expression could be only detected in scattered cells lining the acini or ducts (Figure 4c and Figure 7). In the EGs, α-amylase expression was significantly decreased (*P value* < 0.001) compared to group I. This α-amylase expression was observed in the acinar cells (Figure 4d and Figure 7).

Group III: Two months after treatment with sofosbuvir, the SMGs sections presented statistically significant decrease (*P value* < 0.001) in the expression of α-amylase compared with group I in some acini and ducts. Comparing to group II, the expression was slightly higher with non-significant difference in between them (*P value*= 0.44) (Figure 4e and Figure 7). Concerning the EGs, apparently wider areas of reactivity to α-amylase were elucidated in the acini compared to group II. Thus, the expression was significantly increased (*P value* <0.001) compared to group II. On the other hand, insignificant decrease of α-amylase expression (*P value* = 0.09) was illustrated compared to group I (Figure 4f and Figure7).

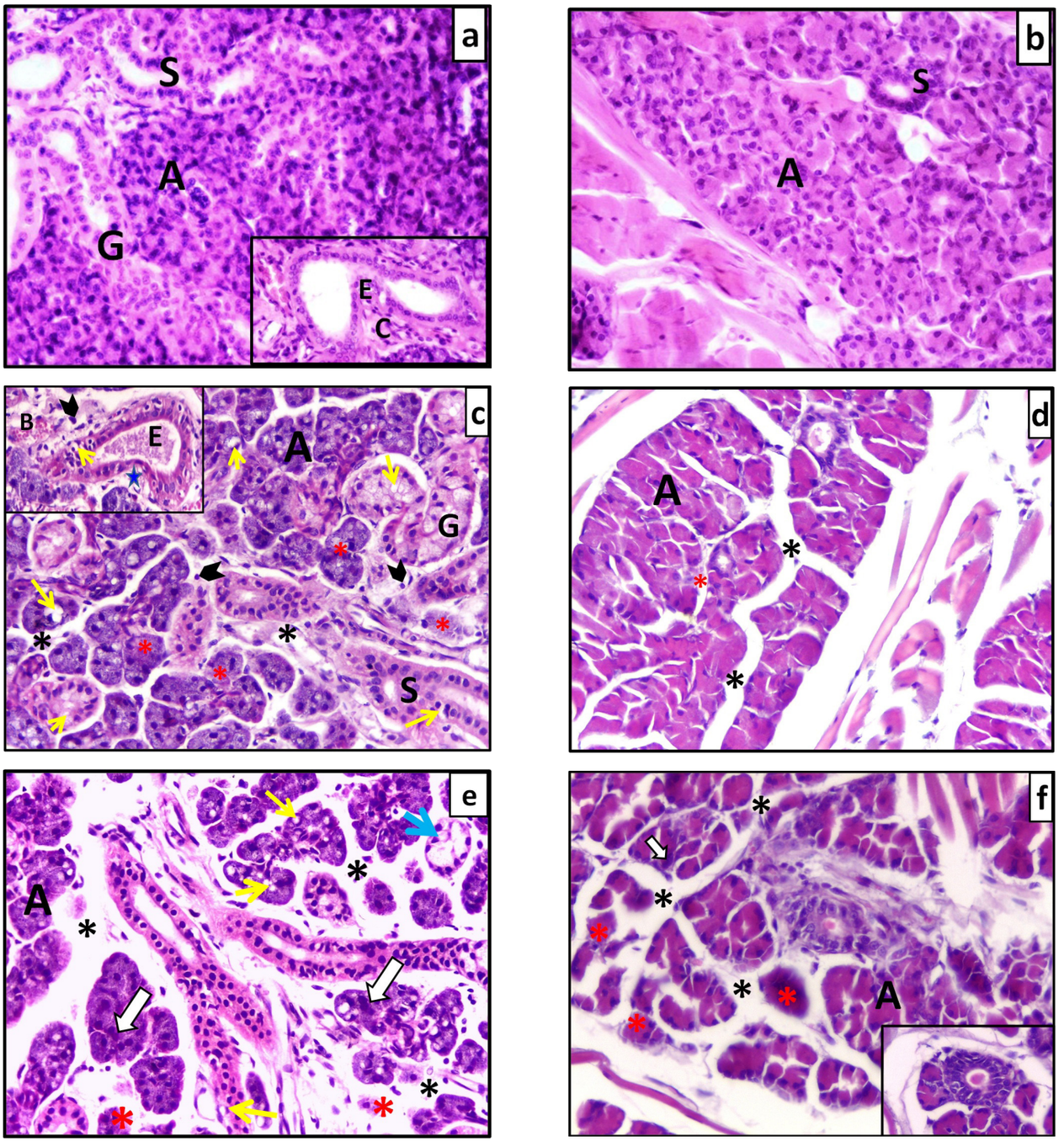

Fig. 1: Photomicrographs of the SMGs (a, c, e) and EGs (b, d, f) (a) SMGs of group I showing well-defined serous acini, striated duct and GCTs. Inset showed excretory duct in normal C.T. septa (H&E X 200).(b) EGs of group I showing serous acini and intralobular striated duct (H&E X 200).(c) SMGs of group II showing marked vacuolization of acinar, striated and GCT cells, degenerating acini, degenerative areas of CT septa with inflammatory cells. Inset showed dilated excretory duct with vacuolizationin in some cells and a dilated blood vessel engorged with RBCs in the adjacent C.T. (H&E X 400).(d) EGs of group II showing degenerating acini, wide degenerative areas of connective tissue septa, thinning of ductal lining and stagnant secretion (H&E X400). (e) SMGs of group III showing marked vacuolization of acinar and ductal cells, mucous transformation of serous glandular cells, degenerating acini, obvious wide degenerated areas of C.T stroma, mitotic figures in few acini (H&E X400). (f) EGs of group III showing acinar degeneration and apparent wide areas of stromal degeneration in between acini, mitotic figures were detected in few acini. Inset: showed stagnant secretion in the stratified duct. (H&E X400).serous acini (A), striated duct (S),GCT (G), excretory duct (E), C.T. septa (C), blood vessel (B), cell vacuolization (yellow arrows), degenerating acini (red asterisks), degenerative areas of C.T. septa (black asterisks), inflammatory cells (arrow heads), mitotic figures (white arrows), mucous transformation (blue arrow).

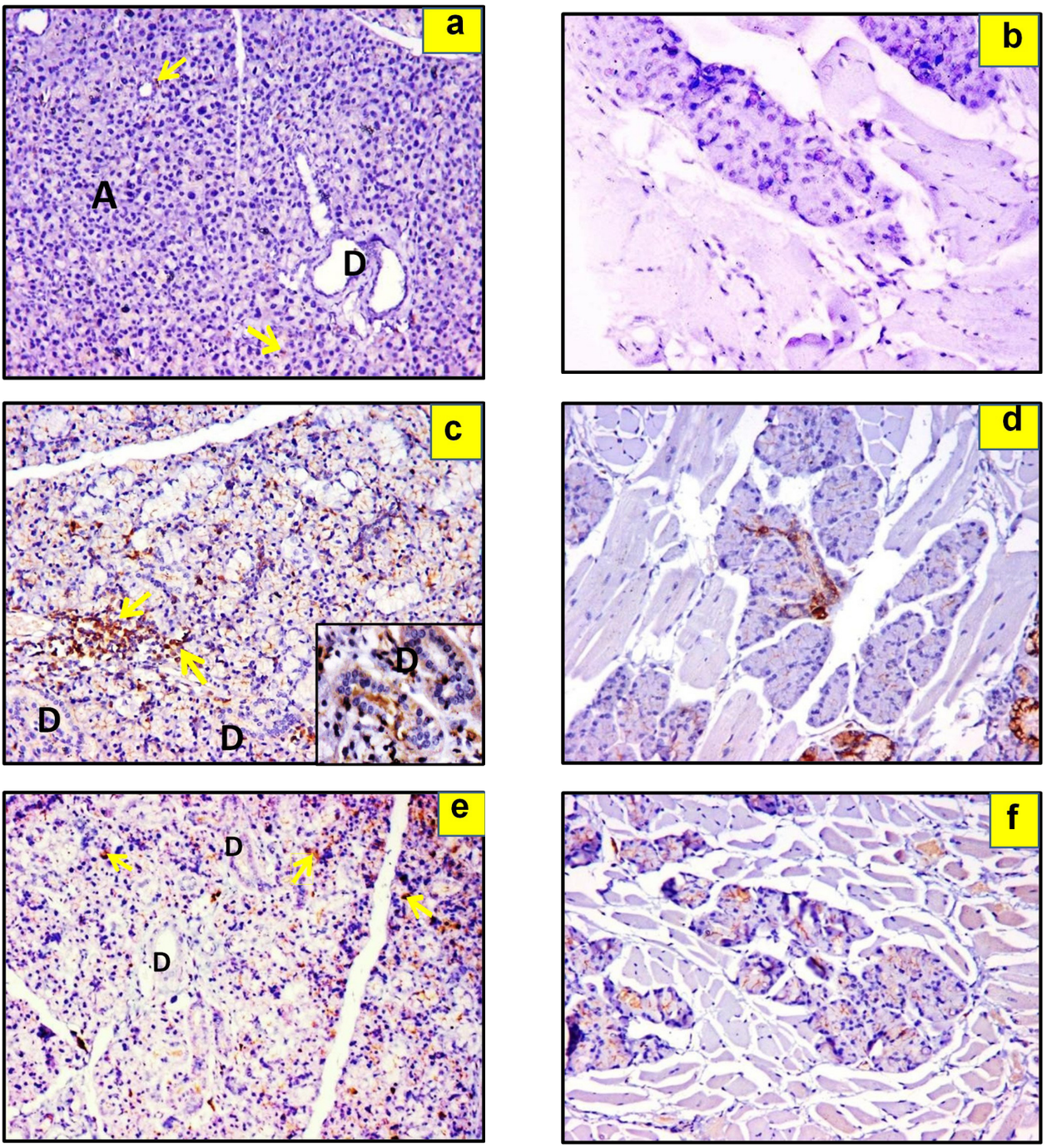

Fig. 2: Photomicrographs of the SMGs (a, c, e) and EGs (b, d, f) (a) SMGs of group I showing negative cytoplasmic immunoreactivity to COX-2 in ducts and most of the acini. Reaction occurred among very few nuclei in the acini (yellow arrows). (b) EGs displayed negative COX-2 reaction. (c) SMGs of group II showing acinar immunoreactivity {cytoplasmic, membranous and nuclear (yellow arrows)} to COX-2, Inset: cytoplasmic reaction was noticed in some ductal cells (D) (d) EGs of group II displayed cytoplasmic and membranous expression in some acini ((e) SMGs of group III showing immunoreactivity to COX-2 in some acinar cells (yellow arrows), negative immunoreactivity in the ducts (D) (f) EGs of group III showing apparently less expression in acinar cells (DAB X200).

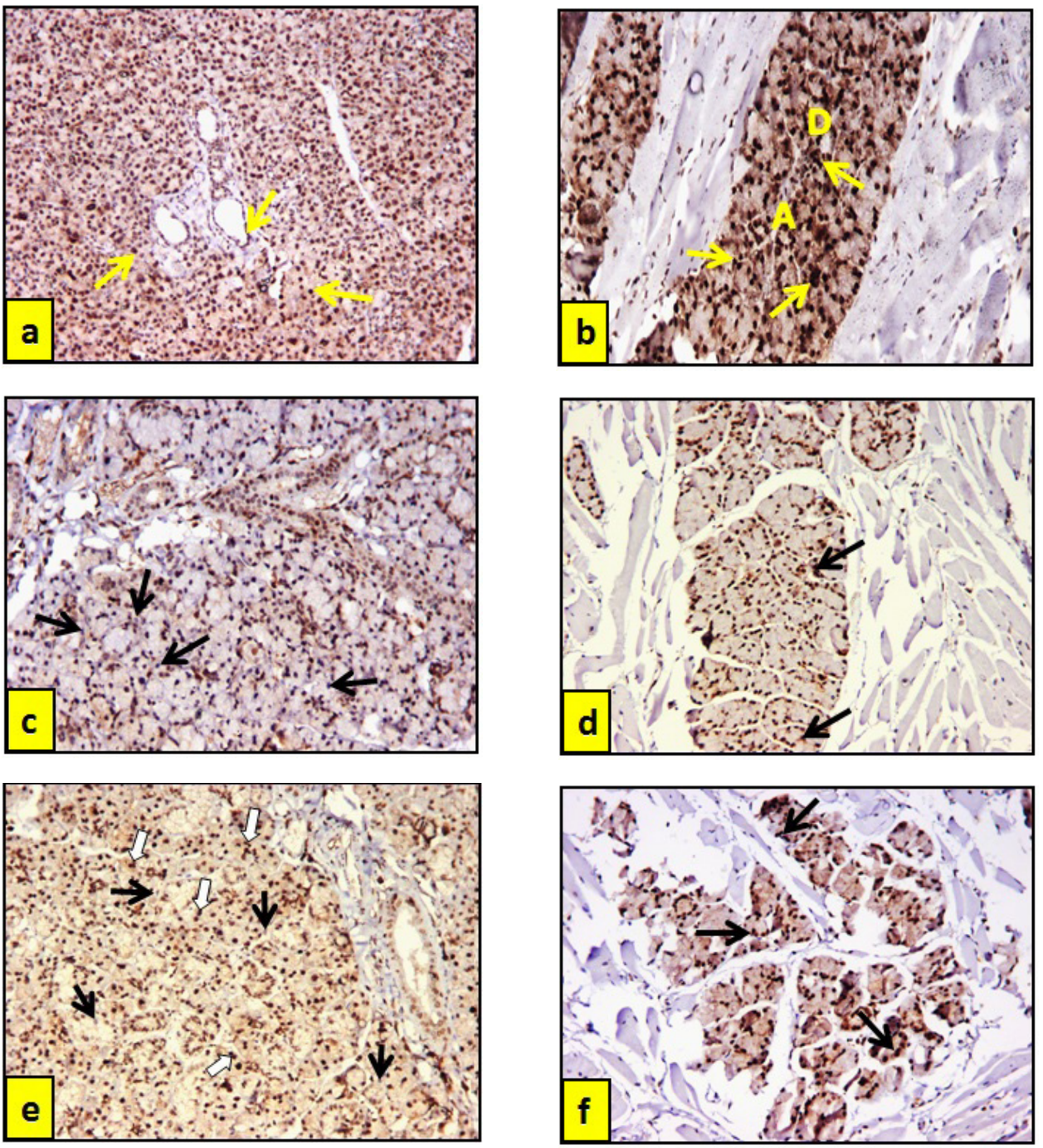

Fig. 3: Photomicrographs of the SMGs (a, c, e) and EGs (b, d, f): (a) SMGs of group I showing both cytoplasmic and nuclear immunoreactivity to PCNA (yellow arrows) in acini and duct system. (b) EGs of group I displayed both cytoplasmic and nuclear immunopositivity (yellow arrows) in all acini (A) and ducts (D). (c) SMGs of group II showing cytoplasmic and nuclear immunoreactivity to PCNA, in addition to the increased number of negative nuclei (black arrows). (d) EGs of group II displayed a weak cytoplasmic and nuclear expression (black arrows) with few negative nuclei. (e) SMGs of group III showing cytoplasmic and nuclear immunoreactivity to PCNA; some mitotic figures were noticed (white arrows) and rarely negative nuclei (black arrows). (f) EGs of group III displayed cytoplasmic expression, strong nuclear immunopositivity and mitotic figures (black arrows) (DAB X200).

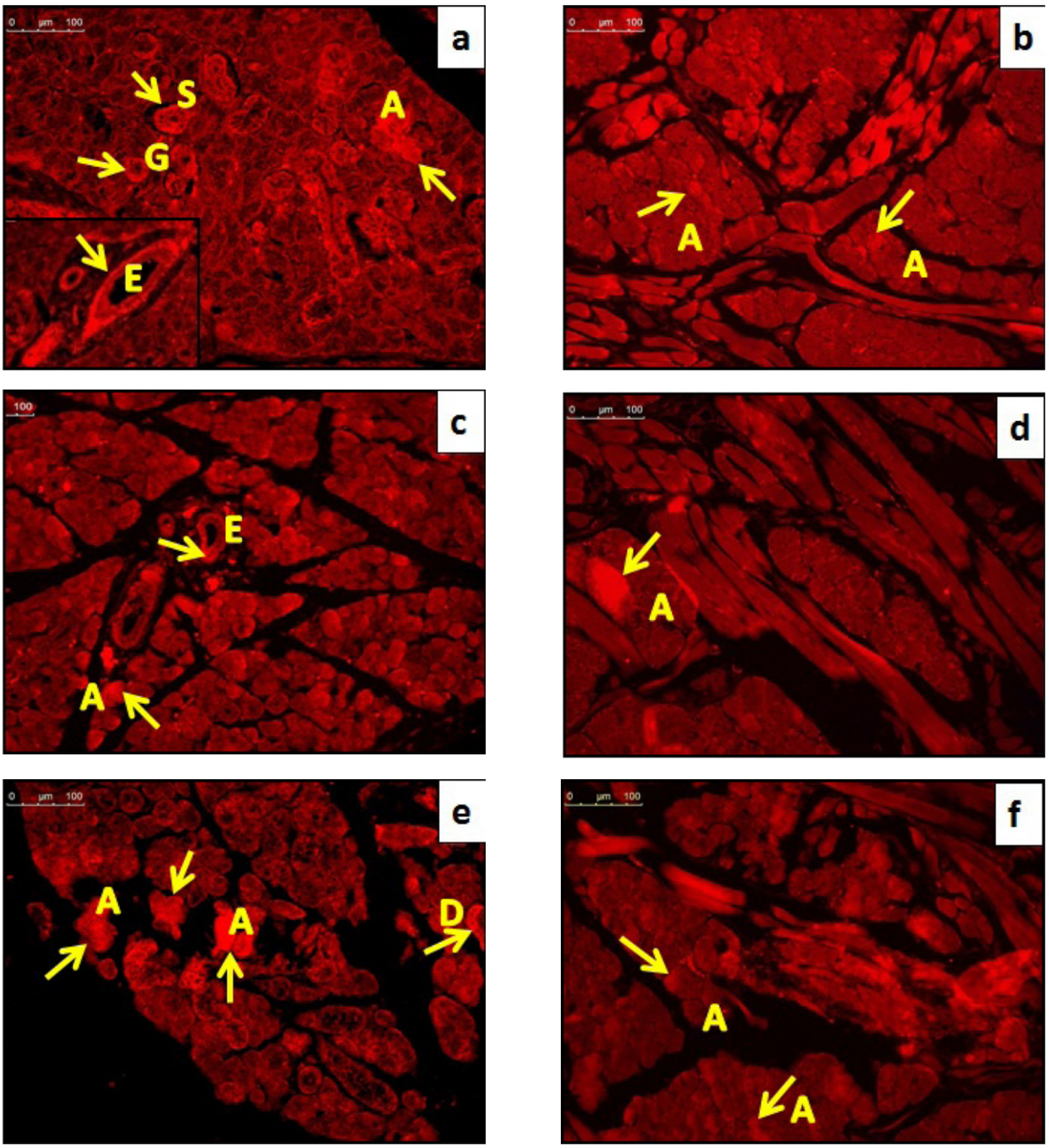

Fig. 4: Photomicrographs of SMGs (a, c, e) and EGs (b, d, f): (a) SMGs of group I showing more obvious α-amylase expression (yellow arrows) in striated ducts and granular convoluted tubules but was less observed in few serous acini. Inset: showing expression of α-amylase (yellow arrows) in the excretory ductal lining. (b) EGs of group I displayed α-amylase expression (yellow arrows) in some acinar cells. (c) SMGs of group II showing weak expression of α-amylase (yellow arrows) in only few glandular cells such as excretory duct and acini. (d) EGs of group II showed apparently less expression of α-amylase in the acini (yellow arrows). (e) SMGs of group III showing apparent increased expression of α-amylase (yellow arrows) in some acini and ducts.(f) EGs of group III revealed apparently wider areas of expression of α-amylase (yellow arrows) in acini. Striated ducts (S), granular convoluted tubules (G), acini (A), excretory duct (E), ducts (D). (X 200).

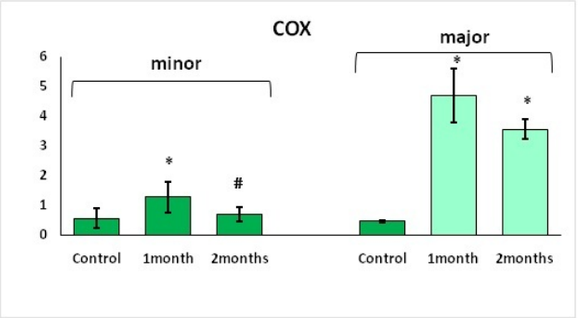

Fig. 5: Bar chart showing mean ±SD of COX-2 in major (SMGs) and minor (EGs) glands among all studied groups.

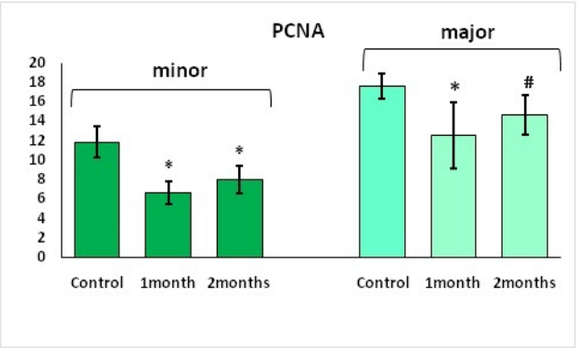

Fig. 6: Bar chart showing mean ±SD of PCNA in major (SMGs) and minor (EGs) glands among all studied groups.

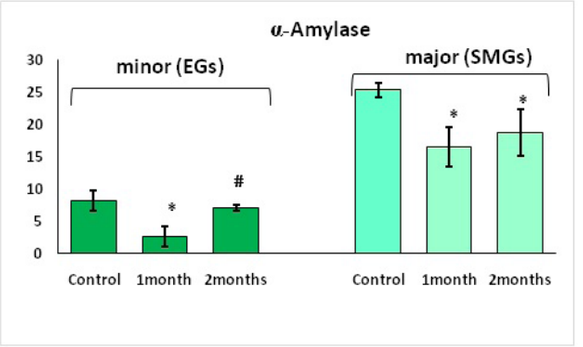

Fig. 7: Bar chart showing mean ±SD of α-amylase in major (SMGs) and minor (EGs) glands among all studied groups.

DISCUSSION

The Egyptian Ministry of Health has proposed a national strategy to control the epidemic HCV in Egypt which was supported by the WHO and other institutes. The plan was titled "The Plan of Action for the Prevention, Care and Treatment of Viral Hepatitis 2014–2018" and promoted sofosbuvir (Sovaldi™, Gilead Sciences, San Francisco, USA) as the fundamental treatment. The direct antiviral drugs; particularly sofosbuvir, could provide up to 90% sustained viral response in HCV genotype-4 showing more tolerable adverse effects, high cure rates and short treatment duration[2].

Although Sofosbuvir was reported as a potent non genotoxic antiviral drug used for the treatment of chronic hepatitis C in adults with safety profile[4,23,24], multiple adverse effects of Sofosbuvir on different body tissues such as heart, muscles[18] as well as salivary glands[6]were the motivated reasons for this ongoing investigation. Accordingly, this study evaluated the extra hepatic effects of sofosbuvir on both submandibular as well as von Ebner salivary glands in adult male rats.

Sofosbuvir administration in the present study for one month caused atrophic changes in the SMGs represented by shrunken acini with wide degenerative areas of C.T. stroma, intracytoplasmic vacuolation and degenerative nuclear signs in some acinar and ductal cells. Similar changes were also noticed in EG acini and C.T. septa while the ductal changes occurred to a less extent. In conformity to these findings, it was affirmed that the intracytoplasmic vacuolation and degeneration of acinar and ductal cells in SMGs mostly resulted from the fatty nature of the secretory portions of the cells so that the decreased cellular activity and metabolism was followed by the utilization of fatty acids and thus accumulation of lipid droplets. During processing, these lipid droplets were dissolved leaving empty vacuoles[25,26]. The authors added that these changes occurred in response to noxious agents and ended with mitochondrial damage due to the peroxides resulting from the reaction of fatty acids in the phospholipid bilayer of acinar and ductal cell membranes with free radicals. This finally gave rise to the insult of cellular membranes which in turn stimulates inflammatory cells migration and initiation of apoptosis[26-28].

In the herein study, all the detected histological changes of group II were also observed in group III, two months after sofosbuvir administration, but to an apparently increased extent in some areas of both SMGs and EGs. Some acini in this group lost their typical form and appeared more shrunken as well as irregular. Completely degenerated acini and ducts were obviously noticed with wider degenerative stromal areas. However; few acini displayed mitotic figures. Likewise, the atrophic changes of SMGs were clearly elucidated in the EG acini and C.T.stroma, while in the ductal cells these changes were less observed. The results of our study were coincident with those of Salem *et al*.[6] who declared that vacuolation, distortion and degeneration of salivary glandular cells were observed after 45 days and were increased after 90 days of the daily administration of sofosbuvir. The authors clarified that these changes could be assigned to the overproduction of reactive oxygen species (ROS) and to their increased levels, which could be proportional to the increased duration of sofosbuvir administration. It was valuable to mention that some serous glandular cells in SMG of group III in this work showed similar features to mucous cells with compressed basal nuclei and foamy cytoplasm. We postulated that these serous cells underwent this transformation to resemble the mucous cells as a protective mechanism in the submandibular

salivary gland. In agreement to this observation, several studies[29-31] demonstrated that the rat SMGs and GCTs formed phenotypically of serous cells. Halawa *et al.*[26] found that some acini of the rat SMGs showed mucous transformation in response to noxious agents. Moreover, Barnes[32], reported that chronic inflammation of major salivary glands could be associated with the mucous transformation of the ductal cells.

In addition, the excretory ducts in both groups II and III displayed lumen dilatation, stagnant secretion as well as areas of flattening in their epithelial lining. This could be explained according to Parlak *et al.*[33] who suggested that this flattening may occur due to metaplasia of the ductal cells along with the accumulated secretion and secondary to glandular injury. Consequently, the resultant impairment of flow rate reflected the glandular dysfunction and xerostomia[25]. Besides, the apparently widened degenerative areas of C.T. stroma in this study could be ascribed to the fibroblasts that showed signs of degeneration and subsequent apoptosis which occurred in chronic inflammation and resulted in suppression of collagen synthesis and increased collagenolytic activity. Furthermore, not only the fibroblasts; but also the inflammatory cells underwent apoptosis[34,35]. The presence of dilated congested blood vessels in sofosbuvir treated groups together with the periductal chronic inflammation could be an inflammatory response to the drug permitting the body to carry more blood to the degenerative areas. Moreover, the proposed increased ROS could stimulate protein kinase C in the vascular tissues; giving rise to loss of permeability and endothelial swelling[36,37].

According to Salem *et al.*[6] who ascribed sofosbuvir effects to ROS production, this study tried to trace this assumption through the detection of COX-2 in both SMGs and EG. Brault *et al.*[8] and Zorov *et al.*[38] demonstrated that mitochondria sense and neutralize cellular stress by controlling and deciding cell growth, proliferation, differentiation as well as apoptosis. Mitochondria act as both a source and a target for free radicals and ROS. Thus, any defect in the mitochondrial transmembranous potentiality gives rise to a cascade of apoptosis-related signals. Moreover, Lin *et al.*[39] declared that COX-2 is expressed in response to diverse inflammatory intracellular and extracellular stimuli such as chemicals, viral infections and ROS.

COX-2 expression is tightly controlled in most tissues[39] and is almost unnoticeable under normal physiologic conditions[7]. For this reason, the cells of SMGs and EGs in the control group of this study revealed a negative cytoplasmic and nuclear immunoreactivity to COX-2 with a moderate nuclear reaction in very few acini of SMGs. In comparison with the control group, SMGs and EGs of group II displayed statistically significant immunoreactivity to COX-2 so that both cytoplasmic and membranous reaction along with the strong nuclear expression was demonstrated. The ductal cells were mostly negative with only moderate cytoplasmic reaction in some ductal areas. On the other hand, EGs exhibited a cytoplasmic and membranous expression in some acini with negative nuclear reaction. In concomitant to these results, previous studies reported that various medications, including ribonucleotide analogues showed mitotoxicity since drugs could provoke mitochondrial damage by impairing the mitochondrial metabolic pathways[9]. Swollen mitochondria with noticeable cristolysis in rat visual cerebral cortex were also illustrated following sofosbuvir administration for 5 weeks[40]. Nucleotide inhibitors, similar to sofosbuvir, were evidenced to inhibit mitochondrial protein synthesis and oxygen utilization in cells resulting in increased ROS, induction of many inflammatory mediators leading to profound changes in the expression of proinflammatory genes including COX-2[8,41,42]. Increased COX-2 expression in several cell types denotes prolonged acceleration of chronic tissue inflammation[43-45]. Yet, some in vitro studies claimed that sofosbuvir did not exhibit cytotoxicity, mitochondrial toxicity and bone marrow toxicity if dosed at multiples beyond the effective dose.

Comparing to group II of the present study, both SMGs and EGs of group III showed significant decrease in the COX-2 immunoreactivity. The diminished COX-2 expression in group III could be attributed to the possibly developing body tolerance after two months of daily sofosbuvir administration. In verification of these observations, Peper[46] had previously reported that if a drug is repeatedly administered, its disturbing effect on the regulation of any physiological process would be gradually and slowly decreased, together with the development of tolerance to this drug. When full tolerance is established to be an autonomous process, the body has adapted to deal with drug disturbances as effectively as possible[46]. On the other hand, the obvious decreased COX-2 reactivity in EGs of both groups in this study comparing to SMGs reactivity was explained by Wanga *et al.*[47] who documented that minor salivary gland function was preserved better than major salivary gland function in hyposalivation related disorders.

To further support the histopathological and COX-2 results of this study, the immunohistochemical assay for PCNA was performed to study cellular proliferation. PCNA is a proliferation-associated antigen, the PCNA synthesis level in the nuclei of proliferating cells was reported to correlate with the degree of DNA synthesis and cell proliferation[21]. Both the SMGs and EGs of the control group revealed significant cytoplasmic and nuclear reactivity to PCNA in the glandular parenchyma. Negative nuclear reaction was rarely observed. In parallel to these observations, some authors described the differentiated acinar, intercalated ducts and GCTs cells of SMGs in adult male rats primarily as the slowly renewing populations in addition to "reserve" or "stem" cell population within the agranular intercalated duct cells and the basal cells of larger ducts. Moreover, the normal non-dividing cells, including quiescent and senescent cells, illustrated very

small amounts of PCNA[13,48,49]. Comparing to the control group in the foregoing study, both SMGs and EGs of group II exhibited a significantly decreased nuclear and cytoplasmic expression of PCNA. This finding proved that sofosbuvir administration caused cell injury in salivary glands by reducing their ability to proliferate. In concurrence, Taha *et al.*[21] proposed that the reduced expression of PCNA was associated with increased rate of apoptosis. Since the elevated ROS production following the unregulated oxidative stress in chronic inflammation, directly contributed to mitochondrial DNA alteration and thus cell injury; this may be severe, ending with unwanted cell death[38,50,51].

On the other side, the cells of SMGs and EGs of group III presented insignificant increase in the cytoplasmic and nuclear reactivity to PCNA comparing to group II. Similar to the histopathological findings of this group (III), nuclear mitotic figures were also observed in some acinar cells of both SMGs and EGs; however, only few negative nuclei were detected in the SMGs. The increased expression of PCNA in this group, compared to group II, assured the COX-2 immuno-expression results; which could be assigned to the fore mentioned tolerability to sofosbuvir following two months of daily intake. Particularly, some reports declared that the positive PCNA staining was most probably an attempt of the cells to repair in order to limit the drug associated cell injury. Moreover, it was mentioned that both acinar and ductal cells were able to proliferate under variable physiological and experimental conditions. Thus, these suggestions could also support the occurrence of mitotic figures despite the presence of accentuated atrophic changes in group III of the herein study. Furthermore, some studies reported that cytoplasmic localization of PCNA raises the possibility of inappropriate intracellular trafficking. Yet, no significant difference was found between the major and minor salivary glands in the expression of PCNA in some experimental studies, it was unclear whether the major or minor salivary glands had a poorer clinical effect secondary to experimental works[11].

It was valuable to use α-amylase in this study as a marker for acinar and ductal cells differentiation and functionality through the detection of salivary amylase activity especially that it was reported as an indicator for clinical diagnosis of some diseases as well as inflammation of salivary glands[14,16,22,52]. In this study, we used adult male rats to exclude hormonal and other histological variations associated with the use of female rats since some in vivo studies elucidated that the estradiol hormonal levels influenced the α-amylase activity in different rat tissues[14]. α-amylase immunofluorescence results in the present work revealed a significant fluorescence in different portions of the SMGs ductal system of the control group particularly in the GCTs compared to the less obvious fluorescence in the serous acini. These findings were consistent with the former results of Yamagishi *et al.*[17] who studied the SMGs in mice and evidenced that α-amylase was produced abundantly by the GCTs serous cells and moderately by the seromucous acinar cells.

Regarding the EGs in the current investigation, some acinar and ductal cells displayed a mild to moderate immunofluorescence for α-amylase. The obvious significant upregulation of α- amylase in the cells of the control SMGs than in those of the control EGs could be ascribed to the endogenous circadian rhythm of the rat EGs. In harmony with these observations, it was formerly elucidated that in normal light conditions, since rats are nocturnal animals, the secretory granules of EGs discharge their contents during feeding at night; with a significant decrease in the stores of secretory proteins in the morning. Accordingly, the secretion and storage of salivary α-amylase in the rat EGs are subjected to endogenous circadian variation which is influenced by the daily light/dark cycle. The morphology and size of the acinar cells along with the amount of secretory granules as well as the digestive enzymes activity including amylase were significantly lesser when the rats were killed in the morning than when they were killed at night. However, it was previously asserted that the secretion of various digestive enzymes is independent of food consumption[53]. In parallel with the prior results of Nater and Rohleder[15], we related our findings in both glands, to the level of cellular differentiation, secretory function and proliferation of the acinar as well as ductal cells[54].

Both SMGs and EGs of group II demonstrated a significant decrease in the α-amylase immuno-fluorescence compared to the control group, so that a weak to mild fluorescence occurred in scattered glandular cells of both SMGs and EGs, while moderate fluorescence of acinar cells was infrequently noticed in the EGs. In synchronization to previous reports[26], we proposed that the degenerative changes of the glandular cells, caused by sofosbuvir-induced oxidative stress, gave rise to biochemical changes in the secretory product. Furthermore, Vag *et al.*[55] explained that the activity of α-amylase (calcium containing metalloenzyme) strongly depends on the intracellular calcium concentration and release from the intracellular stores in salivary cells. Therefore, such disturbances caused by mitochondrial and cellular oxidative stress resulted in changes of amylase expression as well as the morphology of the differentiating glandular cells. Additionally, the deterioration of the glandular structure, including parenchyma, was associated with salivary gland dysfunction, alteration of salivary composition and thus change in the concentration of salivary proteins such as amylase in rat serous glands[56,57].

In comparison to group II, the SMGs sections of group III presented insignificant increase in α-amylase immunofluorescence where a moderate to strong fluorescence was noticed in some acinar and ductal cells. But for the EGs, a significant increase of α-amylase fluorescence was detected. Based on former studies, it was indicated that the increase in the salivary α-amylase under non-stressful conditions followed the increased salivary flow rate[16]. Accordingly; the apparent enhancement in α-amylase expression reflected a functional improvement

in both glands of this group; this might be attributed to the abovementioned acquired tolerance to sofosbuvir which counteract the drug side effects and keep the optimal functions and thus assured all our obtained results[46].

Conclusively, the daily administration of sofosbuvir, the currently selective direct antiviral treatment for chronic HCV infection, resulted in deteriorative histopathological and physiological changes in both rat submandibular and von Ebner's salivary glands. The histopathological changes persisted after two months of sofosbuvir administration, while the functionality of the glands seemed to be improved. This improvement could be an attempt of the body to restore the disturbed physiological processes via developing an acquired tolerance to sofosbuvir. This fact copes with recent clinical studies in which the self-assessed overall tolerance to sofosbuvir was excellent so that patient's fatigue lessened during and also after the end of the treatment[58]. Yet, we could recommend that the ultimate goal is to provide effective anti-oxidants as an adjuvant therapy for HCV to target the COX-2 signaling pathway beside the viral genome. Particularly, it was previously suggested as a promising strategy to eliminate HCV-related disorders, the combination of both anti-HCV and anti-inflammatory agents[39,44].

COFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Nouroz F, Shaheen S, Mujtaba G and Noreen S: An overview on hepatitis C virus genotypes and its control. The Egyptian Journal of Medical Human Genetics. (2015)16: 291-298.
2. Khalifaa RH, Shahina RMH, Aboukhalila REE, Mariec MA, El-deeb AM and Riada N.M: Digging more in the genetic risk prediction of hepatitis C virus epidemic in Egypt: Apoptosis genes polymorphisms in the susceptibility of hepatitis C virus and association with viral load. Virus Research. (2017) 238: 35–39.
3. Simula MS and Re VD: Hepatitis C virus-induced oxidative stress and mitochondrial dysfunction: A focus on recent advances in proteomics. Proteomics Clin. Appl. (2010) 4:782–793.
4. Doss W, Shiha G, Hassany M, Soliman R, Fouad R and Khairy M *et al*.: Sofosbuvir plus ribavirin for treating Egyptian patients with hepatitis C genotype 4. Journal of Hepatology. (2015) 63: 581-585.
5. FDA. U.S. Food and Drug Administration "FDA Drug Safety Communicaation: FDA warns of serious slowing of the heart rate when antiarrhythmic drug amiodarone is used with hepatitis C treatments containing sofosbuvir (Harvoni or Sovaldi) in combination with another Direct Acting Antiviral drug. Ava". (2015) Mar 24.
6. Salem ZA, Elbaz DA and Farag DBE: Effect of an anti-hepatitis c viral drug on rat submandibular salivary gland. E.D.J. (2017) January (63)1.
7. Tominaga M, Migita K, Sano H, Fukui W, Kohno M and Tsubouchi Y *et al*.: Expression of cyclooxygenase-1 (COX-1) in labial salivary glands of SjoÈgren's syndrome. Clin. Exp. Immunol. (2000) 122: 459-463.
8. Brault C, Lévy PL and Bartosch B: Hepatitis C Virus-Induced Mitochondrial Dysfunctions. Viruses. (2013) 5: 954-980.
9. Niyazov DM, Kahler SG and Frye RE: Primary Mitochondrial Disease and Secondary Mitochondrial Dysfunction: Importance of Distinction for Diagnosis and Treatment. Mol Syndromol. (2016) 7:122–137.
10. Xiang HG, Xie X, Hu FQ, Xiao HB, Zhang WJ and Chen L: Cyclooxygenase-2 inhibition as a strategyfor treating gastric adenocarcinoma. Oncology reports. (2014) 32: 1140-1148.
11. El-Sakhawy MA, Al-Sabaa A, Abusaida H, Issa Y and Moheb M: Immunohistochemical Expression of Proliferating Cell Nuclear Antigen (PCNA) In the Parotid Salivary Glands of Male Albino Rats after Long Administration of Nutmeg. RJPBCS. (2016) September–October ,7(5):187.
12. Kintawati S, Darjan M, Yohana W and Rachmawati E: Expression of Pcna in Salivary Gland Adenoid Cystic Carcinoma and Its Correlation with Clinicopathological Parameters. International Journal of Medical Science and Clinical Invention. (2018) 5(06): 3868-3872.
13. Denny PC, Ball WD, Washington DC and Redman RS: Salivary glands: A paradigm for diversity of gland development. Crit Rev Oral Biot Med. (1997) 8 (51):51-75.
14. Fedrowitz M, Hass R, Bertram C and Löscher W: Salivary a-amylase exhibits antiproliferative effects in primary cell cultures of rat mammary epithelial cells and human breast cancer cells. Journal of Experimental and Clinical Cancer Research. (2011) 30:102.
15. Nater UM and Rohleder N:Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. Psychoneuroendocrinology. (2009) 34: 486-496.
16. Bhattarai KR, Lee SW, Kim SH, Kim HR and Chae HJ: Ixeris dentata extract regulates salivary secretion through the activation of aquaporin-5 and prevents diabetes-induced xerostomia. Journal of Experimental Pharmacology. (2017) (9): 81–91.
17. Yamagishi R, Wakayama T, Nakata H, Adthapanyawanich K, Kumchantuek T, Yamamoto M and Iseki S: Expression and localization of α-amylase in the submandibular and sublingual glands of mice. Acta Histochem. Cytochem. (2014) 47 (3): 95–102.

18. Mehanny SS, Salem ZA and Maria MKM: The histopathological effect of sovaldi on masseter muscles in albino rats (an animal study). E.D.J. (2019) July (65):1395-1403.

19. Shin JW, Seol IC and Son CG: Interpretation of Animal Dose and Human Equivalent Dose for Drug Development. The Journal of Korean Oriental Medicine. (2010) 31(3): 1-7.

20. Abdel Moneim RA, El DeebM and Saad FA: Evaluation of the therapeutic potential of Tamarind seeds (Aqueous extract) versus antidiabetic drugs on the histological structure of lingual papillae in diabetic rats. E.D.J. (2018) July, (64): 603-620.

21. Taha NS, Elbaz DA, Farag DBE: Effect of glucocorticoids alone or in combination with h1 receptor antagonist on rat sub mandibular salivary glands. (Histological and immuno-histochemical study). E.D.J. (2015) October, 4 (61):4999-5008.

22. Lim JY, Yi T, Choi JS, Jang YH, Lee S, Kim HJ and Sun U *et al*.: Intraglandular transplantation of bone marrow-derived clonal mesenchymal stem cells for amelioration of post-irradiation salivary gland damage. Oral Oncology. (2013) 49:136–143.

23. Sofia MJ: Beyond sofosbuvir: What opportunity exists for a better nucleoside/nucleotide to treat hepatitis C?. Antiviral Research. (2014) 107:119–124.

24. Beckerich F, Hézode C, Robin C, Beaumont JL, Gautier E, Maury S and Cordonnier C: New nucleotide polymerase inhibitors to rapidly permit hematopoietic stem cell donation from a positive HCV-RNA donor. Blood. (2014)124(16), 2613–2614.

25. El-Sharkawy GZ and Alhazzazi TY: The effect of the commonly used antidepressant drug amitriptyline (TCAs) on the salivary glands. J Dent Oral Disord Ther. (2016) 4(4):1-5.

26. Halawa AM, Mohamed DG, Obeid R: Capsaicin induced histological and ultrastructural changes in the submandibular salivary gland of albino rats. Future Dental Journal. (2016) 2, 22-27.

27. Mahay S, Adeghate E, Lindley MZ, Rolph CE and Singh J: Streptozotocin-induced type 1 diabetes mellitus alters the morphology, secretory function and acyl lipid contents in the isolated rat parotid salivary gland. Mol. Cell. Biochem. (2004) 261: 175-181.

28. Leite MF, Lima AM, Kang SJ, Santos MT and Otton R: Effect of astaxanthin and fish oil on enzymatic antioxidant system and α-amylase activity of salivary glands from rats. Braz. J. Oral Sci. (2014)13 (1): 58-63.

29. Gresik EW: The granular convoluted tubule (gct) cell of rodent submandibular glands. Microscopy research and technique. (1994) 27: l-24.

30. Redman RS: Histologic Changes in the Salivary Glands Following Radiation Therapy. Salivary Gland Development and Regeneration. (2017) chapter 4: 75-91.

31. Moriguchia K, Utsumi M, Jogahara T, Oda S and Honda M: Structural characterization of endogenous peroxidase activity in human, rat, hamster and Suncus murinus salivary glands. Journal of oral biosciences. (2017) 59: 184-19.

32. Barnes L: Surgical Pathology of the Head and Neck, Second Edition, (2000) November, 1:635.

33. Parlak SN, Tatar A, Keles ON, Selli J, Can I and Unal B: Effects of menopause and diabetes on the rat parotid glands: a histopathological and stereological study. Int. J. Med. Sci. Pub. H. (2014) 3(6): 749-755.

34. Riedl SJ and Shi Y: Molecular mechanisms of caspase regulation during apoptosis. Nat. Rev. Mol. Cell Biol. (2004) 5: 897-907.

35. Alikhani Z, Alikhani M, Boyd C, Nagao K, Trackman P and Graves D: Advanced glycation end products enhance expression of pro-apoptotic genes and stimulate fibroblast apoptosis through cytoplasmic and mitochondrial pathways. J. Biol. Chem. (2005) 280: 12087-12095.

36. Evcimen ND and King GL: The role of protein kinase C activation and the vascular complications of diabetes. Pharmacol. Res. (2007) 55 (6), 498-510.

37. Moubarak R: The effect of hypercholesterolemia on the rat parotid salivary glands (histopathological and immuno¬histochemical study. C.D.J. (2008) 24 (I): 19-28.

38. Zorov DB, Juhaszova M and Sollott SJ: Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev. (2014) 94, 909–950.

39. Lin CK, Tseng CK, Liaw CC, Huang CY, Wei CK, Sheu JH and Lee JC: Lobohedleolide suppresses hepatitis C virus replication via JNK/c-Jun-C/EBP-mediated downregulation of cyclooxygenase-2 expression. Scientific reports. (2018) 8: 8676.

40. Issa NM and El-Sherif NM: Light and electronic histological studies to the effect of Sofosbuvir on the visual cerebral cortex of adult male albino rat. J. Am. Sci. (2017) 13(4): 79-87.

41. Khater DS, Kasem RF and Morsy RA: Immuno-histochemical analysis of nf-κb expression and its relation to apoptosis and proliferation in different odontogenic tumors. International Journal of Cancer Research. (2017)13: 76-83.

42. Farag DBE, Elbaz DA and Salem ZA: Alveolar process changes associated with adminstartion of nucleos(t) ide analogue (sofosbuvir) in rat model. Dental and medical problems. (2018) March, 55(1):11-16.

43. Pastor BC, Caram´es B, De´an ML, Garcı´a CV, Blanco FJ and Armada ML: Mitochondrial Dysfunction Activates Cyclooxygenase 2 Expression in Cultured Normal Human Chondrocytes. Arthritis & rheumatism. (2008) August, (58) 8: 2409–2419.

44. Chen KJ, Tseng CK, Chang FR, Yang JI, Yeh CC, Chen WC and Wu SF *et al*.: Aqueous Extract of the Edible Gracilaria tenuistipitata Inhibits Hepatitis C Viral Replication via Cyclooxygenase-2 Suppression and Reduces Virus-Induced Inflammation. PLoS ONE. (2013) 8(2): 57704.

45. Lin CK, Tseng CK, Chen KH, Wu SH, Liaw CC and Lee JC: Betulinic acid exerts anti-hepatitis C virus activity via the suppression of NF-κB- and MAPK-ERK1/2-mediated COX-2 expression. British Journal of Pharmacology. (2015) 172: 4481- 4492.

46. Peper A: Intermittent adaptation: a mathematical model of drug tolerance, dependence and addiction. Computational Neuroscience of Drug Addiction book series, Neurosci. (2012) 10: 19-56.

47. Wanga Z, Lia W, Honga X, Sua JZ, Huab H, Penga X, Lvc L and Yu GW: Minor salivary glands function is decreased in hyposalivation-related diseases. Archives of Oral Biology. (2016) 69: 63–70.

48. Redman RS: Morphologic diversity of the minor salivary glands of the rat: fertile ground for studies in gene function and proteomics. Biotechnic & Histochemistry. (2012) 87(4): 273–287.

49. Kwak M, Alston N and Ghazizadeh S: Identification of Stem Cells in the Secretory Complex of Salivary Glands. Journal of dental research. (2016) 95 (7): 776 -783.

50. Artuso L, Zoccolella S, Favia P, Amati A, Capozzo R and Logroscino G *et al*.: Mitochondrial genome aberrations in skeletal muscle of patients with motor neuron disease. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. (2013) 14: 261–266.

51. Kim HR, Won SJ, Fabian C, Kang MG, Szardenings M and Shin MG: Mitochondrial DNA Aberrations and Pathophysiological Implications in Hematopoietic Diseases, Chronic Inflammatory Diseases, and Cancers. Ann Lab Med. (2015) 35: 1-14.

52. Zheng C, Hoffman MP, Mcmillan T, Kleinman HK and O'connell BC: Growth Factor Regulation of the Amylase Promoter in a Differentiating Salivary Acinar Cell Line. Journal of cellular physiology. (1998) 177: 628-635.

53. Fielda RB, Redmana RS, Callowaya AM and Goldberg WJ: Efect of 24 hours light on circadian rhythms of secretory enzymes and morphology of rat von Ebner's glands. Archives of Oral Biology. (1999) 44: 953-960.

54. Chou YS, Young TH and Lou PJ: Effects of biomaterial-derived fibroblast conditioned medium on the α-amylase expression of parotid gland acinar cells. Acta Biomaterialia. (2015) 27 : 214–223.

55. Vag J, Byrne EM, Hughes DH, Hoffman M, Ambudkar I, Maguire P and O'Connell BC: Morphological and Functional Differentiation of HSG Cells: Roleof Extracellular Matrix and trpc 1. J. Cell. Physiol. (2007) 212: 416-423.

56. Carda C, Lloreda, MN, Salom, L, Gomez de Ferraris ME and Peydro A: A structural and functional salivary disorders in type 2 diabetic patients. Oral Med. Pathol. (2006) 11: 309-314.

57. Negrato CA and Tarzia O: Buccal alterations in diabetes mellitus. Diabetol. Metab. Syndr. (2010) 15, 1-11.

58. Pariente A, Arpurt JP, Rémy AJ, Rosa-Hézode I and Causse X *et al*.: Hepatitis C treatment with all-oral direct-acting antivirals: Effectiveness and tolerance in a multicenter, prospective, observational study from French general hospitals. Presse Med. (2019) Mar; 48 (3 Pt 1): 101-110.

الملخص العربى

التأثيرات الغيركبدية للسوفوسبوفير على نموذج للغدد اللعابية المصلية الرئيسية و الصغيرة في الجرذان

فاطمة عادل سعد[١]، إيمان ابو شادي[٢]، سماح مهني[٣]

[١]مدرس بيولوجيا الفم، كلية طب الفم والاسنان، جامعة المستقبل في مصر، مصر
[٢]أستاذ مساعد بيولوجيا الفم، كلية طب الفم والاسنان، جامعة القاهرة، مصر
[٣]أستاذ بيولوجيا الفم، كلية طب الفم والاسنان، جامعة القاهرة، مصر

نبذة مختصرة: نشأت مؤخرًا تأثيرات ضارة غير كبدية مرتبطة بمعالجة عدوى فيروس التهاب الكبد الوبائي المزمن باستخدام نظم العلاج بالسوفوسبوفير.

الهدف من التجربة: تهدف هذه الدراسة إلى تقييم التأثير الالتهابي للسوفوسبوفير وتأثيره على تكاثر ووظائف وتمايز خلايا كلاً من غدد تحت الفك السفلي اللعابية وغدد فون إبنر اللعابية.

التجربة: تم تقسيم ٢١ من الفئران البيضاء الذكور البالغين إلى ثلاث مجموعات متساوية: المجموعة الاولي الظابطة: تلقت فقط ماء مقطر عن طريق الفم، المجموعة الثانية : تلقت سوفوسبوفير (٤٠ ملجم / كجم / يوم) مذاب في ماء مقطر عن طريق الفم لمدة شهر واحد، المجموعة الثالثة: تلقت سوفوسبوفير لمدة شهرين. تمت معالجة عينات غدد تحت الفك السفلي اللعابية وغدد فون إبنر اللعابية للفحص الهستولوجي الروتيني، وللفحص المناعي باستخدام الأجسام المضادة لكلاً من كوكس2- و بيكنا بالاضافة الي الفحص المناعي المتألق باستخدام الأجسام المضادة لألفا اميليز.

النتائج: مقارنة مع المجموعة الضابطة، أظهرت المجموعة الثانية تغييرات ضارة في الغدد تحت الفك السفلية اللعابية وغدد فون إبنر اللعابية التي زادت في المجموعة الثالثة ؛ انكماش العنيبات المصلية، حدوث فجوات بالخلايا الغدية، تغيرات انحلالية بالانوية، مناطق تنكسية واسعة، تسطيح الخلايا المبطنة لقنوات الاخراج اللعابية مع ركود في الافراز اللعابي، وكذلك تحويل عدد قليل من خلايا الغدية المصلية إلى خلايا شبه مخاطية خاصَّة في الغدد تحت الفك السفلية بالمجموعة الثالثة. وبالمثل، أظهرت كلتا الغدد بالمجموعة الثانية زيادة كبيرة في التفاعل المناعي لكوكس2- في العنيبات المصلية وبعض خلايا القنوات اللعابية ولكن مع انخفاض كبير في ذلك التفاعل بالمجموعة الثالثة. فيما يتعلق بالتفاعل المناعي لبيكنا وايضا بالتألق المناعي لالفا اميليز اظهرت النتائج تراجع ملحوظ في ايجابية التفاعل بالخلايا الغدية في كلتا الغدد بالمجموعة الثانية مقارنة بالمجموعة الظابطة ، بينما وُجد تحسن ضئيل في التفاعل المناعي بالمجموعة الثالثة مقارنة مع المجموعة الثانية باستثناء التحسن الكبير في تفاعل غدد فون إبنر اللعابية للتألق المناعي لالفا اميليز.

الاستنتاج: ومما سبق تم إستنتاج أن الإجهاد التأكسدي المرتبط بالتغيرات التنكسية بالغدد اللعابية بعد شهر واحد من العلاج بالسوسوفوفير قد تضاءل بعد شهرين من العلاج اليومي بسبب القدرة المكتسبة لتحمّل الجسم للعقاقير مما ساعد علي استعادة بعض العمليات الفسيولوجية المضطربة، وبالتالي فإن استخدام مضادات الأكسدة كعلاج مساعد يمكن أن يكون مفيدًا.