Original Article

Effect of Sofosbuvir (Sovaldi) on the Fundic Mucosa of Adult Male Albino Rats and the Possible Protective Role of Fucoidan: Histological, Histochemical, and Immunohistochemical Study

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ABSTRACT

Background: Sofosbuvir (SOF) is a hopeful remedy for chronic hepatitis C virus infection. However, it records some unfavorable influences on gastrointestinal tract. Fucoidan is a sulfated polysaccharide having cytoprotective effect besides anti-inflammatory and antioxidant properties.

Aim: This work has been focused on the possible effect of sofosbuvir on the fundic mucosa and to assess the probable role of protection of fucoidan.

Materials and Methods: We divided 40 adult male albino rats equally into four groups and were received drugs as a single daily dose for five weeks. Control group (I): were received distilled water and normal saline. Fucoidan group (II): were received 80 mg/kg. Sofosbuvir group (III): were received 4 mg/kg. Group IV: were received Fucoidan and Sofosbuvir with the same doses as pervious groups. All rats were sacrificed one day following the last dose. Stomach specimens were obtained and prepared for light and electron microscopic examination.

Results: Sofosbuvir-treated rats showed dilated fundic pits and cystic dilatation of fundic glands with flattening of their epithelial lining. Vacuolation of cytoplasm and shrunken pyknotic nuclei were detected in parietal cells. Dilatation of rough endoplasmic reticulum and loss of secretory granules were detected in the chief cells. Infiltration of inflammatory cells with congested blood vessels and extravasated RBCs were detected in lamina propria. Statistically, there was a highly significant rise in the area% of collagen fibers while area% of Periodic Acid Schieff -Alcian Blue stain (PAS–AB) reaction showed a highly significant reduction comparing with the control. The immunohistochemical results revealed marked increase in proliferating cell nuclear antigen (PCNA) expressions. The administration of Fucoidan with Sofosbuvir minimized these changes.

Conclusion: Sofosbuvir has been proved to induce histological changes in the fundic mucosa and these changes can be attenuated by Fucoidan when given in concomitant with it.

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Key Words: Electron microscopy, fucoidan, fundic mucosa, PCNA, sofosbuvir.

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INTRODUCTION

Chronic hepatitis C has been considered a popular disease and a major cause of chronic liver disorder, hepatocellular carcinoma and liver failure. In the Western countries, it is the main indication for transplantation of liver[1].

Hepatitis C virus infection has its highest prevalence rate (about 14%) in Egypt as one of developing countries in comparison with 2% in the developed countries. Actually, there are about 9% having an active infection among the 14% who carry the antibodies[2].

Before 2011, the pegylated interferon combined with ribavirin was the ideal remedy for chronic hepatitis C[3].

In 2014, some organizations recommended sovaldi and ribavirin plus interferon or not for the management of hepatitis C. They play a vital role in all primary therapies for HCV genotypes 1–6[4].

Sovaldi is the commercial name of sofosbuvir and it is a nucleotide analog given in association to different drugs to manage hepatitis C. When Sofosbuvir-based regimens are compared to the past treatments, it gives better recovery results, little adverse effects and decreased period of treatment[5].

Sofosbuvir has been considered an inactive drug that utilizes a biotechnology plan called ProTide. It is converted into 2'-deoxy-2'-α-fluoro-β-Cmethyluridine-5'-triphosphate which is considered an active antiviral substance. This substance acts as a depressive agent for the RNA polymerase of the virus, resulting in the inhibition of synthesis of its RNA[6].

Sofosbuvir may cause undesirable side effects that need medical attention. Common side effects are recorded as nausea, diarrhea, vomiting, ulcers or sores in mouth. Also, dry mouth, increased lipase, dyspepsia, abdominal discomfort, gastroesophageal reflux and constipation are seen[7,8].

Fucoidan is a sulfate-containing polysaccharide present chiefly in several types of brown sea algae. It was proved that these extracted sulfated polysaccharide

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have beneficial pharmaceutical and biomedical potential actions[9]. Fucoidans have anti-inflammatory, antioxidant, anticoagulant, anti-thrombogenic, anti-tumor and antiviral actions, in addition to immunomodulating activities[9,10].

Fucoidan has been considered as safe substance having possibility for stomach protection and was proved to be efficient in management and prevention of stomach ulcer[11]. Fucoidan polysaccharide is also known to be efficient in proliferation and replacement of cells particularly in digestive tract[12]. Moreover, fucoidan has cytoprotective effect to the gastric mucosa from many irritant substances[13].

Sofosbuvir is valuable remedy for hepatitis C infection; however, its side influences in general and on gastrointestinal tract in particular have not been fully authenticated. So, the purpose of this work is to check the probable effect of sofosbuvir on the stomach of adult male rats and to assess the probable role of protection of fucoidan by histological, histochemical and immunohistochemical techniques.

MATERIALS AND METHODS

Drugs and chemicals

1. Fucoidan powder was bought from Sigma Chemical, Co. Ltd. (St. Louis, MO, USA). We dissolved Fucoidan in normal saline and given to rats intraperitoneally (80 mg was dissolved in 5 ml normal saline to obtain a solution containing 16 mg/ml).
2. Sofosbuvir, a drug produced by Pharco Pharmaceuticals, Alexandria, Egypt. It is present in tablet form having commercial name, Gratisovir. Every tablet consists of sofosbuvir (400 mg). The drug was administrated to rats orally using gastric tube by dissolving the tablets in distilled water (one tablet was dissolved in 500 ml distilled water to obtain a solution containing 0.8 mg/ml).

Animals

We used in this study 40 adult male albino rats weighing 180–200 g. We maintained strict care and hygiene to keep them in normal healthy conditions. They received a balanced diet and drank water freely in animal house of the Faculty of Medicine, Menoufia University. This experiment was done according to the Animal Care and Ethical Committee Guidelines of our Faculty.

Experimental design

We divided the animals into 4 groups, each consisted of 10 rats and were given the drugs as single daily dose for five weeks.

Group I (control group): They were subdivided into 3 subgroups:

Subgroup Ia: Contained four rats, left without treatment during the experiment time.

Subgroup Ib: Contained three rats, they were given normal saline (0.9%) intraperitoneally.

Subgroup Ic: Contained three rats, they were given distilled water by gastric tube.

Group II (fucoidan-treated): Received 80 mg/kg fucoidan intraperitoneally[14].

Group III (sofosbuvir-treated): They were given sofosbuvir 4 mg/kg using gastric tube[15].

Group IV (sofosbuvir and fucoidan-treated): They were given fucoidan 1h before sofosbuvir, with the same doses as pervious groups.

Methods

Twenty four hours after the last dose, the rats were scarified by cervical decapitation. Their stomachs were excised, opened, and washed with saline. Specimens were taken from the fundus and prepared for light and electron microscopic examination.

A- Light microscopic study

Specimens were fixed in 10% formalin and processed in the usual way to obtain the ordinary paraffin blocks. Sections of 4μm thick were cut and underwent the following studies:

1. Histological study: Hematoxylin & eosin (H&E) stain and Masson trichrome[16].
2. Histochemical study: using Periodic Acid Schieff -Alcian Blue stain (PAS-AB stain) for detection of neutral and acidic mucin[17].
3. Immunohistochemical study: for detection of proliferating cell nuclear antigen (PCNA)[18].

B- Electron microscopic study

Specimens for electron microscopy were immediately fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4). Then, they were post fixed in 1% osmium tetroxide at 4°C, dehydrated, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined and photographed using JEOL-JEM-100 transmission electron microscope (Tokyo, Japan) at the Electron Microscopic Unit, Faculty of Medicine, Tanta University[19].

Morphometric measurements

1. The area % of collagen fibers: The area% of collagen fibers was measured in Masson's trichrome-stained sections at a magnification of ×100.
2. The area% of PAS–AB reaction: The area% of PAS–AB reaction was measured in PAS-AB-stained sections at a magnification of ×100.

All measurements were taken using a computerized image analyzer system (Leica Q 500 MC Program; Leica, Cambridge, UK), and were performed in 10 randomly chosen sections from five animals for each group.

Statistical analysis

Morphometric data were statistically analysed using SPSS program, version 17, (IBM Corporation, Somers, New York, USA). The data were presented as mean ± SEM (standard error of mean). We compared the mean of each group with that of the others using one-way analysis of variance (ANOVA) then "Tuckey" post hoc test. Differences were regarded as non-significant if *P-values* were >0.05, significant if *P-values* were $P<0.05$ and highly significant if *P values* were < 0.001[20].

RESULTS

Light microscopic results

Histological study

• Haematoxylin and Eosin staining

Examination of H&E-stained sections of the control group revealed normal structure of the fundic mucosa. The mucosa consisted of surface epithelium, lamina propria containing fundic glands, and muscularis mucosa formed of smooth muscles. The epithelium was formed of surface columnar mucous cells, which were covering the surface and lining the fundic pits and upper part of the glands. The lamina propria was formed of loose connective tissue containing numerous tightly packed parallel running tubular fundic glands which had short narrow pits and was lying perpendicular to the surface. The fundic glands could be divided into three parts: innermost isthmus, middle neck lined by parietal cells and mucous neck cells, and outer base lined mainly by chief cells (Figures 1, 2a&b). The pits and isthmus were lined by surface columnar mucous-secreting cells with basal oval vesicular nuclei and apical pale cytoplasm (Figure 3a). The necks of the glands appeared to be lined with mucous neck cells having vacuolated cytoplasm and flattened basal nuclei and large polyhedral parietal cells. Parietal cells were located all over the glands lying on the basement but not reaching the lumen. They had acidophilic cytoplasm and central rounded vesicular nuclei with prominent nucleoli (Figure 3b). The basal parts of the glands consisted mainly of chief cells and occasional parietal cells. The chief cells appeared low columnar with deeply stained basal basophilic cytoplasm, basal rounded vesicular nuclei, and apical acidophilic or pale stained zymogen granules (Figure 3c). The inspection of the fundic mucosa of fucoidan group (II) revealed a picture similar to the control group (Figures 4 a,b,c). Sofosbuvir treated group (III) revealed that, the fundic glands appeared with wide lumen and fundic pits. Surface mucous cells appeared with little cytoplasm and darkly stained pyknotic thin flattened nuclei. Most of parietal cells had vacuolated cytoplasm and peripheral shrunken pyknotic nuclei. Some parietal cells appeared with lost nucleus (Figure 5). There was cystic dilatation of fundic glands with flattening of their epithelial lining (Figure 7). Areas of the bases of the fundic glands showed disorganization, whereas other areas showed atrophy with degenerated chief cells. Most chief cells showed deeply stained pyknotic nuclei (Figures 6b,7,8). Diffuse inflammatory cellular infiltration was detected in the lamina propria between the fundic glands (Figure 6a). In some areas, the inflammatory cells were aggregated in the form of follicle between the glands (Figure 6b). Also, there were congested blood vessel and extravasated RBCs in the lamina propria between the fundic glands (Figures 7,8). The protected group treated with both sofosbuvir and fucoidan showed a picture nearly similar to the control group except for wide fundic pits and few surface mucous cells appearing with little cytoplasm and thin flattened pyknotic nuclei and few parietal cells appear with vacuolated cytoplasm and shrunken pyknotic nuclei (Figures 9,10).

• Masson's trichrome staining

Masson's trichrome-stained sections of the control group (I) revealed minimal collagen fibers in the lamina propria between the basal parts of fundic glands (Figure 11). Also, the fucoidan treated group (II) showed minimal collagen between the glands (Figure 12). On other hand, the sofosbuvir treated group (III) revealed excessive amount of collagen fibers in the lamina propria at the bases of the fundic glands (Figure 13). Sofosbuvir and fucoidan treated group showed moderate amount of collagen fibers at the bases of the fundic glands (Figure 14).

Histochemical study

• Periodic Acid Schieff -Alcian Blue staining (PAS-AB stain)

PAS–AB-stained sections of the control group (I) revealed a PAS-AB-positive thick mucus film covering the surface epithelium and going to fill the fundic pits. Strong AB-positive staining of mucous neck cells was observed (Figure 15). The fucoidan treated group (II) demonstrated picture similar to the control with a very strong Alcian blue reaction at the pit region, isthmus and necks (Figure 16). Sofosbuvir treated group (III) revealed very thin and faint PAS–AB positive reaction of the surface mucous film. Weak AB-reaction was observed in mucous neck cells with weak PAS-reaction in most of surface columnar mucous cells (Figure 17). Sofosbuvir and fucoidan treated group revealed a continuous thick PAS-AB positive mucous film above the surface epithelium going to fill the fundic pits. Moderate AB-positive reaction is observed at the pit regions, isthmus and necks of the glands (Figure 18).

Immunohistochemical study

• Proliferating cell nuclear antigen (PCNA) staining

The control group (I) and the fucoidan treated group (II) revealed strong positive PCNA reaction in the cells of the isthmus region of the fundic glands (Figures 19,20). The Sofosbuvir group (III) showed strong PCNA reaction in cells lining the whole fundic glands (Figure 21). Sofosbuvir and fucoidan group (IV) showed strong PCNA reaction in the isthmus and neck regions of the fundic glands (Figure 22).

Electron microscope results

Electron microscopic examination of the fundic mucosa of the control group (Group I) showed the surface mucous-secreting cells having multiple, rounded, electron-lucent mucous granules in the cytoplasm with normal apical microvilli projecting within the fundic lumen. Also, they had euchromatic nuclei and mitochondria (Figure 23). Mucous neck cells appeared with apical microvilli, numerous apical secretory granules, and basal oval euchromatic nuclei. Mitochondria were observed (Figure 24). The parietal cells appeared with euchromatic nuclei having regular chromatin distribution. Their cytoplasm had intracellular canaliculi lined with microvilli, numerous rounded to oval mitochondria with intact regular cristae, ribosomes and tubulovesicular structures (Figure 25). Enteroendocrine cells appeared with abundant basal small electron dense secretory granules and euchromatic nuclei (Figure 26). Chief cells revealed many apical electron-dense zymogen secretory granules, euchromatic nuclei and well-developed rough endoplasmic reticulum (Figure 27). The fundic mucosa of the fucoidan treated group (II) revealed picture similar to the control except that most surface mucous cells were full with large rounded mucoid granules (Figures 28-31). The fundic mucosa of the Sofosbuvir treated group (Group III) revealed the surface mucous cells appearing with few apical microvilli, few apical mucous granules and areas of cytoplasmic loss. Some of their nuclei appeared shrunken and indented. Disrupted mitochondria were observed (Figure 32). Also, the mucous neck cells appeared with shrunken irregular heterochromatic nuclei and few mucous granules. Disrupted mitochondria and areas of cytoplasmic loss were observed (Figure 33). The parietal cells showed vacuolated cytoplasm and irregular heterochromatic nucleus. Fibroblast, macrophage and eosinophil were observed. The nucleus of eosinophil was bilobed. Characteristic eosinophil granules were seen (Figure 34). The enteroendocrine cells appeared with irregular shrunken heterochromatic nuclei, areas of cytoplasmic loss and few basal small secretory granules of variable density and underlying inflammatory cell was seen (Figure 35). Many chief cells had shrunken electron-dense nuclei with condensed heterochromatin, rough endoplasmic reticulum dilatation, cytoplasmic vacuoles. Disrupted mitochondria and loss of secretory granules were observed (Figure 36). Sofosbuvir and fucoidan treated group (Group IV) revealed that, most of surface mucous cells and mucous neck cells appeared nearly normal with apical microvilli, some rounded mucous granules and basal euchromatic nuclei with few cytoplasmic vacuoles were still seen (Figures 37,38). The parietal cells had rounded euchromatic nuclei, abundant normal mitochondria and typical tubulovesicular structures. Most of the intracellular canaliculi appeared with microvilli, while few canaliculi had deficient microvilli (Figure 39). The enteroendocrine cells had slightly irregular euchromatic nuclei and basal secretory granules with few cytoplasmic vacuoles (Figure 40). The chief cells had relatively regular euchromatic nuclei, some electron-dense secretory granules and rough endoplasmic reticulum and few cytoplasmic vacuoles were still seen (Figure 41).

Morphometric and Statistical Results

1- Area% of collagen fibers

In comparison with control group, the area% of collagen fibers in fucoidan group (II) revealed non-significant difference ($P>0.05$). Sofosbuvir treated group (III) revealed highly significant rise ($P<0.001$) in the area% of collagen fibers in comparison with control group. Sofosbuvir and fucoidan treated group (IV) showed highly significant reduction ($P<0.001$) in comparison with Sofosbuvir treated group (III) (Table 1, Histogram 1).

2- Area% of PAS-AB reaction

The mean area% of PAS-AB reaction in group II showed a significant rise ($P<0.05$) in comparison with the control group. Sofosbuvir treated group (III) showed a highly significant decrease ($P<0.001$) in the area% of PAS-AB reaction when compared with the control group. Sofosbuvir and fucoidan treated group (IV) revealed highly significant rise ($P<0.001$) in comparison with sofosbuvir group (III) (Table 1, Histogram 2).

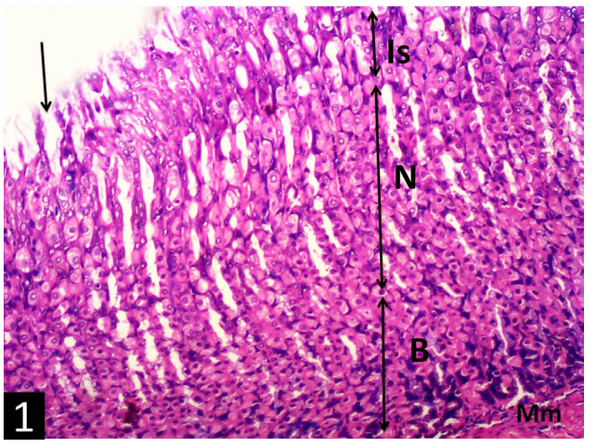


Fig. 1: A photomicrograph of a section in the fundic mucosa of a control rat showing numerous tightly packed parallel running tubular fundic glands lying perpendicular to the surface with short narrow pits (↑). The glands are divided into inner isthmus (Is), middle neck (N), and outer or deep base (B) regions occupying the lamina propria. Muscularis mucosa (Mm) can be seen. H&E X 100.

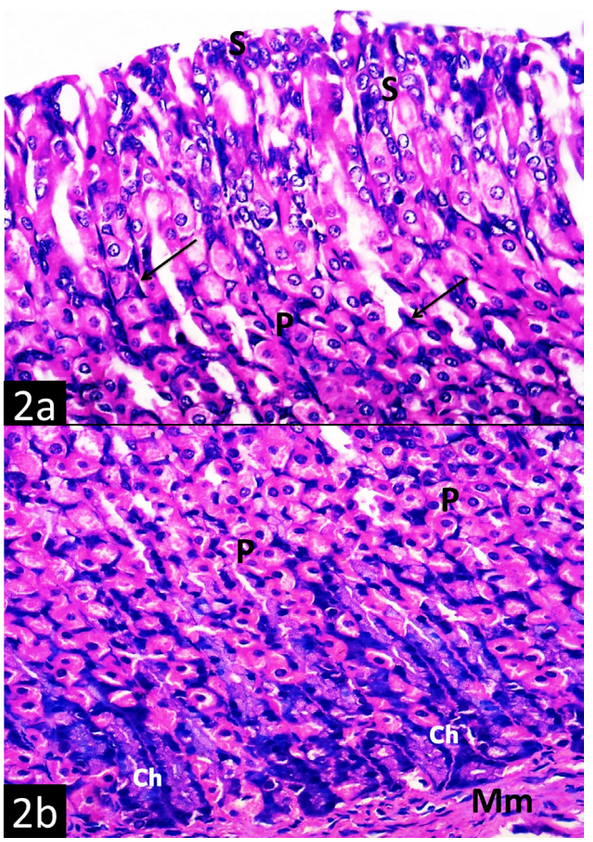


Fig. 2: A photomicrograph of a section in the fundic mucosa of a control rat showing (a) the upper part of the fundic glands formed of surface epithelial columnar mucous secreting cells (S) covering the surface and extending down into fundic pits and isthmus. The neck regions of the fundic glands show large polyhedral parietal cells (P) and mucous neck cells (↑).(b) the basal part of the fundic glands lined mainly by many chief cells (Ch) with basal nuclei, a basal basophilic cytoplasm, and an apical pale part. Parietal cells (P) can also be seen in the basal part. Note muscularis mucosa (Mm). H&E X 200.

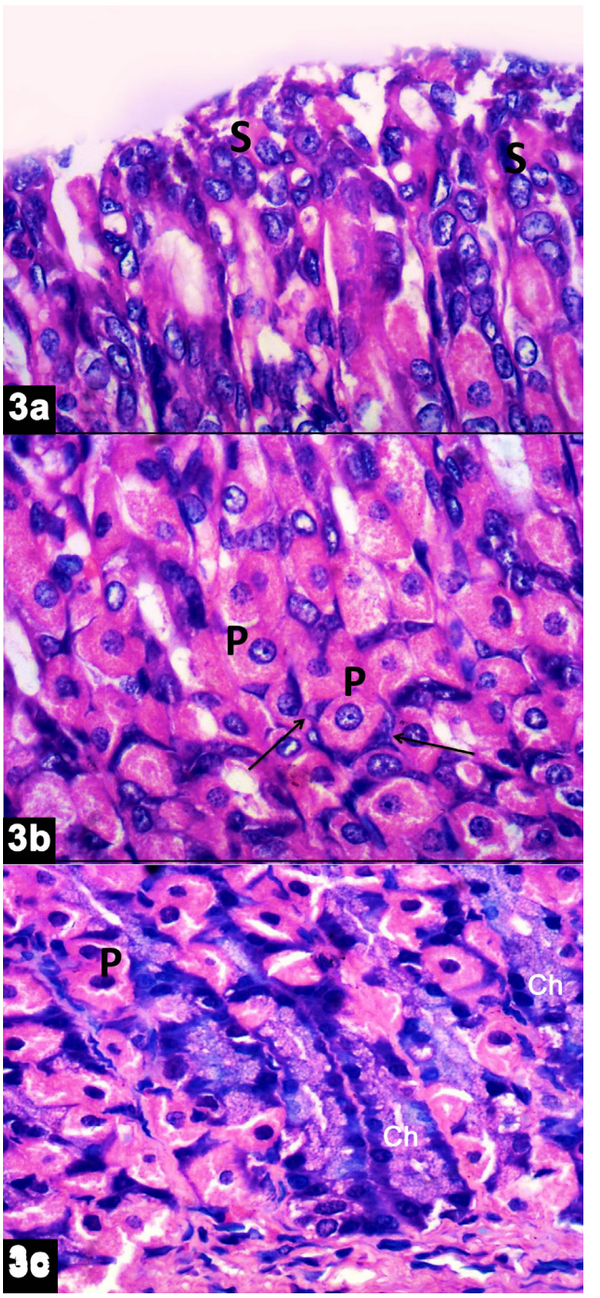


Fig. 3. A photomicrograph of a section in the fundic mucosa of a control rat showing (a) surface epithelial columnar mucous secreting cells (S) with basal oval vesicular nuclei and apical pale cytoplasm. (b) The large polyhedral parietal cells (P) with acidophilic cytoplasm and central rounded vesicular nuclei having prominent nucleoli and mucous neck cells (↑) with vacuolated cytoplasm and flattened basal nuclei. (c) at the base many chief cells (Ch) with basal basophilic cytoplasm, basal rounded vesicular nuclei, and an apical acidophilic or pale stained zymogen granules. Note parietal cells (P).. H&E X 400.

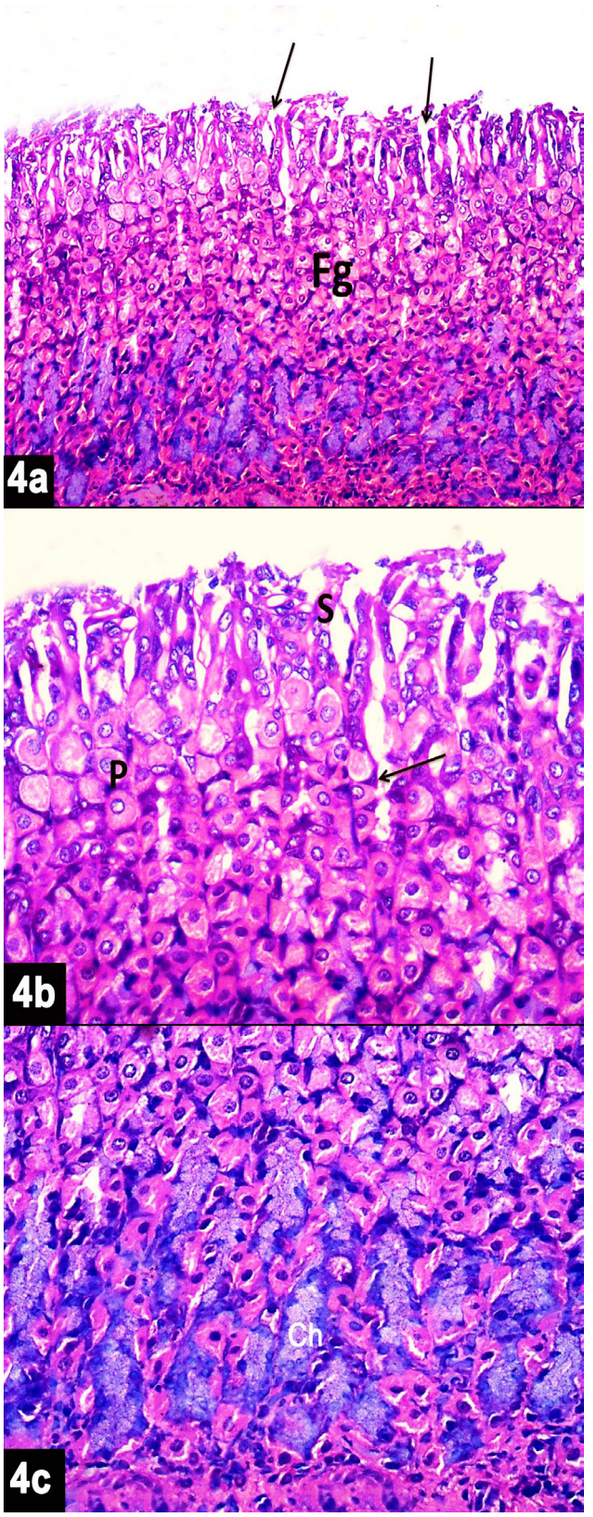


Fig. 4. A photomicrograph of a section in the fundic mucosa of fucoidan treated showing (a) tubular fundic glands (Fg) with short narrow pits (↑). H&E, × 100. (b) A higher magnification of (a) the normal surface mucous secreting cells (S), mucous neck cells (↑) and parietal cells (P). (c) the basal part of the fundic glands lined mainly by chief cells (Ch). H&E, × 200

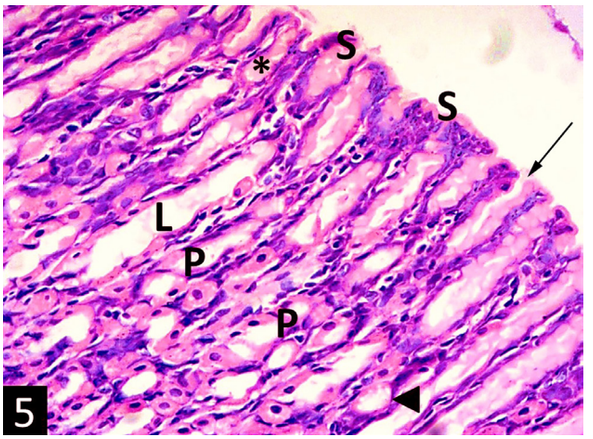


Fig. 5: A photomicrograph of a section in rat's fundic mucosa of sofosbuvir-treated group showing fundic glands with wide lumen (L) and fundic pits (arrow). Surface mucous cells (S) appear with little cytoplasm and pyknotic flattened nuclei (*). Most of parietal cells (P) have vacuolated cytoplasm and peripheral shrunken pyknotic nuclei, some cells appear with karyolitic nuclei (▲). H&E, × 200

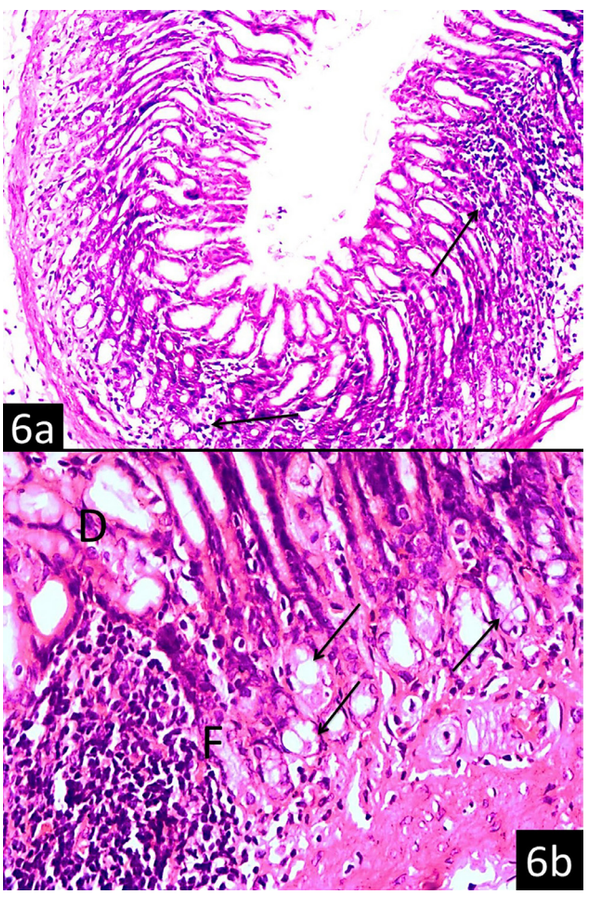


Fig. 6: A photomicrograph of a section in rat's fundic mucosa of sofosbuvir-treated group showing (a) diffuse mononuclear cellular infiltration (↑) in the lamina propria between the fundic glands. H&E, × 100. (b) showing aggregated inflammatory cells in the form of follicle (F) between glands which appear disrupted (D) especially at the bases with degenerated chief cells (↑). H&E, × 200.

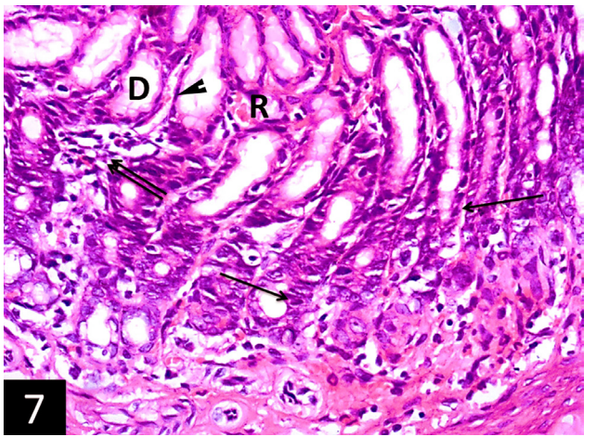


Fig. 7: A photomicrograph of a section in rat's fundic mucosa of sofosbuvir-treated group showing disorganization of the bases of the fundic glands; most of the chief cells show degeneration with deeply stained pyknotic nuclei (↑). Glandular cystic dilatation (D) with flattening of the epithelial lining is observed (arrow head). Inflammatory cells (↑↑) and extravasated RBCs (R) are seen between glands. H&E, × 200.

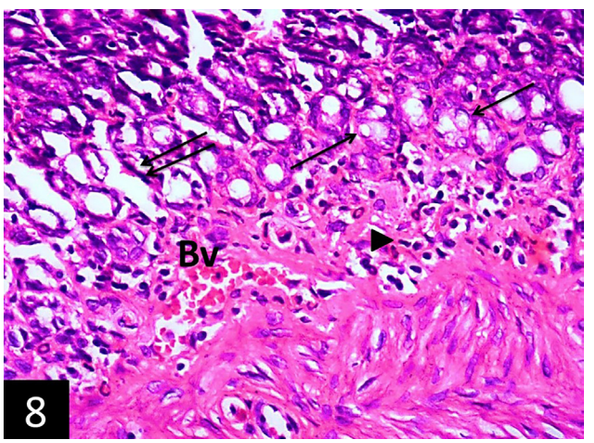


Fig. 8: A photomicrograph of a section in rat's fundic mucosa of sofosbuvir-treated group showing distorted shape of fundic glands. Necrotic chief cells with vacuolated cytoplasm and karyolytic nuclei are observed (↑). Other chief cells appear thin with flattened pyknotic nuclei (↑↑). Congested blood vessel (Bv) and some inflammatory cells (▲) are seen in the lamina propria at the base of the glands. H&E, × 200.

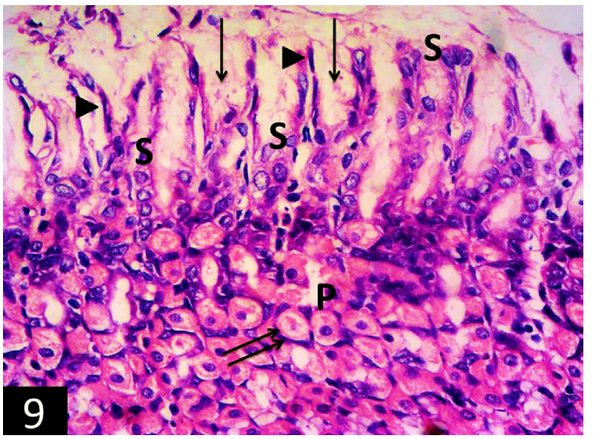


Fig. 9: A photomicrograph of a section in rat's fundic mucosa of sofosbuvir and fucoidan-treated group showing fundic glands with wide fundic pits (↑). Most of surface mucous secreting cells (S) appear normal with pale cytoplasm and basal oval vesicular nuclei. Only few cells appear with little cytoplasm and thin flattened pyknotic nuclei (▲). Most of parietal cells (P) appear with acidophilic cytoplasm and central rounded vesicular nuclei, few cells appear with vacuolated cytoplasm and shrunken pyknotic nuclei (↑↑). H&E, × 200.

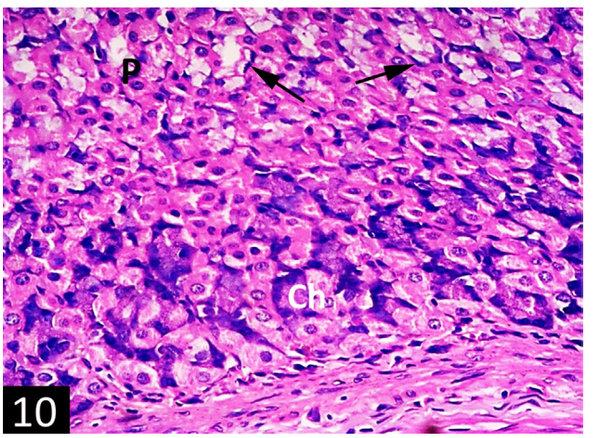


Fig. 10: A photomicrograph of a section in rat's fundic mucosa of sofosbuvir and fucoidan-treated group showing the neck and basal parts of the fundic glands. Mucous neck cells appear with flat basal nuclei and a pale foamy cytoplasm (↑). The basal columnar chief cells appear nearly normal with basal oval nuclei, a basal basophilic cytoplasm, and an apical pale part (Ch). Parietal cells (P) appear nearly normal. H&E, × 200.

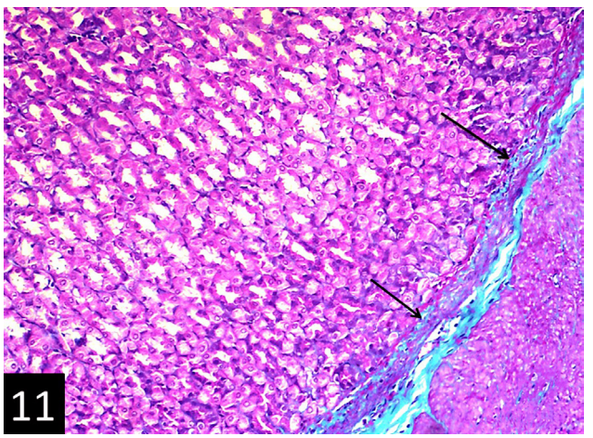


Fig. 11: A photomicrograph of a section in the fundic mucosa of a control rat showing minimal amount of collagen fibers (↑) in the lamina propria between the basal parts of fundic glands. Masson's trichrome, × 100

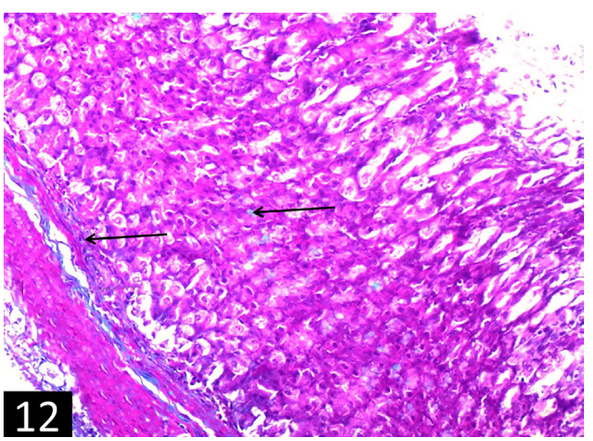


Fig. 12: A photomicrograph of a section in the fundic mucosa of Fucoidan treated group showing minimal amount of collagen fibers (↑) in the lamina propria . Masson's trichrome, × 100

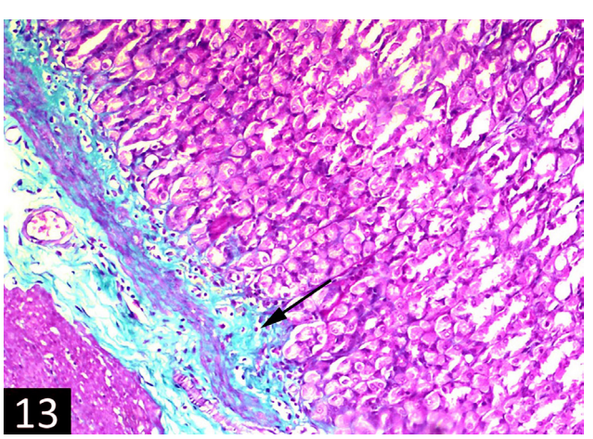


Fig. 13: A photomicrograph of a section in rat's fundic mucosa of sofosbuvir-treated group showing excessive amount of collagen fibers in the lamina propria at the bases of the fundic glands (↑). Masson's trichrome, × 100

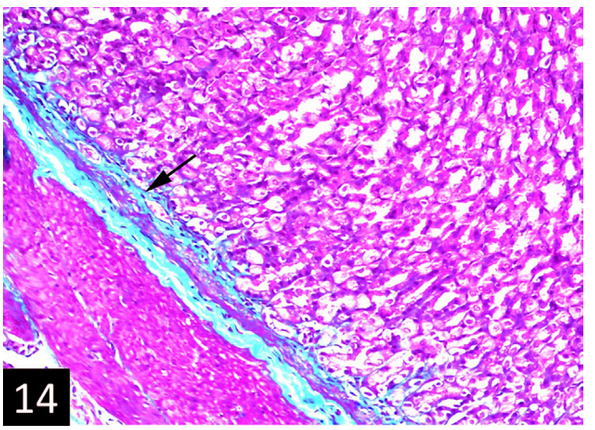


Fig. 14: A photomicrograph of a section in rat's fundic mucosa of sofosbuvir and fucoidan-treated group showing moderate amount of collagen fibers in the lamina propria at the bases of the fundic glands (↑). Masson's trichrome, × 100

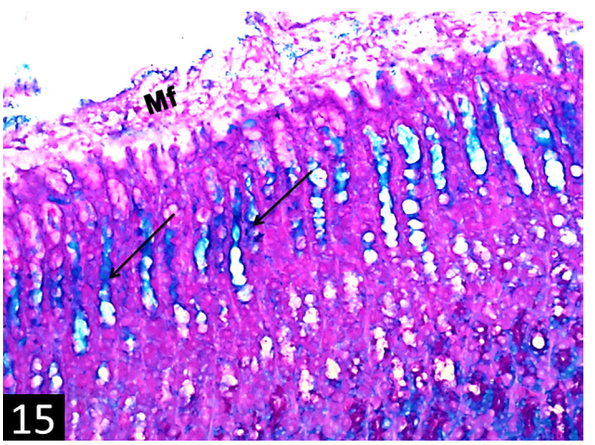


Fig. 15: A photomicrograph of a section in the fundic mucosa of a control rat showing PAS–AB-positive thick mucous film (Mf) over the surface epithelium extending to fill the fundic pits. Strong AB-positive staining of mucous neck cells is observed (↑). PAS–AB, ×100.

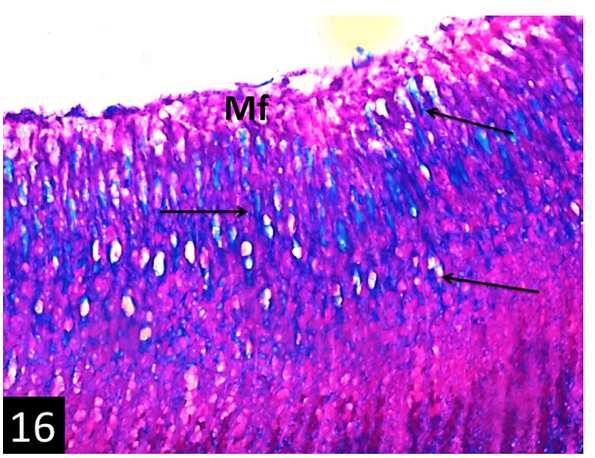


Fig. 16: A photomicrograph of a section in the fundic mucosa of fuocidan treated group showing PAS–AB-positive thick mucous film (Mf) over the surface epithelium extending to fill the fundic pits. Very strong AB-positive reaction is observed at the pit regions, isthmus and necks of the glands (↑). PAS–AB, ×100.

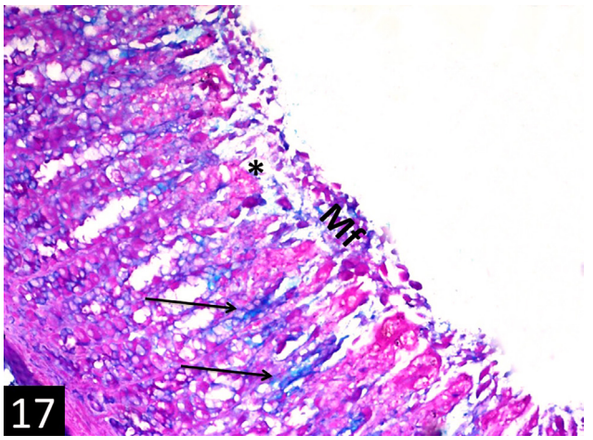


Fig. 17: A photomicrograph of a section in the fundic mucosa of sofosbuvir-treated group showing very thin, faint and interrupted PAS–AB positive reaction of the surface mucous film (Mf). Note the weak AB-reaction in mucous neck cells (↑) and the weak PAS-reaction in most of surface columnar mucous cells (*). PAS–AB, ×100.

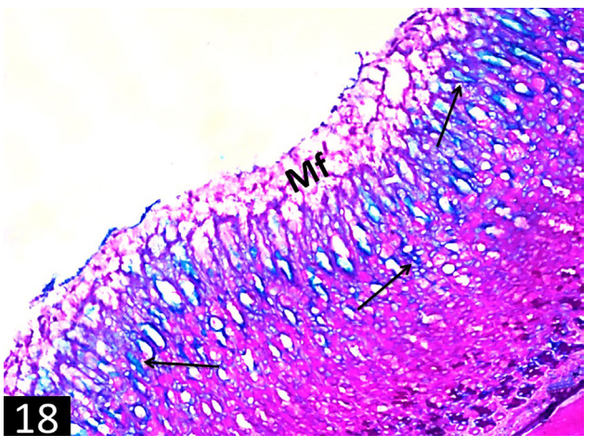


Fig. 18: A photomicrograph of a section in the fundic mucosa of sofosbuvir and fucoidan-treated group showing a continuous thick PAS-AB positive mucous film (Mf) over the surface epithelium extending to fill the fundic pits. Moderate AB-positive reaction is observed at the pit regions, isthmus and necks of the glands (↑). PAS–AB, ×100.

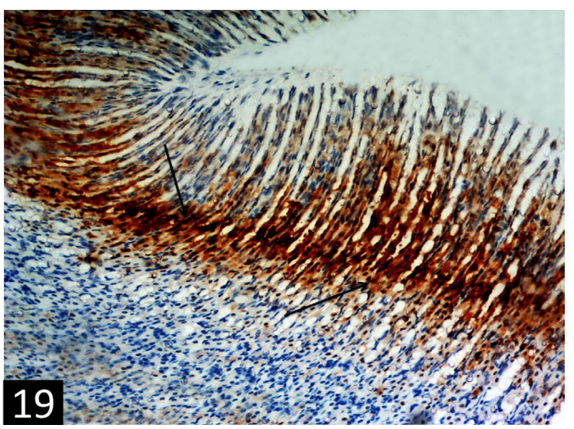


Fig. 19: A photomicrograph of a section in the fundic mucosa of a control rat showing strong positive reaction for proliferating cell nuclear antigen (PCNA) in the cells of the isthmus region of the fundic gland (↑). PCNA, × 100

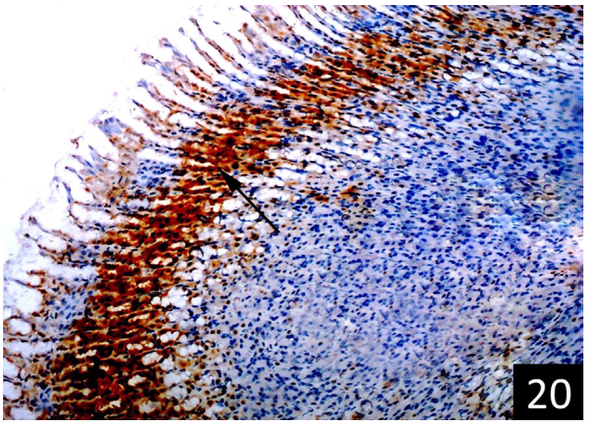


Fig. 20: A photomicrograph of a section in the fundic mucosa of fucoidan treated group showing strong positive reaction for proliferating cell nuclear antigen (PCNA) in the cells of the isthmus region of the fundic glands (↑). PCNA, × 100

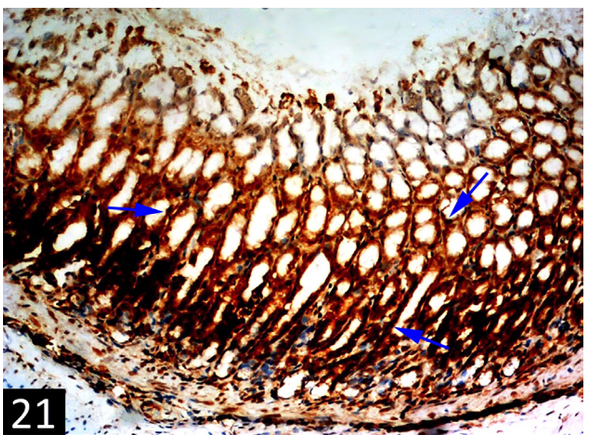


Fig. 21: A photomicrograph of a section in the fundic mucosa of sofosbuvir-treated group showing strong positive reaction for proliferating cell nuclear antigen (PCNA) in the cells lining the whole fundic glands (↑). PCNA, × 100

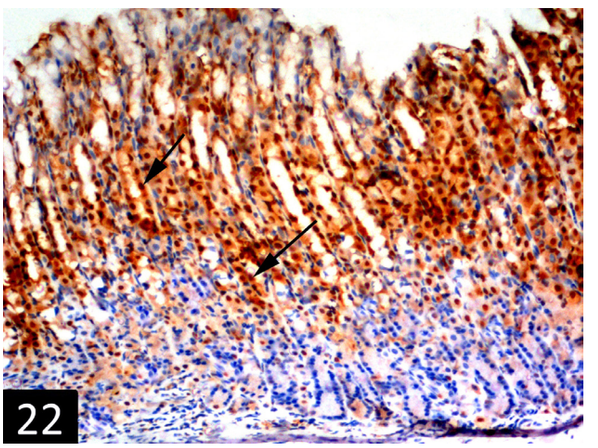


Fig. 22: A photomicrograph of a section in the fundic mucosa of sofosbuvir and fucoidan-treated group showing strong positive reaction for proliferating cell nuclear antigen (PCNA) in the cells of the isthmus and neck regions of the fundic glands (↑). PCNA, × 100

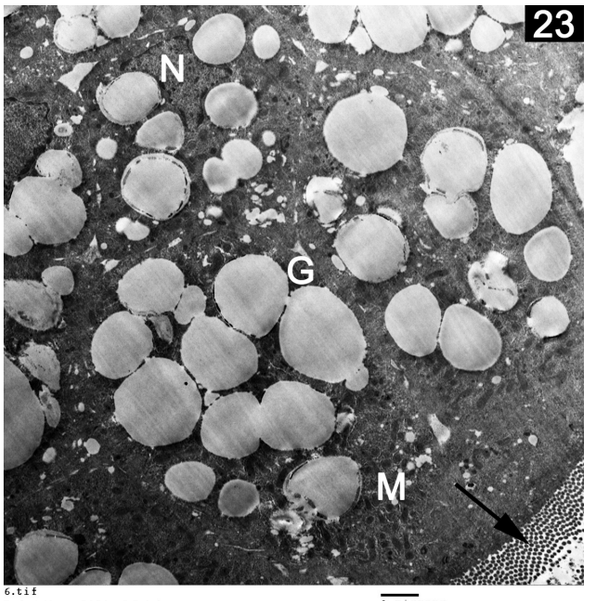


Fig. 23: An electron micrograph from the control group showing surface mucous cells with many rounded electron-lucent mucous granules (G) and apical microvilli (↑). Euchromatic nuclei (N) and mitochondria (M) are observed. Mag X 1000

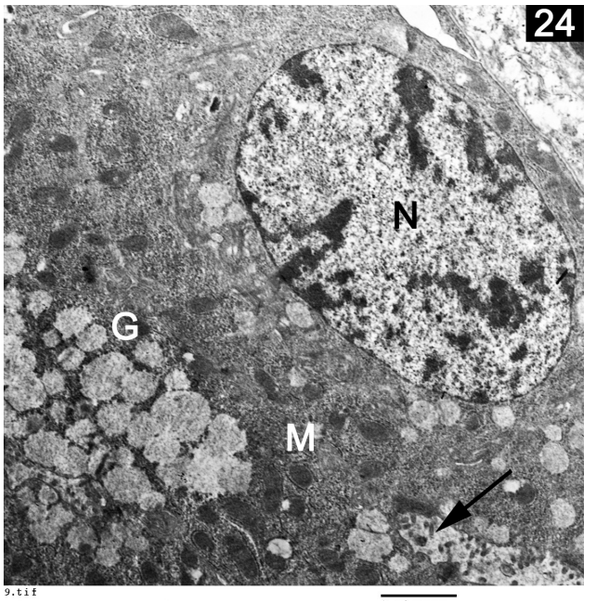


Fig. 24: An electron micrograph from the control group showing mucous neck cell with basal oval euchromatic nucleus (N), apical microvilli (↑) and many apical mucous granules (G). Mitochondria (M) are observed. Mag X 2000

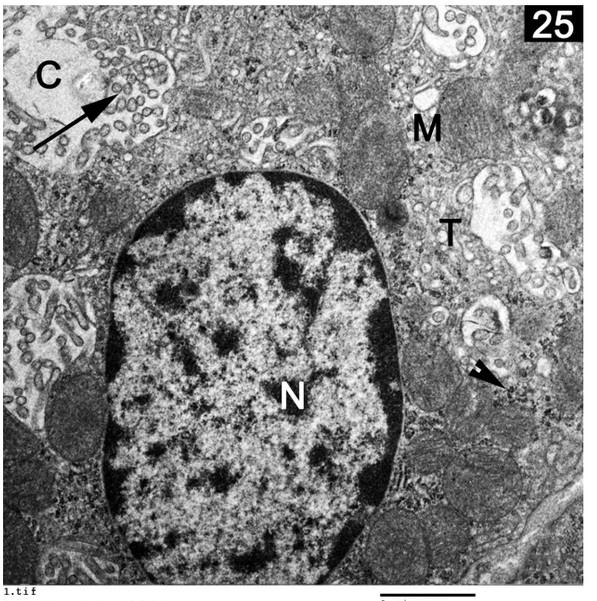


Fig. 25: An electron micrograph from the control group showing parietal cell with an euchromatic nucleus (N) having regular distribution of chromatin. The cytoplasm shows intracellular canaliculi (C) lined with microvilli (↑), numerous rounded to oval mitochondria (M) with intact regular cristae, ribosomes (arrow head) and tubulovesicular structures (T). Mag. X 2500.

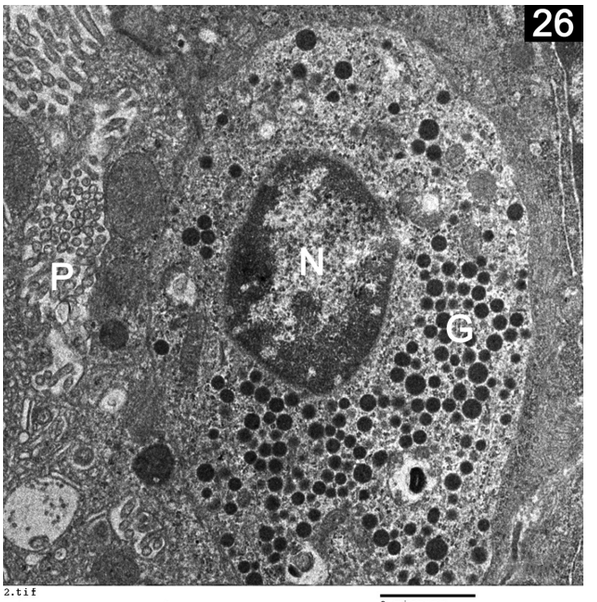


Fig. 26: An electron micrograph from the control group showing an enteroendocrine cell with an euchromatic nucleus (N) and numerous basal small electron-dense secretory granules (G). Part of parietal cell (P) is also seen. Mag. X 2500.

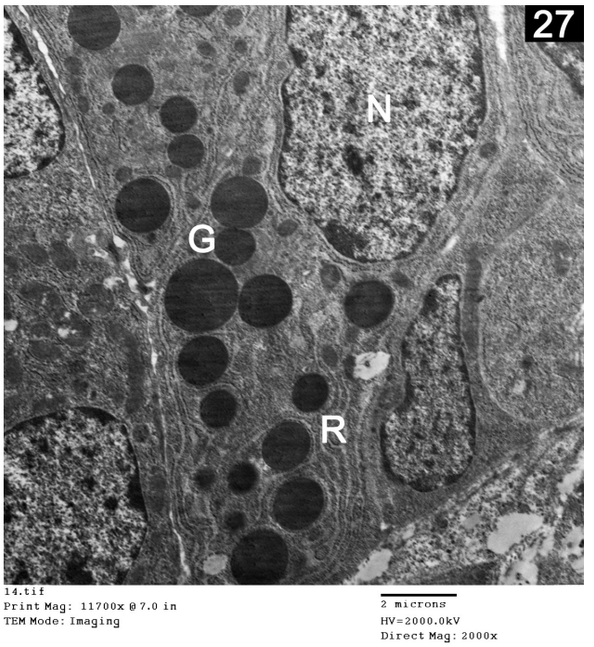


Fig. 27: An electron micrograph from the control group showing a chief cell with euchromatic nuclei (N), many apical electron dense secretory granules (G), and well-developed rough endoplasmic reticulum (R). Mag. X 2000.

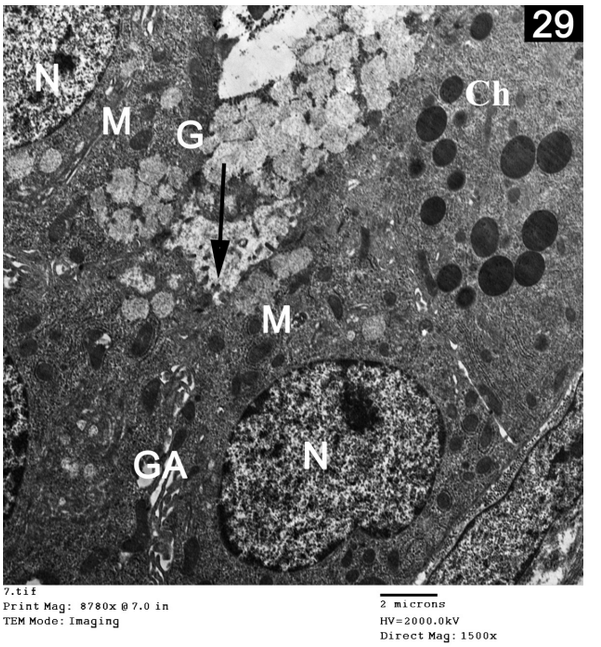


Fig. 29: An electron micrograph from the fucoidan treated group showing mucous neck cells with basal oval euchromatic nuclei (N) and, apical microvilli (↑) and many apical mucous granules (G). Golgi apparatus (GA) and mitochondria (M) are observed. Notice: a part of a chief cell (Ch) with its secretory granules. Mag X 1500

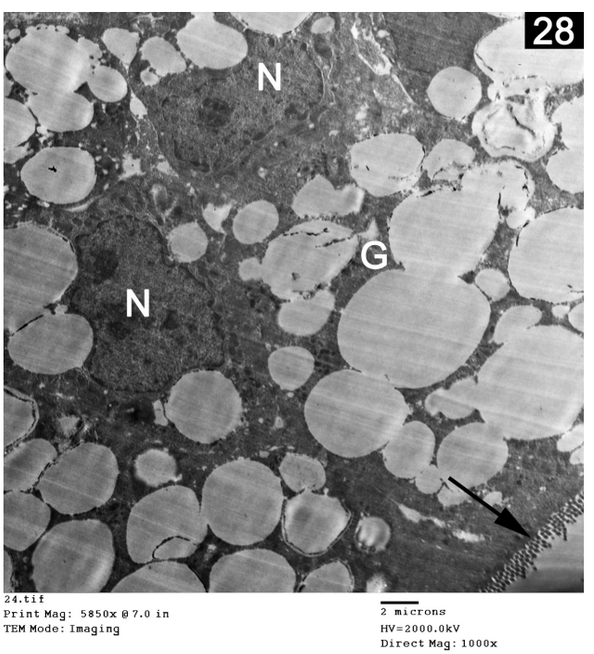


Fig. 28: An electron micrograph from the fucoidan treated group showing surface mucous cells with more numerous large rounded electron-lucent mucous granules (G) and normal apical microvilli (↑) within the gastric lumen. Euchromatic nuclei (N) with prominent nucleoli are observed. Mag X 1000

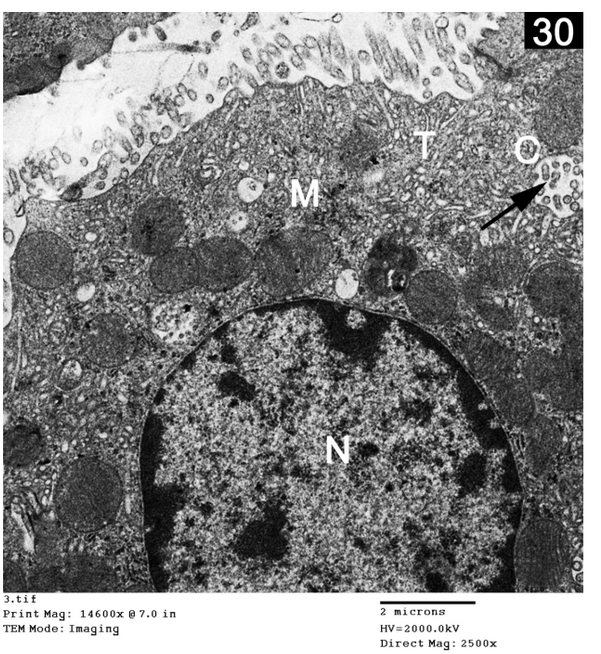


Fig. 30: An electron photomicrograph from fucoidan treated group showing a parietal cell with rounded euchromatic nucleus (N). The cytoplasm shows intracellular canaliculi (C) with microvilli (↑), numerous normal mitochondria (M) and multiple tubulovesicular structures (T). Mag X 2500.

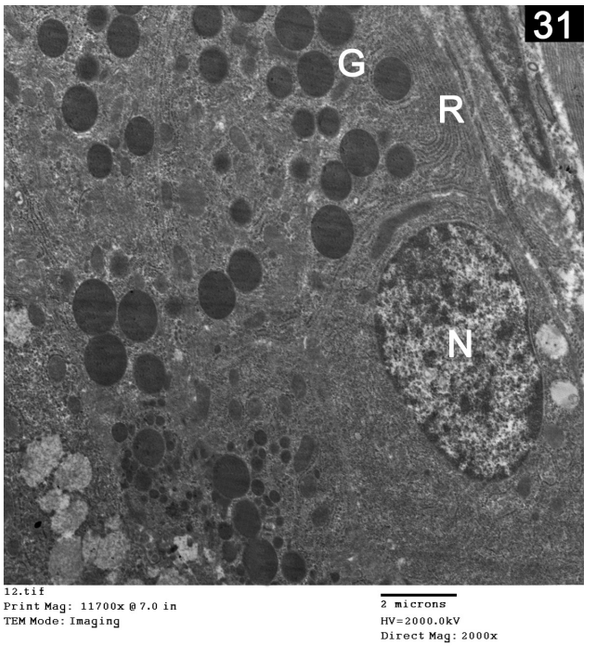


Fig. 31: An electron micrograph from the fucoidan treated group showing a chief cell with euchromatic nuclei (N), many apical electron dense secretory granules (G), and well-developed rough endoplasmic reticulum (R). Mag. X. 2000

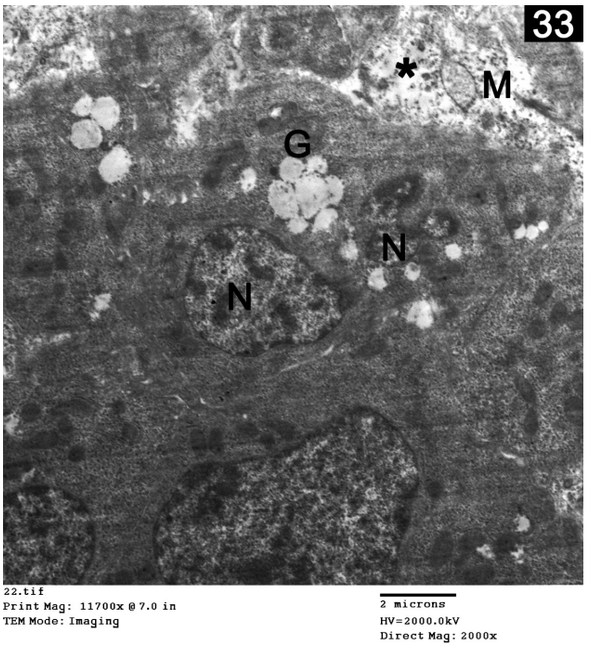


Fig. 33: An electron micrograph of the sofosbuvir-treated group showing mucous neck cells with shrunken irregular heterochromatic nuclei (N) and few electron-lucent mucous granules (G). Disrupted mitochondria (M) and areas of cytoplasmic loss (*) are observed. Mag. X 2000

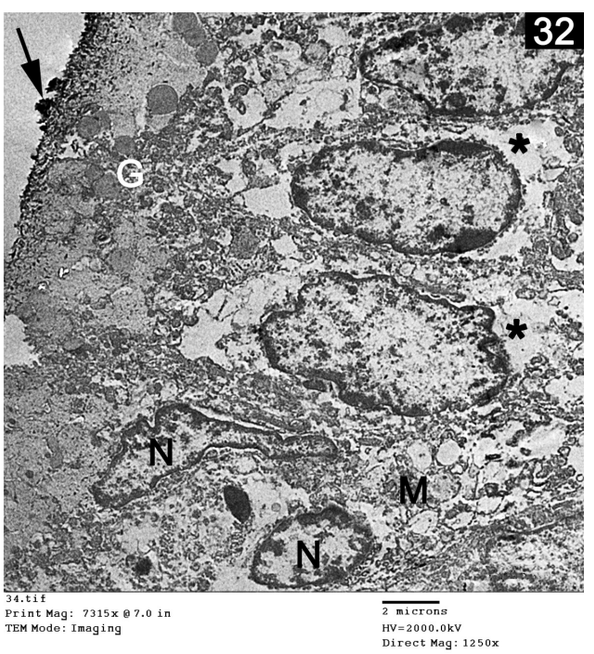


Fig. 32: An electron micrograph of the sofosbuvir-treated group showing surface mucous cells with few sparse apical microvilli (↑),few apical mucous granules (G), areas of cytoplasmic loss (*). Some nuclei appear shrunken and indented (N). Disrupted mitochondria (M) are observed. Mag. X 1250

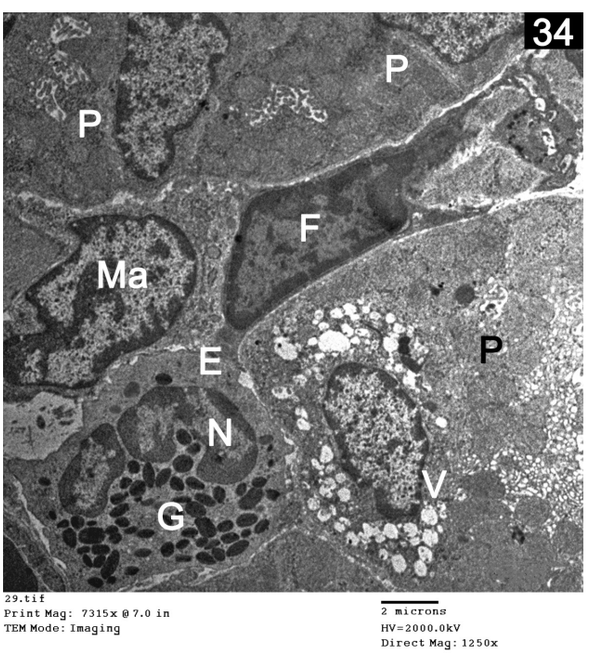


Fig. 34: An electron micrograph of the sofosbuvir-treated group showing multiple parietal cells (P) with irregular heterochromatic nuclei. One parietal cell shows many cytoplasmic vacuoles (V). Fibroblast (F), macrophage (Ma) and eosinophil (E) are observed. The eosinophil has bilobed nucleus (N) and characteristic eosinophil granules (G) . (Mag. X 1250)

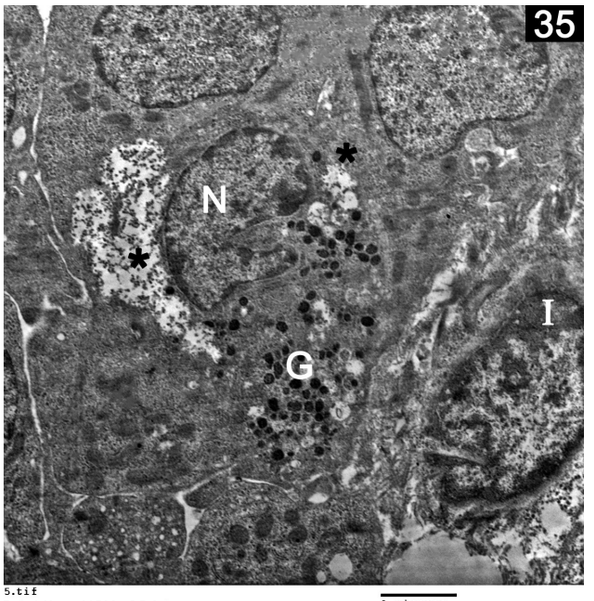


Fig. 35: An electron micrograph of the sofosbuvir-treated group showing an enteroendocrine cell with irregular shrunken heterochromatic nucleus (N), areas of cytoplasmic loss (*) and few basal small secretory granules (G) of variable density. An inflammatory cell (I) is observed. (Mag. X 2000)

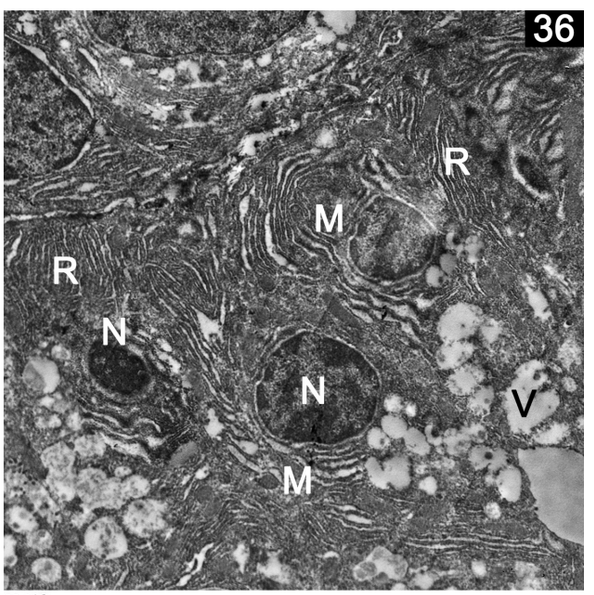


Fig. 36: An electron micrograph of the sofosbuvir-treated group showing multiple chief cells having shrunken electron-dense nuclei with condensed heterochromatin (N), dilated cisternae of rough endoplasmic reticulum (R), cytoplasmic vacuoles (V) and disrupted mitochondria (M). Loss of secretory granules is observed. (Mag. X 1250)

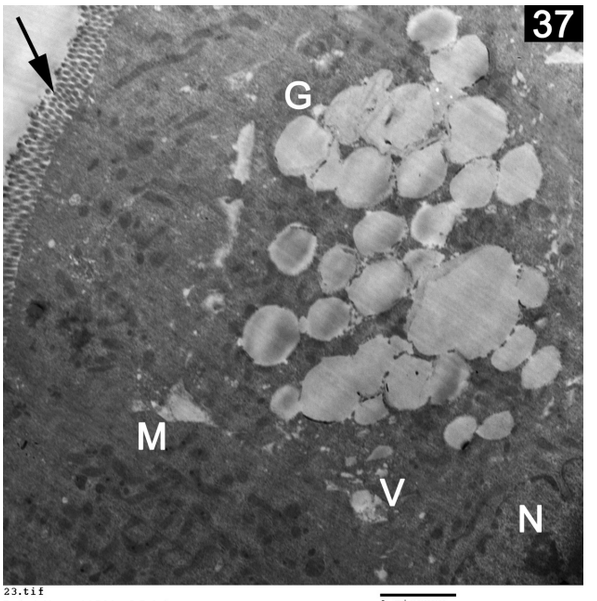


Fig. 37: An electron micrograph from the sofosbuvir and fucoidan treated group showing apparently normal surface mucous cells with mucous granules (G) and apical microvilli (↑). Euchromatic nucleus (N) with prominent nucleolus and mitochondria (M) are observed. Few vacuoles (V) are seen. (Mag. X 2000)

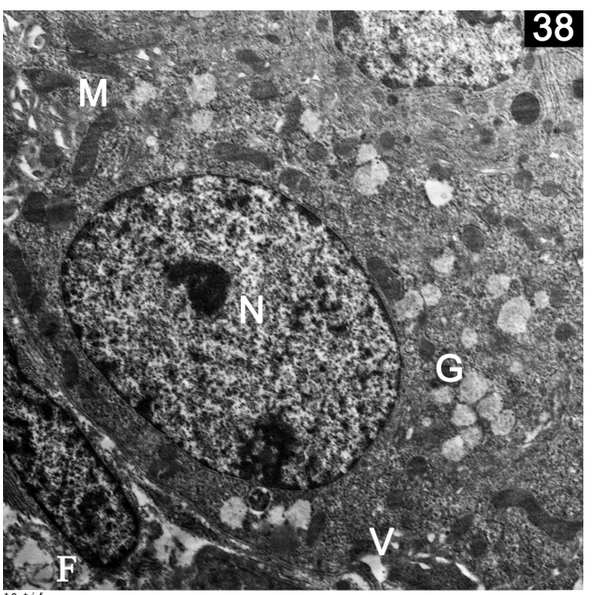


Fig. 38: An electron micrograph from the sofosbuvir and fucoidan treated group showing mucous neck cell with basal oval euchromatic nucleus (N) and some mucous granules (G). Mitochondria (M) and few vacuoles (V) are observed. Notice: fibroblast (F) in underlying corium. Mag X 2000

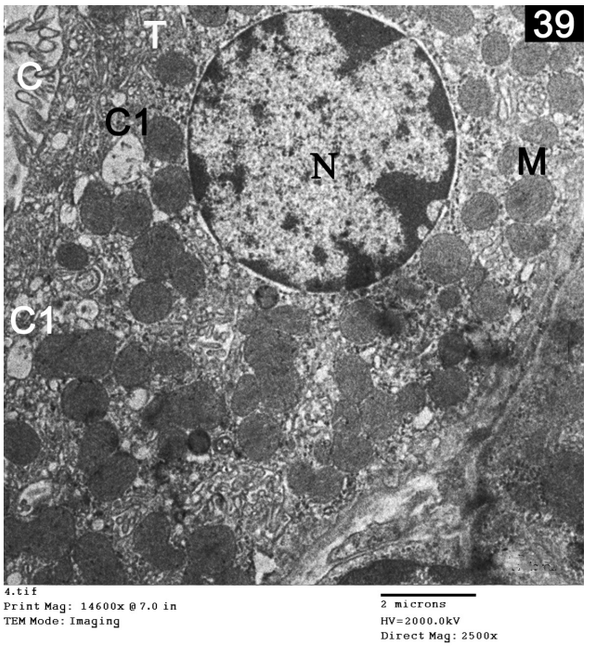


Fig. 39: An electron micrograph from the sofosbuvir and fucoidan treated group showing apparently normal parietal cell with rounded euchromatic nucleus (N). The cytoplasm shows intracellular canaliculi (C) with microvilli, few canaliculi (C1) appear with deficient microvilli, numerous mitochondria (M) and tubulovesicular structures (T). Mag X 2500

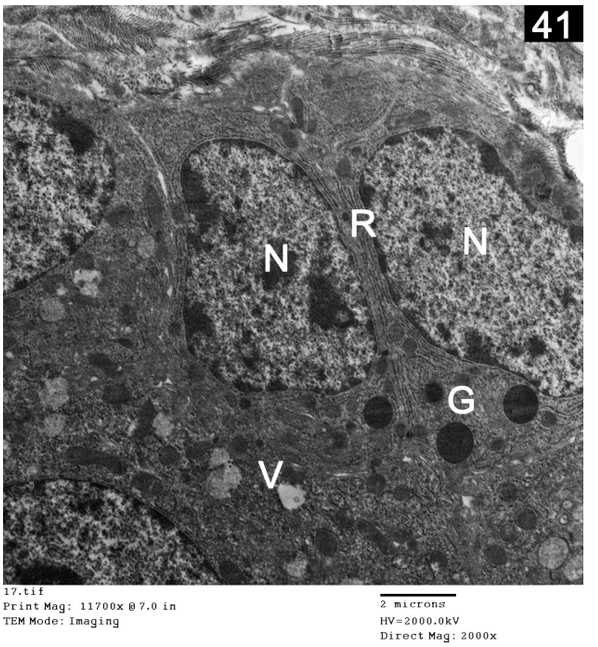


Fig. 41: An electron micrograph from the sofosbuvir and fucoidan treated group showing chief cells with some electron-dense secretory granules (G), rough endoplasmic reticulum (R), and euchromatic nuclei (N). Few vacuoles (V) are seen. Mag X 2000

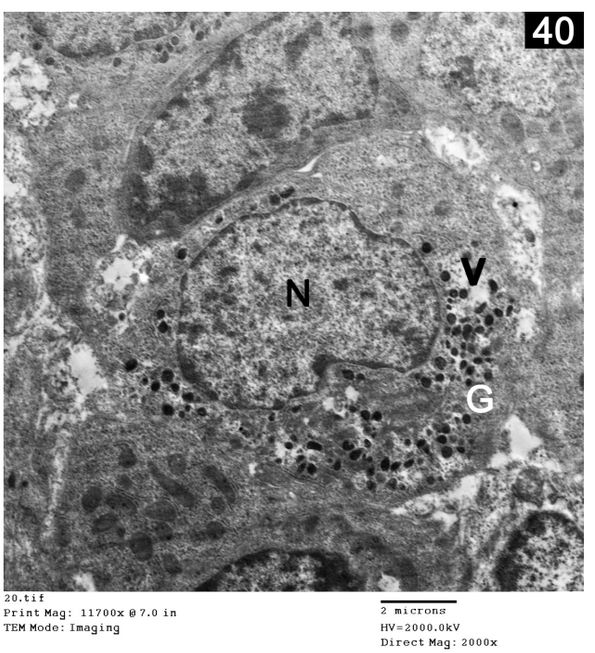
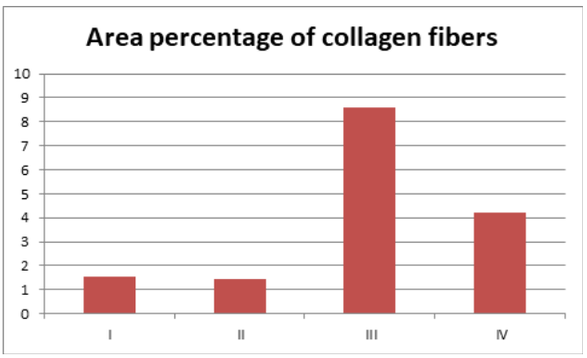


Fig. 40: An electron micrograph from the sofosbuvir and fucoidan treated group showing an enteroendocrine cell with slightly irregular euchromatic nucleus (N) and basal secretory granules (G). Few cytoplasmic vacuoles are observed (V). Mag X 2000

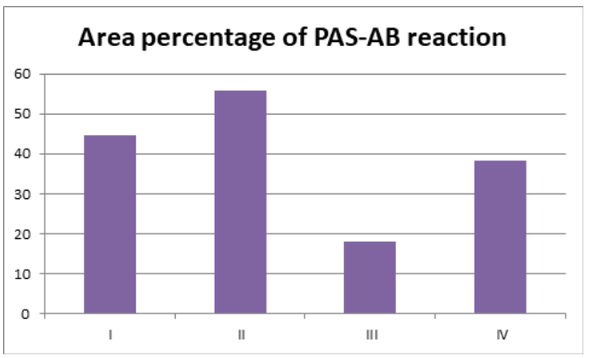
Table 1: The area% of collagen fibers and PAS-AB reaction in the control and experimental groups

	Group I	Group II	Group III	Group IV	*P.value*
area% of collagen fibers Mean ± SD	1.53±0.41	1.44±0.43	8.58±1.03	4.21±0.73	$P1>0.05)^{*}$ $(P2<0.001)^{***}$ $(P3<0.001)^{***}$
area% of PAS-AB reaction Mean ± SD	44.75±5.55	55.67±6.29	17.92±3.71	38.42±4.44	$(P1<0.05)^{**}$ $(P2<0.001)^{***}$ $(P3<0.001)^{***}$

P1: Group I V Group II P2:Group I V Group III P3: Group III V Group IV
Non-significant * (P> 0.05) Significant** (P<0.05) Highly significant*** (P<0.001)



Histogram 1: The mean area% of collagen fibers in the control and experimental groups.



Histogram 2: The mean area% of PAS-AB reaction in the control and experimental groups.

DISCUSSION

Sofosbuvir (SOF) is a new drug candidate for hepatitis C treatment, with hopeful outcomes in many in-*vitro* experiments against all the genotypes of HCV. This drug has proved great efficiency in conjunction with multiple other drugs against HCV[21]. Limited data is obtainable about the effect of sofosbuvir on the stomach. Therefore, this work was designed to explore the possible effect of sofosbuvir on the fundus mucosa of the rats and to assess the potential role of protection of fucoidan histologically and immunohistochemically.

Fucoidan, a nutritional substance, forms a category of fructose-containing sulfated polysaccharides present in the brown algae. Fucoidans have anti-coagulant, anti-inflammatory, anti-angiogenic, and antiadhesive effects[9]. Thus, considerable attention has been directed to estimate the potential protective effect of fucoidan on SOF-induced changes in fundic mucosa.

In our work, the microscopic examination of fucoidan group showed no difference in comparison with control group except for increase in mucous production. This was supported by Sinurat and dan Rosmawaty[11] who added that fucoidan didn't induce any adverse effects in rats with the standard doses.

The increase in mucous production of fucoidan group was evident by significant rise in PAS-AB reaction. Such finding was confirmed by examining the ultrathin sections of this group in which the cytoplasm of most of the surface mucous cells were full with large rounded mucous granules. This could be explained by ability of fucoidan to increase the mucus secretion of gastric glands[11]. This effect is gained through the cytoprotective effect of fucoidan and its capability to raise growth factor production of epithelial cells of the gastric mucosa and regulate gut homeostasis[22]. In a similar way, the epithelial cell regeneration will be enhanced by the presence of growth factors, so that better mucus production by new epithelial cells will be obtained[23].

The gastric mucus is glycoprotein which presents two main functions: food greasing to ease motion in the stomach and acting as a protective cover above the epithelium that line the stomach cavity. This mucous cover is a defense way that prevents digestion of stomach by its enzymes[11].

Both light and electron microscopic histological findings of the fundic mucosa of sofosbuvir treated group revealed many histopathological changes. Surface mucous cells with little cytoplasm and pyknotic thin nuclei, vacuolated parietal cells with shrunken pyknotic nuclei and degenerated chief cells with dilated RER were detected. Also, degenerated enteroendocrine cells with heterochromatic nuclei, cystic dilatation of fundic glands with flattening of their epithelial lining, diffuse inflammatory cell infiltration and congested vessels were seen. These findings were in agreement with the results of Salem *et al*.,[24] in their study on the effect of sofosbuvir on the rat submandibular salivary gland. They attributed these changes to the great affinity of sofosbuvir as a nucleotide analogue for polymerases of mitochondria leading to mitochondrial injury and dying of cell. They added that damaged mitochondria cause oxidative stress by secreting higher levels of reactive oxygen species (ROS) which in

turn induces cellular damage. Ultrastructural picture of this group confirmed this mitochondrial damage in many cells of fundus.

Recent studies stated that mitochondrial reactive oxygen species (mROS) act as signaling molecules to stimulate proinflammatory cytokine release through NFκB signaling activation[25]. The results of Salem *et al*.,[24] confirmed this by detecting strong immunoreaction for NF-κBa in the submandibular salivary gland of the sofosbuvir treated rats. Our results were in harmony with such explanation due to the presence of different signs of inflammation manifested as mononuclear cell infiltration and congested dilated blood vessels in the mucosa of SOF-treated group. Moreover, the inducible nitric oxide synthase (iNOS) is activated by NFκB leading to the production of great amount of ROS in form of nitric oxide (NO). The cytotoxic compound peroxy-nitrite is produced by combination of NO with superoxide anions & hence leading to lipid peroxidation and interstitial fibrosis[26,27].

The dilated fundic glands that were detected in G III in the current study could be due to the degeneration of cells lining these fundic glands giving the appearance of wide lumen[28].

In our study, the masson's trichrome-stained sections of the sovaldi treated group showed a highly significant increase in the collagen content in comparison with the control group. This could be explained by many growth factors released by the inflammatory cells that reached the affected area in the lamina propria of the fundic mucosa. The basic fibroblastic growth factor is one of these growth factors, which stimulates fibroblast to proliferate and to deposit collagen in the area of inflammation[7].

The histochemical results of the current work revealed that sofosbuvir suppressed the mucous production as manifested by thin, faint and interrupted PAS-AB positive mucous film with weak PAS-reaction in the surface mucous cells and weak AB-reaction in mucous neck cells, this was confirmed by the statistical analysis that revealed a highly significant decrease in the area percentage of PAS-AB- positive reaction in the SOF-treated group compared to control. The mucous production disorders could be explained by the oxidative stress and the development of inflammation as has been previously reported by Suzuki *et al*.,[29]. Also, Mohamed A,[30] attributed the decrease in the mucous production to decreased prostaglandin release and the harm of the mucus cells. Such explanation was supported by ultrastructural picture of the mucous cells which revealed areas of cytoplasmic loss, shrunken & indented nucleus and reduction in the mucous granules as compared to control.

The sofosbuvir treated group revealed increased reaction for PCNA in the cells lining the whole fundic glands. PCNA is used as a marker of cell proliferation[31] and the increase in this proliferative marker may indicate the presence of stem cells that proliferate and differentiate to allow for the regeneration of damaged epithelial and mucus-producing cells thus permitting the healing of the degenerated areas of the gastric mucosa[32]. This was in agreement with the results of Issa and El-Sherif,[33] who detected increase in the proliferative marker ki67 in the cerebral cortex of sovaldi treated rats and attributed this to the stimulation of progenitor cells to proliferate in order to restore cell populations that were lost by injury caused by sovaldi.

Another explanation for increased PCNA reaction in the cells was mentioned by some investigators[31] who stated that the increase of PCNA expression could be induced as a response to damaged DNA even after the cell is no longer active in the cell cycle. They also added that PCNA is involved in the excision and replacement of abnormal nucleotides and is thus also expressed in non-proliferating cells undergoing DNA repair.

In our study, the administration of fucoidan with sofosbuvir highly protect the mucosa against histological, histochemical and immunohistochemical changes except for wide gastric pits, few surface mucous cells with thin flattened pyknotic nuclei, and few cytoplasmic vacuoles in some fundic cells. That may indicate that fucoidan has protective effect against sofosbuvir induced mucosal changes. This finding was in consistence with some studies[34] which reported that orally fucoidan is efficient in the treatment and prevention of stomach ulcer in experimental animals. The protective effect of fucoidan could be explained by its ability to increase the mucus secretion of gastric mucosa with no side effects as has been previously discussed in our study. In addition, the protective effect of fucoidan could be referred to its anti-inflammatory and anti-oxidant properties. This could be confirmed by other reports[35] which found that fucoidan could protect against gastric ulceration by preventing pro-inflammatory cytokines release. Also, Wang *et al*[36] have demonstrated that the administration of fucoidan ameliorated chronic colitis via decreasing the release of interleukin-6 in the cells. Also, El-Naggar and Hussein[37] reported that fucoidan protected and attenuated the side effects of NSAIDs by scavenging the oxidative stress and decreasing proinflammatory cytokines. Moreover, Che *et al*,[14] detected that, fucoidan altered the amounts of inflammation-related cytokines, apoptosis-correlating proteins and oxidative stress-associated protein and hence can significantly relieve the inflammatory and oxidative stress responses.

In group IV in this study, masson's trichrome-stained sections showed moderate amount of collagen fibers in the lamina propria and this was in harmony with Charboneau *et al*.,[38]. Van Weelden *et al*.,[12] added that fucoidan has cytoprotective effects on the fibroblast which explained the reducing amount of collagen fiber in group IV. This result was confirmed statically by highly significant decrease of collagen amount of this group when compared with the Sofosbuvir treated group.

As regarding PAS-AB staining in group IV, there was continuous thick mucous layer above the surface epithelium and this was in accordance with Sinurat and dan Rosmawaty,[11] who explained that by its sulphate group. This finding was confirmed statically by highly significant increase of PAS-AB intensity when compared with the sofosbuvir treated group.

PCNA immunohistochemical staining in group IV, revealed decreased reaction in comparison with group III. This was in accordance with You *et al*.,[10] who explained this by the role of fucoidan in reducing mucosal cells damage and crypt destruction. Wang *et al*[36] added that fucoidan plays a role in increasing the tight junction protein claudin-1 leading to the protection of epithelial barrier from damage by oxidative stress and decreasing proinflammatory cytokine levels in mucosal epithelium.

Ultrastructural results of group IV showed improved histological results as compared to group III, but few cytoplasmic vacuolation were still seen. This was in agreement with Wang *et al*., 2019[36] who explained this with enhancement effects of fucoidan on immunity.

CONCLUSION

From the present study, sofosbuvir has been proved to induce obvious histological changes in the fundic mucosa. So, hepatitis C patients treated with sofosbuvir should take care of their stomach. Fucoidan can attenuate these changes when given with it. Fucoidan is a promising protective agent for patients who receive sofosbuvir as antihepatitic therapy.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تاثيرالسوفوسبوفير(السوفالدي) على الغشاء المخاطى لقعر المعدة فى ذكور الفئران البيضاء البالغة و الدور الوقائي المحتمل للفوكويدان: (دراسة هستولوجية، هستوكيميائية و هستوكيميائية مناعية)

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الخلفية: سوفوسبوفير هو علاج واعد لالتهاب الكبد الوبائي المزمن بعدوى فيروس سي. ومع ذلك ، فإن له بعض الآثار السلبية على الجهاز الهضمي. الفوكويدان هو عديد السكاريد الكبريتي يعمل على الحمايه الخلويه للغشاء المخاطي في المعدة إلى جانب خصائصه المضادة للالتهاب و الأكسدة.

الهدف: ركز هذا العمل علي التأثير المحتمل للسوفوسبوفير على الغشاء المخاطي لقعرالمعدة وتقييم الدور الوقائي المحتمل للفوكويدان.

مواد وطرق البحث: تم تقسيم أربعين من ذكور الفئران البيضاء البالغة إلى أربع مجموعات متساوية و تم تلقيهم الأدوية كجرعة يومية واحدة لمدة خمسة أسابيع. المجموعة الضابطة (الاولي): تلقت الماء المقطر و محلول الملح. مجموعة الفوكويدان (الثانية): تلقت جرعة ٨٠ مجم/كجم. مجموعة السوفوسبوفير (الثالثة): تلقت جرعة ٤ مجم/كجم. المجموعة الرابعة: تلقت الفوكويدان و السوفوسبوفير بنفس الجرعات كالمجموعات السابقة. تم ذبح جميع الفئران بعد يوم واحد من آخر جرعة. تم الحصول على عينات من قعر المعدة وتم تحضيرها من أجل الفحص بالمجهر الضوئى والإلكتروني.

النتائج: أظهرت المجموعة المعالجة بالسوفوسبوفير حفر معدية متسعة و إتساع حويصلى للغدد القاعية مع تسطيح بطانتهم الطلائية. أظهرت الخلايا الجداريه فجوات سيتوبلازمية و انوية منكمشة داكنة. أظهرت الخلايا الرئيسية اتساع فى الشبكة االاندوبلازمية الخشنة و انتقاص فى الحبيبات الافرازية.كما يوجد تسلل خلوى إلتهابى منتشر ، أوعية دموية محتقنة ، و خلايا دم حمراء خارج الأوعية الدموية. كانت هناك زيادة كبيرة ذات دلالة إحصائيه فى النسبه المئويه لمساحة ألياف الكولاجين ، بينما أظهرت النسبه المئويه لمساحة تفاعل البيريوديك أسيد شيف و الألشين االازرق انخفاضا كبيرا ذو دلالة إحصائية وذلك مقارنة بالمجموعة الضابطة. أظهرت النتائج الهستوكيميائية المناعية زيادة كبيرة في مستوي تفاعل بكنا. إعطاء الفوكويدان مع السوفوسبوفير قد قلل من هذه التغيرات الي حد كبير.

الخلاصة: قد ثبت ان السوفوسبوفير يحدث تغيرات نسيجية ملحوظة في الغشاء المخاطى لقعر المعدة، وهذه التغيرات يمكن تخفيفها الي حد كبير بالفوكويدان عندما يعطى معه