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THE EFFECT OF ANTIHEPATITIS C DRUG (SOFOSBUVIR) ON TONGUE PAPILLAE OF ALBINO RATS AND THE POSSIBLE MODULATORY ACTION OF GRAPE SEEDS EXTRACT (HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY)

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ABSTRACT

Hepatitis c virus (HCV) is the major cause for chronic hepatitis, Sofosbuvir (SOF) is a primary treatment choice for HCV patients however it has many side effects, Grape seed extract (GSE) is a dietary supplement proven to consist of phenolic compounds leading to an antioxidant property.

Aim of the study: This work has been focused on the possible effect of SOF on the tongue Papillae of male Albino rats and assessment of the probable protective role of GSE as an antioxidant.

Materials & methods: adult male Albino rats were utilized in the work (n=30), receiving drugs orally as a single daily dose for 6 weeks. The animals were arbitrarily divided into three groups. Group I (control group): Rats received distilled water only. Group II (SOF group): Rats received 40 mg\kg\day of SOF dissolved in distilled water (8mg\ml) via gastric tube. Group III (combination SOF& GSE group): Rats received SOF with the same concentration as mentioned before in combination with GSE at a dose of 200mg \kg\day dissolved in distilled water given via gastric tube. After 6 weeks, all animals were sacrificed by ketamine overdose; tongues were carefully dissected and prepared to be examined under light microscope using Hematoxylin and Eosin (H & E) and immunohistochemical staining using ki67, CD20.

Results: SOF-treated group showed degenerative changes to the papillae including torn and detached keratin layer, numerous intracellular epithelial vacuolations, degenerative areas in the connective tissue with leucocytic infiltrations. Immunohistochemically and statistically, there was highly significant reduction in the ki67 count, and also decrease in CD20 area % in Gp II papillae compared to Gp I and Gp III. The administration of GSE with SOF minimized these changes.

Conclusions: SOF has been proved to induce degenerative changes in the tongue papillae and these changes can be attenuated by GSE when it is co-administered with it.

KEYWORD: CD20, Grape seed extract, Ki67, Sofosbuvir, tongue papillae.

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INTRODUCTION

The liver is a multi-functional organ with different metabolic activities that have a critical role to maintain the body and sustain life [1]. There are liver diseases caused by infection with viruses, such as hepatitis A, hepatitis B, hepatitis C [2].

Hepatitis c virus (HCV) is judged as a substantial worldwide health problem. It presently infects more than 170 million people. The great bulk of patients develop chronic HCV infection, which can eventually result in hepatic cirrhosis, failure and/or hepatocellular carcinoma, leading to about 350,000 losses each year. Egypt showed the highest incidence (15%) of hepatitis C virus worldwide. It also has the highest predominance of specific genotype that is HCV- 4 by (67%) [3]. **HCV** is the main cause of chronic hepatitis and liver diseases worldwide [4,5,6].

The anti-hepatitis C viral drug has undergone a lot of deleterious histopathological changes. Sofosbuvir was discovered in 2007 by Michael Sofia, the drug was first tested on people in 2010. It was approved in the American States in November 2014 and in European Union in December 2014 under the trademark of (Sovaldi™) [7].

Sofosbuvir (SOF) is a nucleotide analogue it's mechanism that it inhibits the RNA-dependent RNA-polymerase (NS5B protein) of HCV. The most common systemic side effects of SOF are fatigue, mouth ulcers, headache, muscle pain, diarrhea, dry mouth, muscle spasms [8]. Some histopathological changes of salivary glands and fundic mucosa following SOF administration were also reported [9,10].

Grape seed extract (GSE) is a powerful dietary supplement that is extracted by drying, removing and pulverizing any bitter-tasting seeds of grapes. GSE was proven to consist of phenolic compounds leading to an antioxidant, anti-inflammatory properties [11,12,13].

Monoclonal antibodies are essential tools for various molecular immunology investigations, moreover, monoclonal antibodies have become key components in many clinical laboratory diagnostic tests [14]. In the present study, ki67 and CD20 immunohistochemical markers were used as a tool to evaluate the potential change in the tissue caused by SOF. Ki-67 is a non- histone nuclear and nucleolar protein encoded by MKI-67 gene mapping to chromosome 10q26.2 in humans [15]. Ki-67 is present in the G1, S and G2 phases of the cell cycle but not in the quiescent or resting cells G0 as they do not express this antigen [16]. So, it's present is an excellent operating marker to determine the growth fraction of a given cell population [17]. For CD20, it is expressed in B cells from pre- to mature phases of cell development. It has a part in the regulation of B-cell activation, proliferation and differentiation is also identified in many types of non-Hodgkin lymphoma [18].

The tongue of Albino laboratory rats is a well-developed muscular organ, its dorsal surface has four types of papillae; filiform, fungiform, circumvallate, foliate papillae [19].

This study was conducted to assess the action of SOF on tongue papillae and the possible herbal therapy represented in GSE which could minimize these supposedly adverse effects of SOF administration.

MATERIALS AND METHODS

Animals

Thirty adult male Albino rats weigh up between 150-200mg were used in the study. These rats were obtained from the animal house of Ain Shams University. The animals were housed into sterile, controlled environment (temperature +\- 2°C. and 12-hour dark and light cycles) and were fed with soft bread diet and tap water. The size of the cage was 20 cm width and 40 cm length. The work was performed according to the guidelines of animal experimentation and was reviewed and approved

by the Faculty of Dentistry's research ethical committee, Ain Shams University, Cairo, Egypt (final approval number is FDASU-RecIM111905)

Medications

Sofosbuvir (SOF) was manufactured by Gilead Sciences pharmaceutical company. It is present in a tablet form in the market under commercial name, Sovaldi ™. Grape seeds extract (GSE) was obtained from Sigma Pharmaceutical Industries, Egypt.

Methodology

Study design: The animals were at random divided into three groups (10 rats each).

- Control group (Gp I): Rats received Only distilled water daily orally via gastric tube for 60 days.
- SOF group (Gp II): Rats received 40 mg\kg\day of SOF dissolved in distilled water (8mg\ml) orally via gastric tube for 60 days (20).

Combination SOF & GSE group (Gp III): Rats received 40 mg\kg\day of SOF dissolved in distilled water (8 mg\ml) in combination with GSE at a dose of 200mg \kg\day dissolved in distilled water orally via gastric tube for 60 days [21] **(table 1).**

TABLE (1): Showing different groups, dosage of different drugs.

Groups	Number of rats/groups	Dosage of Sofosbuvir	Dosage of grape seeds
Control group	10	Non	Non
SOF group	10	40mg/kg/day	Non
Combination SOFand GSE group	10	40mg/kg/day	200mg/kg/day

Tissue preparation for light microscopic examination

At the end of the experiment, rats were sacrificed separately by Ketamine overdose. Tongue specimens were immediately fixed in 10% phosphate buffered formalin solution then washed properly under running water to remove all fixative residues. Then specimens were dehydrated and embedded in paraffin blocks to be cut by microtome to 4 to 5 microns thickness. Slides were stained by hematoxylin and eosin (H&E) stains for routine histological examination [22].

Tissue preparation for immunohistochemical examination using ki67 and CD20:

Paraffin set in tissue sections were prepared. According to the manufacturer's protocol, Deparaffinized retrieved tissue sections were handled by 0.3% H_2O_2 for 20 Mins. Then were incubated with Anti-CD20antibody (Abcam- ab64088 - 1:100), Anti Ki-67 Antibody (thermofisher- MA5-14520 - 1:100) at 4C overnight. Then washed out by PBS followed by incubation with secondary antibody HRP Envision kit (DAKO) 20 mins; washed out and incubated with diaminobenzidine (DAB) for 15 mins. Washed by PBS then counter staining with hematoxylin, dehydrated and clearing in xylene then cover slipped for microscopic examination. [23].

Histomorphometric analysis

H & E-stained sections were examined by a light microscope (Model BX40F, 7E12569) Olympus Optical Co., LTD. Japan. Photographing was carried out using a attached camera (Olympus soft imaging solutions, Munster, Germany, Model LC20,59001227). This was done at the department of Oral Biology, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.

Sections (200X) were randomly selected and examined by light microscope and the most homogenous areas of the positive reaction in both circumvallate and filiform papillae were chosen for evaluation. The image analyzer computer system applying Image j software (Version 1.41a, NIH, USA) was used for automated measurement of area percent of CD20 positivity and counting of Ki-67

index. It was performed in a standard frame area of $17.9X10^6 \mu m^2$, five fields were measured per case**.**

Statistical analysis:

Statistical analysis of the results was performed using SPSS software (version 20.0, SPSS Inc., Chicago, Illinois, USA). Shapiro-Wilk test of normality was used to test normality hypothesis of all quantitative variables. Aanalysis of variance (ANOVA) test was used for evaluation of statistical significance of each marker within the studied groups, followed by Tukey Kramer Post hoc test for the statistically significant results. P-values ≤ 0.05 were considered statistically significant.

RESULTS

Light microscopic results for the sections stained with H & E:

Filiform papillae

- **Gp I:** showed papillae in anterior two thirds of the tongue; appeared long and slender with (thread like shape), with concave and convex sides having their tips pointing backwards towards the base of tongue. These papillae were typically covered by orthokeratinized stratified squamous epithelium with a well-formed connective tissue (Ct) core. **(fig.1A).**

- **GpII:** showed distorted papillae with loss of their normal thread like shape. Most of them appeared apparently short with rounded tips. Separated torn keratin was found covering the papillae with many intracellular vacuolations in addition to some leucocytic cells infiltration in Ct. Other papillae showed atrophy with apparent loss of keratin layer. Loose Ct papillae with areas of degeneration were found. **(fig.1B).**

- **Gp III:** Most of the papillae showed thread like shaped projections, but some papillae still showed rounded tip papillae. There was less intracellular vacuolation, with apparent less keratin detachment and apparent less leucocytic cells infiltration in epithelium. lamina propria showed areas Ct destruction. **(fig.1C).**

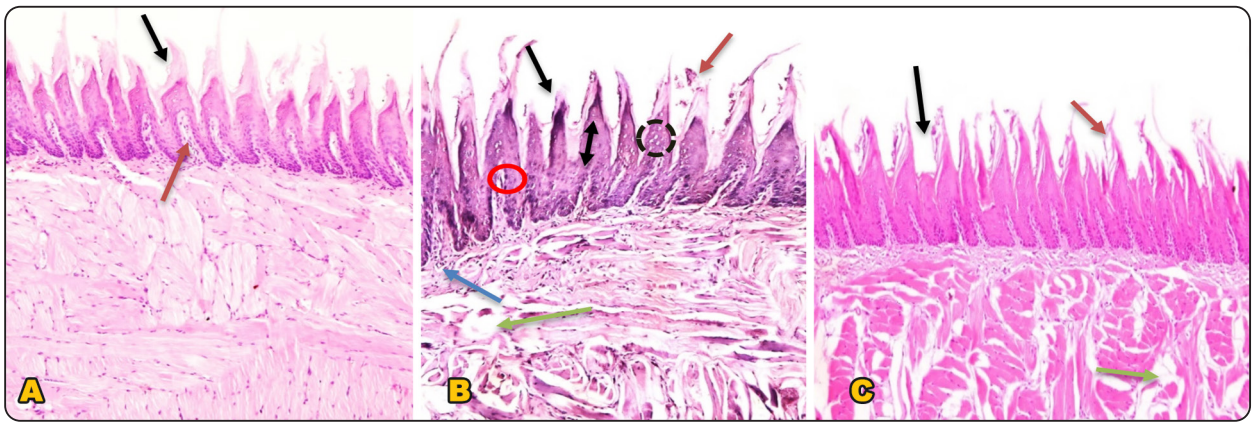

Fig. (1) : ***1A*****:** A photomicrograph of the true filiform papillae of Gp I showing orthokeratinzed thread like shaped projections **(black arrow),** Ct **(red arrow) (H&E original mag. × 200),** ***1B*****:** A photomicrograph of true filiform papillae of Gp II showing disfigured papillae **(black arrow),** with epithelial hyperplasia **(double head arrow),** detached keratin **(red arrow),** vacuolated cells in epithelium **(black circle),** pyknotic nuclei **(red circle)**, leucocytic cells infiltrations in the C.t **(blue arrow)** and areas of Ct degeneration **(green arrow) (H&E, original mag. × 200),** ***1C*****:** A photomicrograph of true filiform papillae of Gp III showing some papillae with rounded tips **(black arrow),** detached keratin **(red arrow),** areas **C**.t destruction **(green arrow) (H&E original mag.× 200).**

Fungiform papillae:

- **Gp I:** papillae appeared mushroom shaped, elevated above the surface of the tongue and were covered by orthokeratinized stratified squamous epithelium showing a single well defined barrel shaped taste bud, located at the center of the dorsal surface of papillae. Ct core was well defined, intact, dense and fibrous **(fig.2A).**
- **Gp II:** papillae appeared atrophic, dome shaped with detached keratin layer, vacuolated cells in the epithelial lining and other cells with deeply stained nuclei. Having infiltration of leucocytes in the Ct core with areas of degenerations in the lamina propria. Taste buds appeared distorted, vacuolated with peripheral arrangement of cells and empty center. **(fig.2B).**
- **Gp III:** some papillae were well formed with normal taste buds, however, some areas showed detached keratin. Intact Ct was found. **(fig.2C).**

Circumvallate papillae:

Gp I: An inverted cone-shaped papilla was detected that is surrounded by a well-developed deep trough. The papilla was covered by orthokeratinized stratified squamous epithelium with numerous well-defined taste buds in the epithelial wall of the trough. A central core of dense intact fibrous Ct. **(fig.3A).**

- **Gp II:** The papilla was markedly shrunken with wide trough. It covered by torn and separated keratin. In addition, the taste buds appeared shrunken and distorted. The Ct revealed degenerated areas with leucocytic cells infiltrate. **(fig.3B).**
- **Gp III:** The trough around the papilla was narrow and taste buds in its wall were well-defined. The Ct showed few leucocytic cell infiltration. **(fig.3C).**

Foliate papillae:

Gp I: The papillae appeared as series of parallel ridges, covered by orthokeratinized stratified squamous epithelium. The troughs between papillae appeared narrow and uniform with several taste buds along their wall. Intact fibrous c.t cores were observed with many secondary Ct papillae. **(fig.4A).**

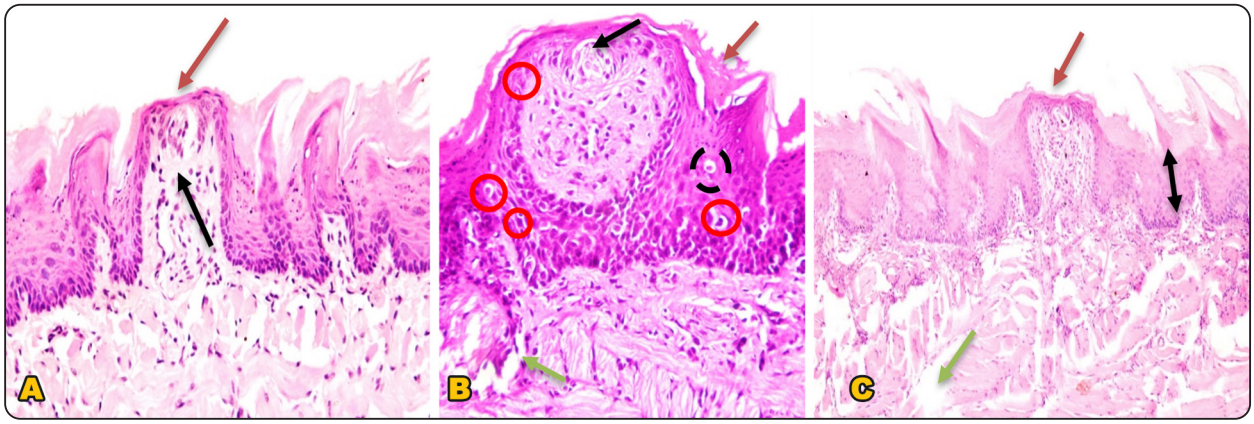

Fig. (2): *2A*: A photomicrograph of fungiform papilla of Gp I showing mushroom shaped papilla with a well-defined **C**t core **(black arrow)** and barrel shaped taste bud **(red arrow) (H&E original mag. × 400), *2B*:** A photomicrograph of fungiform papilla of GpII showing; disfigured papilla, detached keratin layer **(red arrow),** intracellular vacuolations **(black circle),** pyknotic nuclei **(red circle),** distorted taste bud **(black arrow)** and C.t destruction **(green arrow) (H&E original mag. x400), *2C*:** A photomicrograph of fungiform papilla of Gp III showing almost normal mushroom shaped appearance and apparently normal taste bud **(red arrow),** with hyperkeratosis **(double head arrows)** and apparent c.t destruction (**green arrow**) **(H&E original mag. X 400).**

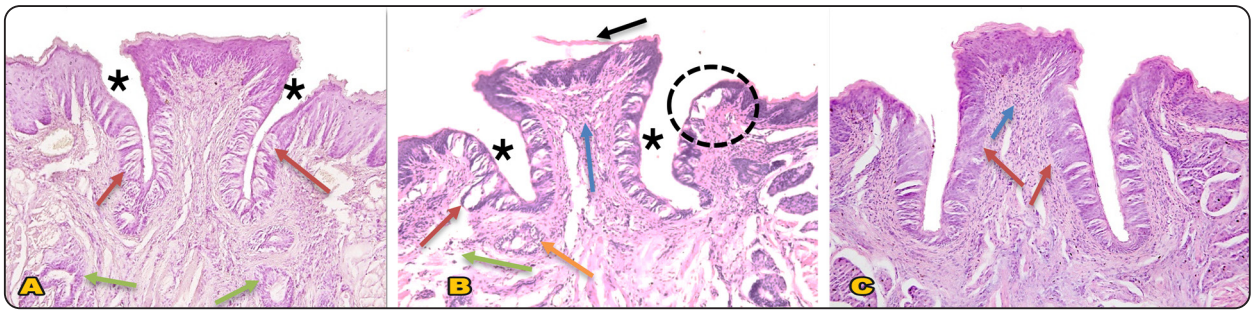

Fig. (3): 3A: A photomicrograph of circumvallate papilla of GpI showing an inverted cone-shaped papilla that is surrounded by a well-developed deep trough **(black Asterix),** numerous well-defined taste buds in the epithelial wall of the trough **(red arrows),** Von Ebner salivary gland **(green arrow) (H&E original mag.×200),** ***3B*:** A photomicrograph of circumvallate papilla of GpII showing apparently shrunken circumvallate papillae with wide trough **(black Asterix)** with almost no or detached thin keratin layer **(black arrow),** taste buds were distorted and show degeneration **(red arrow),** disruption in the epithelial lining **(circle),** C.t core was dense having lymphocytic infiltrations **(blue arrow),** areas of C.t tissue destruction **(green arrow),** Von Ebner salivary gland showing vacuolations and widely separated acini **(orange arrow) (H&E original mag.× 200),** ***3C*:** A photomicrograph of circumvallate papilla of Gp III showing some taste buds regained its shape while others remained degenerated **(red arrows)**, Aggregation of leucocytic cells in the lamina propria **(blue arrow) (H&E original mag. X200).**

- **Gp II:** The papillae appeared shrunken with apparently wide troughs with areas of torn and separated keratin layer. The taste buds were distorted, and the Ct had degenerated areas. **(fig.4B).**
- **Gp III:** The papillae appeared as parallel ridges, separated by narrow troughs and the taste buds were well-defined. Fibrous and intact Ct cores were seen. **(fig.4C).**

Light microscopic results for immunohistochemical results:

Anti-ki67 stain was used for the detection of cell proliferation. Positive reactions appeared as brown nuclear staining. Anti-CD20 to detect lymphocytic infiltration which was also revealed as a well-defined brown ring at the periphery of the cell or in the cells themselves.

Filiform papillae

- **Gp I:** For ki67, the epithelial covering of the papillae showed a positive reaction in the basal as well as the parabasal cells and positive reaction in Ct, and for CD20, the papillae showed almost negative reactions in the Ct. **(fig.5A).**
- **Gp II:** For ki67, the papillae showed minimal positive reactions confined to the basal layer of the epithelial covering. For CD20, the papillae showed minimal positive reactions confined between the muscles and in deeper Ct with almost no reaction in lamina propria of papillae. **(fig.5B).**
- **Gp III:** For ki67, the epithelial covering of the papillae showed positive reactions in the basal layer and few in para basal layer. For CD20, papillae showed few positive reactions found in the lamina propria of the papillae, in between the muscles and in deeper Ct. **(fig.5C).**

Circumvallate papillae:

Gp I: For ki67, the epithelial covering of the papillae has shown positive reactions in all the basal and para basal cells with the presence of a noticeable reaction at the von Ebner salivary gland. As for CD20, the papillae showed almost negative reaction in Ct. **(fig.6A).**

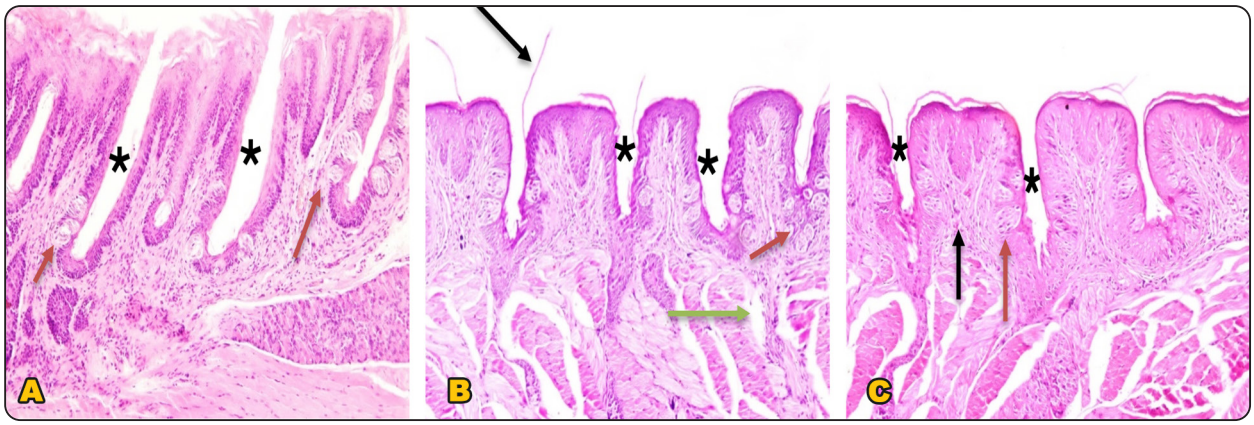

Fig (4) : 4A: A photomicrograph of foliate papillae of GpI showing foliate papillae as series of parallel ridges with alternating grooves **(black Asterix)** with normal taste buds along their lateral walls **(red arrow) (H&E original mag. × 200), *4B*:** A photomicrograph of foliate papillae of GpII showing shrunken papillae with wide troughs in some areas **(black Asterix),** detached keratin **(black arrow)** distorted taste buds **(red arrows)** with degenerative areas in the C.t **(green arrow) (H&E original mag.× 200), *4C*:** A photomicrograph of foliate papillae of GpIII showing parallel ridges separated by narrow troughs **(black Asterix)** and intact fibrous C.t cores **(black arrow**) and uniform taste buds (**red arrow) (H&E original mag.× 200)**

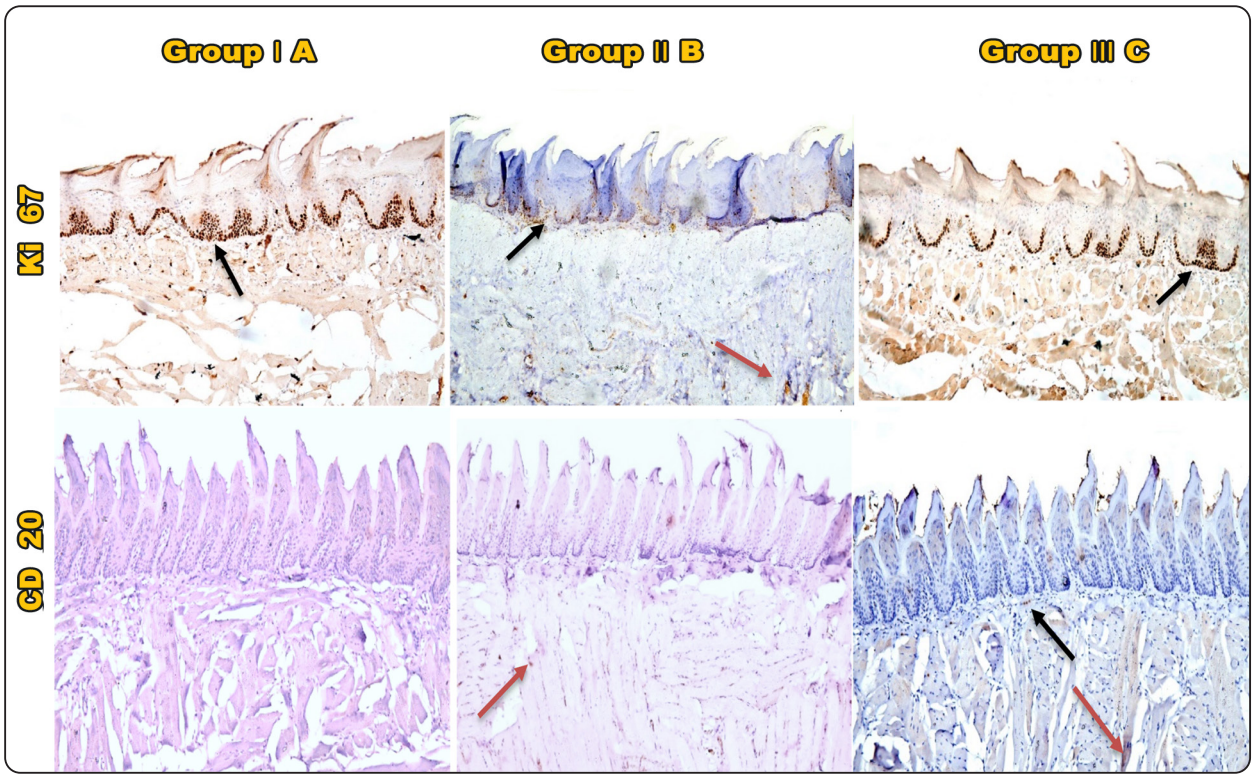

Fig 5: 5A: A photomicrograph of filiform papilla of GpI showing positive reaction toward ki67 in the basal and parabasal cells of the epithelial covering **(black arrow) (Anti-ki67 original mag. ×200)** and almost negative reaction toward CD20 **(Anti-CD20 original mag. × 200), 5B:** A photomicrograph of filiform papilla of GpII showing minimal positive reaction toward ki67 in the epithelial covering **(black arrow)** and in C.t**(red arrow) (anti-ki67 original mag. × 200)** and minimal positive reaction toward CD20 in muscles **(red arrow) (Anti-CD20 original mag. × 200), 5C:** A photomicrograph of filiform papilla of Gp III showing positive reaction toward ki67 confined in the basal cells of the epithelium covering **(black arrow) (anti-KI67 original mag.× 200)** and showing positive reaction toward CD20 in lamina propria of the papillae **(black arrow),** and in between muscles **(red arrow) (Anti-CD20 original mag. × 200)**

- **Gp II:** For ki67, the papillae showed noticeable decrease in the detected positive reactions in basal cells of the epithelium, in Von Ebner salivary gland and negative reactions in others. As for CD20, the papilla showed minimal positive reactions, mainly confined in deeper Ct. **(fig.6B).**
- **Gp III:** For ki67, the papillae showed an increase in the positive reactions in all basal cells as well as few para-basal cells of epithelium in comparison to Gp II with the presence of a noticeable reaction in Ct. As for CD20, the papillae showed positive reactions scattered throughout the Ct. **(fig.6C).**

Statistical results:

Ki 67 count

Filiform papillae

There was a statistically significant difference between filiform groups according to counting of positive reaction to ki67 with p-value ($p<0.05$). The highest value was in control group (Gp I) (52), followed with combination group (Gp III) (34) and the lowest was in SOF group (Gp II) (19.4) **(table 2, fig.7).**

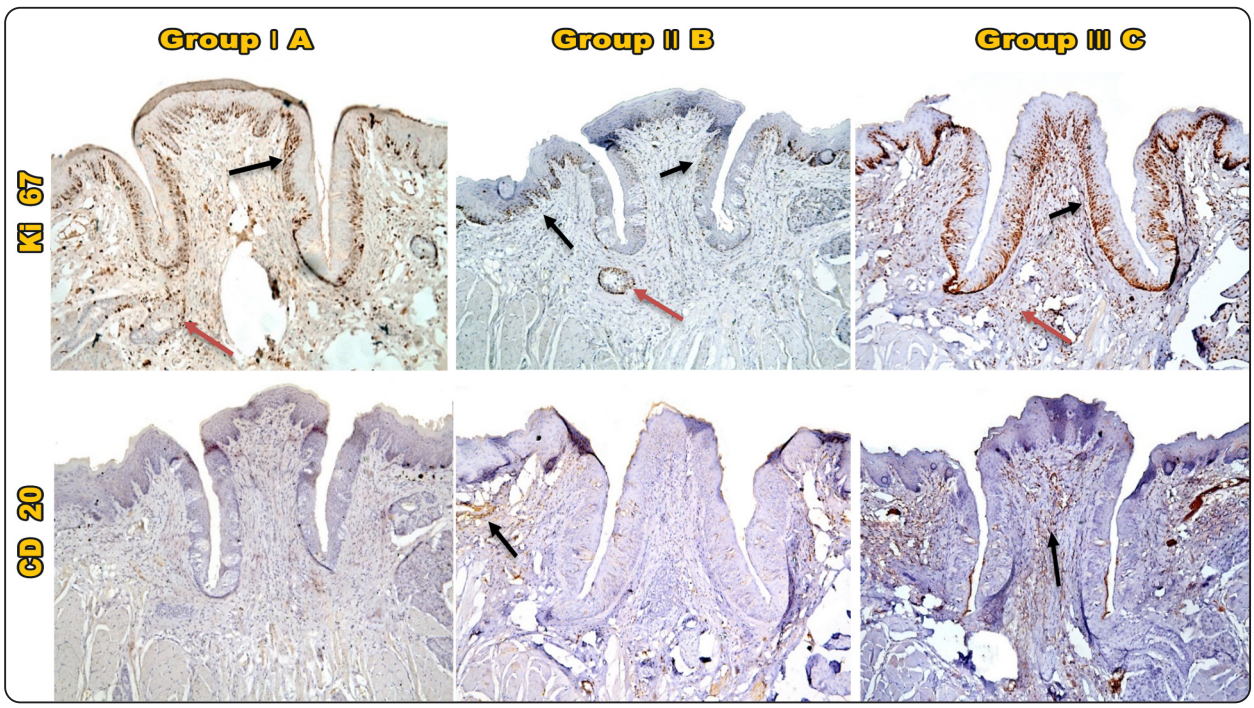

Fig *6: 6A*: A photomicrograph of circumvallate papilla of GpI showing positive reactions against KI67 in basal and parabasal layer **(black arrow),** and in Von Ebner salivary gland **(red arrow) (anti-KI67 original mag.× 200**) and showing almost negative reactions against CD20 **(anti-CD20 original mag. × 200), *6B*:** A photomicrograph of circumvallate papilla of GpII showing some positive reactions against KI67 only in basal layer **(black arrow)** and in Von Ebner salivary gland **(red arrow) (anti-KI67 original mag.×200) and** positive reactions toward CD20 in **C**.t **(black arrow) (anti-CD20 original mag. × 200), *6C*:** A photomicrograph of circumvallate papilla of GpIII showing positive reaction toward KI67 in basal and parabasal cells of the epithelial covering **(black arrow)** and positive reaction in C.t **(red arrow) (anti-KI67 original mag.×200**).and showing positive reaction against CD20 scattered in C.t **(black arrow) (anti-CD20 original mag. ×200).**

TABLE (2): Ki67 cell count in all groups of filiform papillae and significance. of the difference using (ANOVA) test

P.O.C	Control	SOF	GSE
Mean	52[a]	19.4[c]	34[b]
Std Dev	9.82	2.70	9.27
Std error	4.40	1.21	4.15
Max	61	22	43
Min	40	15	21
F-value		21.090	
P-value		< 0.001*	

Significant at p<0.05** * a b c: **Tukey's post hoc test means sharing the same superscript letter are not significantly different.

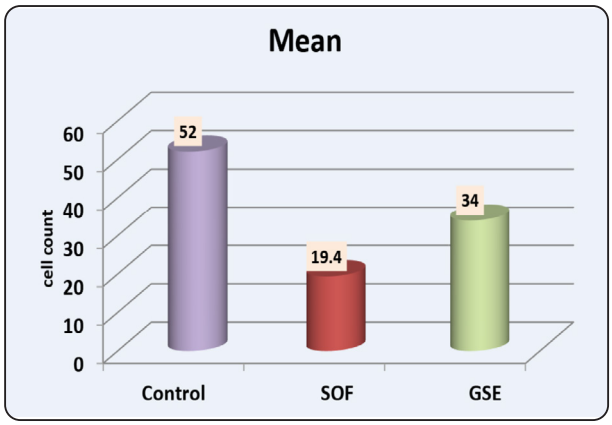

Fig. (7): Column chart showing mean Ki67 cell count in all groups.

TABLE (3): Ki67 cell count in all groups of circumvallate papillae and significance of the difference using (ANOVA) test

P.O.C	Control	SOF	GSE
Mean	448.8[a]	41.4[c]	320.4[b]
Std Dev	71.01	6.58	47.40
Std error	31.76	2.94	25.67
Max	507	47	386
Min	364	30	248
F-value		88.750	
P-value		< 0.001*	

Significant at p<0.05** * a b c: **Tukey's post hoc test means sharing the same superscript letter are not significantly different.

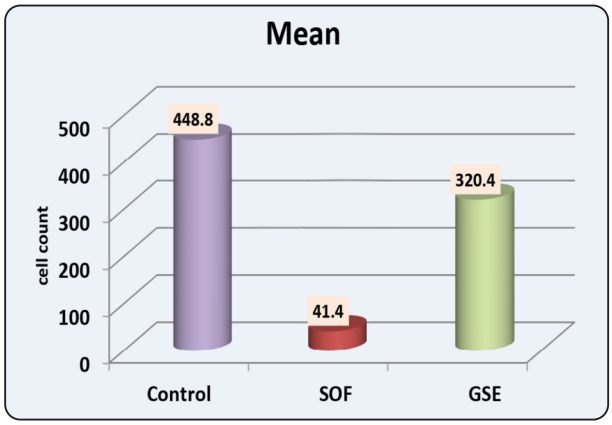

Fig. (8): Column chart showing mean Ki67 cell count in all groups.

Circumvallate papillae:

There was a statistically significant difference between circumvallate groups according to counting of positive reaction to ki67 with p-value (p<0.05). The highest value was in control group (Gp I) (448.8), followed by combination group (Gp III) (320.4) and the lowest was SOF group (Gp II) (41.4) **(table 3, fig.8).**

CD20 area percent:

Filiform papillae

There was a statistically significant difference between filiform groups according to area percentage (%) of positive reaction to CD20 with p-value (p<0.05). the highest value was in combination group (Gp III) (1.51), followed by SOF group (Gp II) (0.32) and the lowest was control group (Gp I) (0.13) **(table 4, fig.9).**

Table (4): Area percent of CD20 in all groups of filiform papillae and significance of the difference using (ANOVA) test

P.O.C	Control	SOF	GSE
Mean	0.13[a]	0.32[c]	1.51[b]
Std Dev	0.04	0.07	0.24
Std error	0.02	0.03	0.11
Max	0.18	0.41	1.84
Min	0.08	0.23	1.30
F-value		130.91	
P-value		< 0.001*	

Significant at p<0.05** * a b c: **Tukey's post hoc test means sharing the same superscript letter are not significantly different.

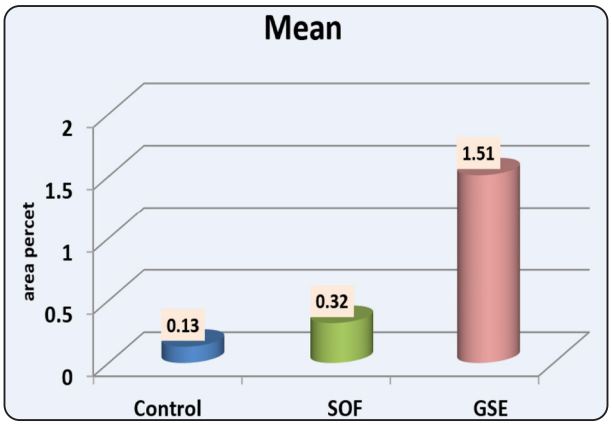

Fig. (9): Column chart showing mean area percent of CD20 in all groups.

Circumvallate papillae

There was a statistically significant difference between circumvallate groups according to area percentage (%) of positive reaction to CD20 with p-value (p<0.05). the highest value was in combination group (Gp III) (2.18), followed with control group (Gp I) (0.95) and the lowest was SOF group (Gp II) (0.06) **(table 5, fig.10).**

TABLE (5): Area percentage of CD20 in all groups of circumvallate papillae and significance of the difference using (ANOVA) test

P.O.C	Control	SOF	GSE
Mean	0.95[a]	0.06[c]	2.18[b]
Std Dev	0.26	0.02	0.22
Std error	0.10	0.01	0.10
Max	1.36	0.08	2.44
Min	0.60	0.04	1.88
F-value		132.3	
P-value		< 0.001*	

Significant at p<0.05 * a b c: Tukey's post hoc test means sharing the same superscript letter are not significantly different.

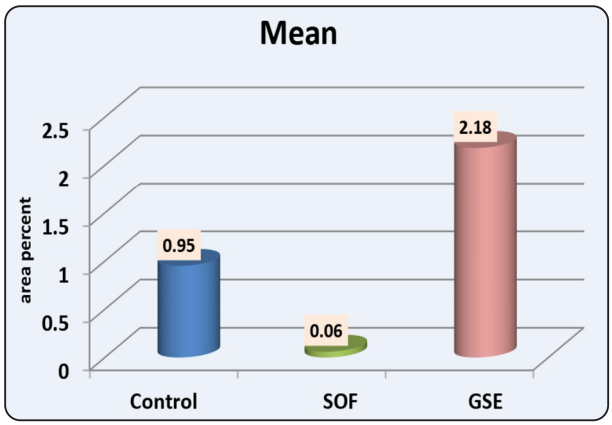

Fig. (10): Column chart showing mean area percent of CD20 in all groups.

DISCUSSION

In this study, Sofosbuvir (Sovaldi™) was the primary treatment of choice as the Egyptian Ministry of Health and Population decided for HCV patients in a plan titled as "The Plan of Action for the Prevention, Care and Treatment of Viral Hepatitis 2014–2018" [24]. Drugs were administrated orally because the oral route is economical, convenient, relatively safe, and mimics the most used mode of administration of substances to humans [25]. Rat's tongue was chosen as it provides wide surface

area for sufficient tissue sampling. Tongue also is considered as the mirror of the general health particularly filiform papillae [26,27].

Grape seeds extract (GSE) was used in this experiment as a potent antioxidant that scavenges reactive oxygen species (ROS) [28] and to assess its efficacy during HCV therapy as recommended by **Saad et al.,** [29]. CD 20 was known to be used as an immunomarker as being the core lineage markers expressed on the surface of normal and neoplastic B cells [30].

In the current study, the histology of the lingual papillae in the SOF group showed degenerative changes especially in filiform papillae. This explained by **Osman et al.,** [26], as filiform papillae are of high metabolic activity, so any enzymatic disturbance, vascular insufficiency, or nutritional deficiency result in changes in those papillae particularly.

These changes were in the form of torn, detached keratin layer and numerous intercellular vacuolations within the epithelium thickness covering the lingual papillae. These findings were in accordance with [31], [32] and [33]. These researchers attributed these findings to that SOF is a member of the Nucleotide inhibitors (NIs). NIs interact with mitochondrial RNA polymerase, inhibiting mitochondrial protein synthesis leading to decrease in mitochondrial oxygen consumption in cells and this, in turn, lead to hypoxia, mitochondrial toxicity and production of ROS, so the imbalance between these ROS and natural antioxidants cause oxidative stress which also did not only lead to cellular death but also hypoxia mediated disassembly and degradation of keratin intermediate filaments as declared by [34].

Another explanation to intracellular vacuolations within epithelium thickness in the current results was mentioned by **Elghazouly & Yassien,** [10], who attributed this to the production of mitochondrial reactive oxygen species (mROS) acting as signaling molecules stimulating proinflammatory cytokine release through NF-B signaling activation Inducible nitric oxide synthase (iNOS) is activated by NF-B, producing great amount of ROS in form of nitric oxide (NO). The cytotoxic compound peroxy-nitrite is formed by combination of NO with superoxide anions leading to lipid peroxidation in the epithelium as explained by [35].

These results were statistically confirmed in our study by a highly significant decrease in ki67 in Gp II when compared to Gp I which was found in harmony with **Coussens & Werb,** [36] who had related these changes to the production of inflammatory cytokines which inhibit taste progenitor cell proliferation through the generation of ROS, leading to DNA damage.

In this research Gp II also exhibited degenerative changes in the Ct in addition to leucocytic infiltration with the decrease of the height of the papillary layer and the presence of wide degenerative areas. These results were found in agreement with [32] and [9], and was explained by **Salem et al.,** [20], as the researchers related the presence of vacuolization and shrinkage in salivary gland to the increased levels of mitochondrial ROS following SOF administration. These findings were also in consistence with [37], who declared that SOF inhibits mitochondrial protein synthesis and increase the induction of many leucocytic mediators causing prolonged acceleration of chronic tissue inflammation.

On the other hand, **Riedl & Shi,** [38] explained these degenerative areas in C.t to be due to that fibroblast show signs of degeneration and subsequent apoptosis in chronic inflammation state and in turn resulted in suppression of collagen synthesis and increase collagenolytic activity. Furthermore, **Alikhani et al.,** [39] declared that not only the fibroblasts; but also, the inflammatory cells undergo apoptosis.

In the present study, Gp III revealed that GSE appeared to ameliorate SOF-induced histological

alterations on the lingual papillae especially filiform papillae which showed minimal changes, epithelium is normal with hyperkeratosis and pointed tips with few blunt ones with Less cell vacuolations, less inflammatory infiltration. These findings were found in agreement with **Houde et al.,**[40] who declared that GSE exhibit a strong free-radical scavenging effects with a combined inhibitory effect on both ROS and RNS generation, also because of its proanthocyanidin-rich extracts which have different types of polyphenolic components, which makes it owns a powerful antioxidant, anticancerogenic, and anti-inflammatory properties.

In addition, Von Ebner salivary gland showed some improvement in the acinar and ductal histological architecture. Fewer vacuolations were seen in the serous acinar cells as compared to the group only taking SOF. These findings were explained by [41] and [42] to be due to radical scavenging activity of the extract and was attributed to its hydrogen donating ability**,** giving hydrogen ion from the phenolic hydroxyl groups and forming stable end products which stops further oxidation of lipids which is another support for the existing study. This was also proven statistically as the expression of ki67 in both papillae in Gp III was increased due to the effect of GSE and this was in harmony with **Sen et al.,** [43] who stated that GSE treatment increase proliferation in epithelial region, showing higher cell density and also improving the histological architecture.

Concerning the expression of CD20 in GpII and Gp III, it was found that there was a significant statistical increase in Gp III than Gp II , this was explained by [44] who found a significant increase in the antibody titer after GSE administration, and suggested that GSE immunostimulatory function was due to its antioxidant and free radical scavenging properties that could increase the integrity and proliferation of B-lymphocytes and its differentiation into antibodies producing plasma cells [45]**.** This was also explained by [46] who found out that treatment with SOF resulted in a significant decrease in the concentration of total lymphocytes during treatment ,which is due to the decrease in the expression of interferon stimulated genes (ISG) during DAA therapy (ISGs, cell-secreted protein factors which play a key role in the regulation of cell growth and differentiation), this was also found in agreement with **Arai et al.,**[47] who concluded by examining HCV patients after DAA therapy that there was a down-regulating in the endogenous ISGs in host cells like B lymphocytes and therefore decrease in expression of B lymphocytes which also supports the current study.

CONCLUSIONS

Sofosbuvir has been proved to induce obvious histological changes in the various tongue papillae through increased ROS levels in cells leading to degenerative effect, Grape seeds extract can attenuate these changes when given with SOF. GSE is a promising antioxidant, anti-inflammatory agent for patients who receive SOF as an antihepatitic therapy.

Recommendations

Further clinical and experimental studies are recommended to evaluate the effect of using SOF on various hard dental and oral tissues. Also, more clinical trials were needed to assess the safest way for GSE coadministration with HCV drugs.

Conflict of interests

There are no conflicts of interests.

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