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Antiviral Drugs Used to Treat COVID-19 are not Liver Safe: A Comparative Experimental Study

COVID-19 Tedavisinde Kullanılan Antiviral Ajanlar Karaciğer İçin Güvenilir Değil: Karşılaştırmalı Deneysel Çalışma

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ABSTRACT

Objective: This study aimed to determine whether the medication alone might be effective in the drug-induced liver damage that was reported during coronavirus disease-2019 (COVID-19) in healthy rats.

Methods: Thirty-three 8-10 week old Wistar albino male rats were separated into seven groups: the sham control groups for intravenous, subcutaneous, and gavage stress; the acyclovir, hydroxychloroquine, anakinra, and favipiravir groups. At the end of the experimental period, hematoxylin-eosin and silver impregnation histochemical, tumour necrosis factor-alpha (TNF-α)/interleukin-1 beta (IL-1β)/IL-6 immunohistochemical stainings were performed on liver tissue. Data were supported statistically.

Results: Morphological degeneration were observed in both the classic liver lobule and portal triad regions with drug administration. The intensity of the reticular fibers was found to be decreasing in the medicament groups, especially around the vena centralis. TNF-α, IL-1β, and IL-6 immunoreactivities were found to be significantly higher in the antiviral drug-administered groups than in the sham control groups.

Conclusion: It is concluded that liver damage was reported for treating COVID-19 triggered by the medicines applied.

Keywords: Antiviral agents, coronavirus, liver, TNF-α, IL-1β, IL-6

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) hastalarında ilaca-bağlı rapor edilen karaciğer hasarında, tedavi süresince kullanılan ajanların tek başlarına etken oluşturup oluşturmadıklarını sağlıklı sıçanlar üzerinde incelenmesi, çalışmamızda amaçlamıştır.

Yöntemler: Otuz üç adet 8-10 haftalık Wistar albino cinsi erkek sıçan intravenöz, subkutan ve gavaj stresi sham kontrol grupları, asiklovir grubu, hidroksiklorokin grubu, anakinra grubu ve favipiravir grubu olmak üzere yedi gruba ayrılmıştır. Hematoksilen-eozin ile gümüş impregnasyon yöntemleri ile histokimyasal; tümör nekroz faktörü-alfa (TNF-α)/interlökin-1 beta (IL-1β)/IL-6 ile de immünohistokimyasal boyamalar deney bitiminde alınan karaciğer dokuları üzerinde uygulanmıştır. Veriler istatistiksel olarak değerlendirilmiştir.

Bulgular: Çalışma sonucunda, klasik karaciğer lobülünde ve portal triad alanlarında ilaç uygulamalarına bağlı dejenerasyonlar tespit edilmiştir. Retiküler lif yoğunluğunun vena centralis çevresinde azaldığı; TNF-α, IL-1β ve IL-6 immün pozitivitelerinin ise sham kontrol gruplarına karşın, antiviral ajan uygulanan gruplarda arttığı görülmüştür.

Sonuç: COVID-19 tedavisi sırasında rapor edilen karaciğer hasarının, uygulanan ajanlar tarafından tetiklendiği kanısına varılmıştır.

Anahtar Sözcükler: Antiviral ajanlar, koronavirüs, karaciğer, TNF-α, IL-1β, IL-6

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INTRODUCTION

One of the most significant worldwide health crises at present is coronavirus disease-2019 (COVID-19), a respiratory viral infection brought on by a coronavirus (1,2). Globally, there have been more than 700 million COVID-19 cases, resulting in six million fatalities (3). Public health is being threatened by the COVID-19 pandemic, which has led to severe acute respiratory syndrome (SARS). Although this virus, according to existing clinical data, not only causes respiratory disorders and affects the lungs, but severe patients may exhibit a systemic and multi-organ disease. It also causes histopathological alterations in many non-respiratory organs, including the kidney, liver, brain, and heart (4,5).

Given these facts, antiviral drugs are in the spotlight because of their crucial role in treating SARS, but they are also required to stop the virus from spreading throughout the body, which will result in non-respiratory damage (6). Drug repurposing is another effective strategy for finding new clinical uses for already approved applications, in addition to drug development initiatives (7). In that case, antiviral medicaments that have been prescribed for other viral infections were acutely administrated to patients for a predefined period until COVID-19 vaccines were created (8).

One of the antiviral drugs administered to patients during that time was acyclovir, which is mostly used to treat herpes virus infections. This medicine inhibits viral DNA polymerase through the phosphorylation of the acyclovir compound (9,10). Another is the immunosuppressive drug anakinra, which is used to treat rheumatoid arthritis (RA). The cytokine storm cascade is significantly inhibited by this interleukin-1 (IL-1) receptor antagonist, which binds to both IL-1 alpha (α) and IL-1 beta (β) receptors (11-13). Owing to its immunosuppressive effects, hydroxychloroquine has been used to treat autoimmune diseases like lupus and RA and to prevent and treat malaria. It has also been suggested as a treatment for COVID-19 (14-16). It is well known that this medicine affects endosomal activity and raises intracellular pH. Wide-ranging secondary effects result from this action, which also alters membrane stability, interferes with lysosomal function and autophagy, disrupts signaling pathways, and inhibits transcriptional activity. By inhibiting toll-like receptor signaling and cytokine synthesis at the cellular level, this drug can decrease immunological activation (15,17). Finally, favipiravir, the first antiviral medicine utilized against COVID-19, was specifically used to treat influenza virus. It is a RNA-dependent RNA polymerase inhibitor that is activated in cells in its phosphoribosylated form and suppresses viral RNA polymerase activity (18-20).

Along with all this information, research has shown that the use of medicaments (particularly anakinra and favipiravir) (21,22) in the treatment of COVID-19 patients has been linked to drug-induced liver or hepatocellular damage (23,24). Direct viral damage, drug-induced hepatotoxicity, systemic inflammation, underlying liver disease, or hypoxia are some of the mechanisms of liver injury that have been proposed but not yet proven (25).

In this research, we sought to determine whether the medication alone might be effective in the drug-induced liver damage that was observed in COVID-19 patients through healthy rats. Using literature reviews as our guide, we chose the most widely used antiviral drugs for the treatment of COVID-19 patients. To achieve this, we compared the possible structural degenerations throughout the liver and examined the expression of tumor necrosis factor-alpha (TNF-α)/IL-1β/IL-6 in the tissue, which are known to be effective in many pathological pathways in the liver from fibrosis to necrosis.

MATERIALS AND METHODS

Animal studies were conducted in accordance with ethical standards and were approved by the Institutional Animal Ethics Committee Guidelines of Gazi University (approval number: G.Ü.ET-22.038).

Chemicals

All medications used in the experimental procedure were purchased from local pharmacies.

Animals

In this study, thirty-three 8-10 weeks old Wistar albino male rats were employed (Gazi University Faculty of Medicine Experimental Animal Breeding and Experimental Research Center, Ankara, Türkiye). They were housed in clean, sterile polypropylene cages with access to water and normal rat food under a 12-h light/dark cycle. They were housed six to a cage in an air-conditioned animal room with a temperature of 22±3 °C and 55±10% humidity. Before the start of the research, the animals were exposed to laboratory conditions for 4 weeks.

Experimental Design

The animals were separated independently into seven groups. A detailed summary of the administrations submitted to the experimental groups is shown in Table 1.

The administration doses were proportional to the masses of the experimental animals, even though the drug doses and termination of an experiment for the study were the same as those used in humans. On the basis of the COVID-19 Guidelines of the National Ministry of Health and the medicine prospectuses, dosages and durations were chosen. The sham control groups were initially planned to have six subjects for each; however, this was reduced to three at the ethics committee's request.

The animals were sedated with 45 mg/kg ketamine and 5 mg/kg xylazine, and liver tissue were harvested at the end of the experiment.

Histochemical Analysis

Liver tissue samples were preserved for 72 h in 10% neutral formaldehyde. The samples were washed in tap water, dehydrated with ascending alcohols, clarified in xylene, and embedded in paraffin. Hematoxylin-eosin and silver impregnation were performed on 4 µm thick liver sections. For the silver impregnation, a silver impregnation for reticulum kit (04-040801 Bio Optica, Lot: .0210, Milano, Italy) was used, which was obtained from standard commercial suppliers.

Immuno-Histochemical Analysis

4 µm cross sections of the liver tissue were taken on slides. The pieces were rehydrated after the paraffin was removed. A citrate buffer was used to achieve heat-induced antigen retrieval at pH 6.0. Endogenous peroxidase activity was blocked with 3% H2O2 (Lot: O2Q46013, Thermo Scientific, Waltham, MA) and a serum

blocking solution (Lot: PHL547, Thermo Scientific, Waltham, MA) were performed for 10 min. The sections were incubated with primary antibodies against TNF-α (SantaCruz: sc130349; 1:100); IL-1β (SantaCruz: sc7884; 1:100); and IL-6 (Santa Cruz: sc1265; 1:100) overnight at +4 °C. All primary antibodies were diluted with Large Volume UltrAb Diluent (Lot: UD51273, Thermo Scientific, Waltham, MA). Biotinylated secondary antibody and streptavidin peroxidase (Lot: PHL547, Thermo Scientific, Waltham, MA) were incubated with tissues for 15 min at room temperature. Phosphate buffer saline was used to wash the slides between each step. The binding sites of the antibody were visualized using DAB (Lot: HDX57664, Thermo Scientific, Waltham, MA). After washing in water and alcohol and clarifying in xylene, the slides were counterstained with Harris' hematoxylin and coated with balsam.

The density and intensity of the TNF-α, IL-1β, and IL-6 stainings were evaluated in the liver using a light microscope equipped with a digital camera (DM4000B Image Analyze System; Leica, Wetzlar, Germany), a Leica DFC280 plus camera, and a LAS software program (Leica). We used the following semi-quantitative IHC scoring system to assess the TNF-α, IL-1β, and IL-6 staining intensity; (0) no staining, (1) weak staining, (2) moderate to weak staining, (3) moderate staining, (4) moderate to strong staining, and (5) strong staining. Two independent observers, blinded to the treatment protocol, evaluated the immunostaining scores separately. The H-score was calculated as H-score = Σpi (i + 1), where is the intensity of the TNF-α, IL-1β, and IL-6 staining with values of 0-5, and Pi is the percentage of stained cells for each "i" intensity (26).

To create a single sham control group with six subjects for statistical analysis, two subjects were randomly chosen from each of the sham control groups (from the sham control groups 1-3), and their data were collected.

Statistical Analysis

H-score values derived from immunohistochemical scores were evaluated using the SPSS version 20.0 Software (SPSS Inc., Chicago, IL). All data were provided as mean ± standard deviation. ANOVA and Duncan's post-hoc tests were used to analyze the differences between the groups. A p-value of 0.05 was considered statistically significant.

RESULTS

Histochemical Results

The classic liver lobule (that includes vena centralis, endothelium, radially arranged hepatocytes, sinusoidal structures) and the portal triad (that includes portal vein, hepatic artery, bile excretory duct extension, and intermediate connective tissue) were both observed in their normal appearance through all three sham control groups, in accordance with microscopic examinations (Figures 1, 2, Table 2).

Dilatation was observed in the vena centralis of the acyclovir group. Degeneration was observed in some of the hepatocytes, and it was found that the nuclei of these types of hepatocytes were abolished, and the cell borders were also difficult to detect. The presence of condensed/pyknotic nuclei associated with the necrotic alteration identified in some hepatocytes was the most remarkable observation in this group. The vena centralis was also dilated in the hydroxychloroquine group, and it was remarkable that the normal radial organization of the hepatocytes, particularly in the regions around the vena centralis, was disrupted and a mass of cells was created. The cell morphologies of this type of hepatocyte were not readily evident. In these regions, the endothelium-lining the vena centralis was not easily visible and the sinusoids were dilated. This group also included hepatocytes with pyknotic nuclei. Around the

Table 1. Experimental design

Experimental groups	Number of subjects	Administrations	Termination of the experiment
Sham control-1 (gavage stress)	(n=3)	0.5 cc 0.09% sterile saline by gavage	5 days
Sham control-2 [intravenous (i.v.) injection stress]	(n=3)	0.5 cc 0.09% sterile saline by i.v. injection	7 days
Sham control-3 (subcutaneous [s.c.] injection stress)	(n=3)	0.5 cc 0.09% sterile saline by s. c. injection	7 days
Acyclovir	(n=6)	2x10 mg/kg acyclovir (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 hours) i.v. injection	7 days
Hydroxychloroquine	(n=6)	2x400 mg/kg hydroxychloroquine (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 h for the day 1) 2x200 mg/kg hydroxychloroquine (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 h for the day 2-5) gavage	5 days
Anakinra	(n=6)	4x100 mg/kg anakinra (dissolved in 0.09% sterile saline) (4 times a day, seperated by 6 hours) s.c. injection	7 days
Favipiravir	(n=6)	2x1600 mg/kg favipiravir (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 h for the day 1) 2x600 mg/kg favipiravir (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 h for the day 2-5) gavage	5 days

Table 2. Scores for each group based on degenerative criteria

	Sham control-1	Sham control-2	Sham control-3	Acyclovir	Hydroxychloroquine	Anakinra	Favipiravir
Dilatation in V. centralis				++	+	++	++
Dilatation in the sinusoids					+	+	+
Congestion				+	++	++	+
Radial disorganization					++	+	++
Degeneration in hepatocyte morphology				+	+	+	+
Pyknotic nuclei				++	+		
Karyolysis						+	++
Vacuolar degeneration						+	+++
Infiltration					+	+	
Fibrosis						++	++
Dilatation in the portal vein				+	++	++	+++
Hyalinization						+	+
Reticular fibers	+++	+++	+++	++	++	+	+

vena centralis, infiltration was visible. In the anakinra-administered group, vena centralis and sinusoids were dilated, and infiltration and an increase in the connective tissue that extended to the sinusoids were also distinguished. A few cells had pyknotic nuclei that could be observed. The karyolysis in this group that was compatible with the deletion of the intranuclear chromatin material seen in some hepatocytes was the most distinguishing characteristic of this group. Some hepatocytes were found to have vacuolar degeneration. The vena centralis and sinusoids exhibited significant dilatation in the favipiravir group, and a hyalinized structure that extended to the sinusoids. The typical radial arrangement of the hepatocytes was disrupted, and a mass of cells was formed in almost all of the hepatocytes. A loss of nuclei indicating karyolysis was observed in certain hepatocytes. Hepatocytes displayed a vacuous structure that may have been caused by organelle degeneration (Figure 1, Table 2).

When the portal triad region was examined for each group, the group that received acyclovir show minimal portal vein dilatation. The portal vein in the hydroxychloroquine group had severe dilatation and congestion. In addition to the severe dilatation and congestion through the portal vein after anakinra administration, hyalinization of the connective tissue in this region was observed. Administration of favipiravir led to intense dilatation of the portal vein and an increase in connective tissue in this region. However, it was noted that the fibers were not yet organized in this connective tissue area (Figure 2, Table 2).

The distribution of reticular fibers in the sinusoidal regions, particularly around the vena centralis, was found to be intense in all three sham control groups during the silver impregnation tests. The intensity of the reticular fibers was found to decrease in the experimental groups from acyclovir to hydroxychloroquine, anakinra, and favipiravir at a discontinuous level. The gold-yellow staining in the anakinra and favipiravir groups caused by increased collagen fibers and potential fibrosis despite decreased reticular fibers was another remarkable discovery of silver impregnation (Figure 3, Table 2).

Immuno-Histochemical Results

Immunoreactivities were distinguished at the cytoplasmic level through the classic liver lobule cells in TNF-α, IL-1β, and IL-6 immunohistochemical staining. Very weak TNF-α immuno-reactivity was observed in some hepatocytes in all three sham control groups. Weak immunoreactivity was observed in the acylovir and hydroxychloroquine groups, whereas the anakinra group showed weak to moderate immunoreactivity. In addition, more intense immunoreactivity was observed in the hepatocytes located around the vena centralis. Moderate immunoreactivity was observed in the hepatocytes around the vena centralis, whereas other hepatocytes showed weak immunoreactivity in certain places and moderate immunoreactivity in others (Figure 4). According to statistical findings, TNF-α immuno-reactivities were found to be significantly higher in the antiviral drug-administered groups than in the sham control group ($p<0.05$). However, no statistically significant difference was detected between the antiviral drug-administered groups ($p>0.05$) (Graphic 1).

Very weak IL-1β immuno-reactivity was observed in some hepatocytes in all three sham control groups. In the acyclovir group, hepatocytes had weak to moderate immunoreactivity, whereas strong immunoreactivity was observed in Kupffer cells. Moderate immunoreactivity in the hydroxychloroquine group and moderate to strong immunoreactivity were observed in the anakinra group. In addition, strong IL-1β immuno-reactivity was observed in relatively few Kupffer cells in the anakinra group compared with the acyclovir group. In the favipiravir group, strong immunoreactivity was observed in the hepatocytes around the vena centralis, whereas other hepatocytes showed moderate to strong immunoreactivity in certain places. Immunoreactive Kupffer cell distributions were the same as those in the acyclovir group (Figure 5). According to statistical findings, IL-1β immuno-reactivities were found to be significantly higher in the antiviral drug-administered groups than in the sham control group ($p<0.05$). Additionally, significantly higher IL-1β immuno-reactivities were observed in the anakinra

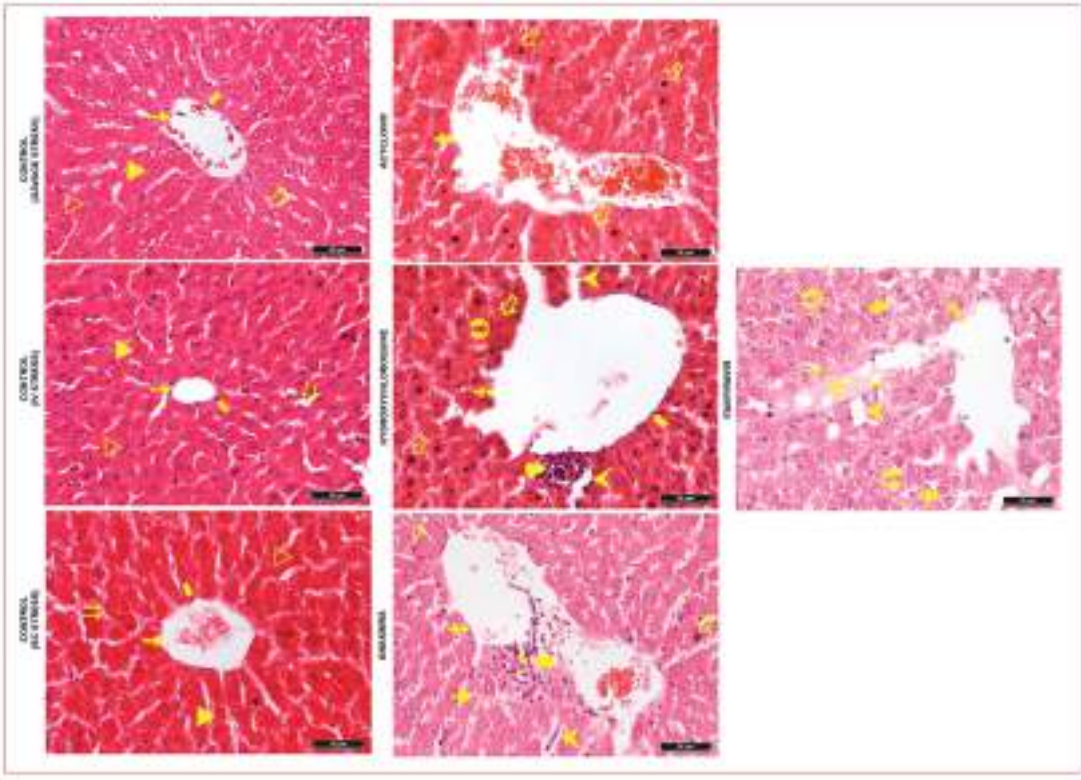

Figure 1. Liver sections (classical lobule) for each group showed ➙: Vena centralis, ▶: Hepatocyte, ⇉: Sinusoid, ➡: Endothelium, ▷: Nucleus, ‡: Dilatation in vena centralis, ⇨: Degeneration in hepatocyte morphology, ⇪: Pyknotic nucleus, ⇩: Radial disorganization, ➤: Dilatation in sinusoids, ➡: Infiltration, *: Fibrosis, ⇢: Karyolysis, ➧: Vacuolar degeneration, ⇔: Congestion, H: Hyalinization (hematoxylin-eosin x400).

and favipiravir groups than in the acyclovir and hydroxychloroquine groups (p<0,05) (Graphic 1).

For all three sham control groups, very weak IL-6 immunoreactivity was observed in a few hepatocytes. Moderate immunoreactivity in hepatocytes and strong immunoreactivity in Kupffer cells were determined in the acyclovir group. The hepatocytes around the vena centralis showed moderate to strong immunoreactivities for the other three groups. The hydroxychloroquine group had weak to moderate immunoreactivity of hepatocytes in certain places, whereas the final two groups showed moderate immunoreactivities in these regions (Figure 6). According to statistical findings, IL-6 immunoreactivities were found to be significantly higher in the antiviral drug-administered groups than in the sham control group (p<0.05). However, no statistically significant difference was detected between the antiviral drug-administered groups (p>0.05) (Graphic 1).

DISCUSSION

COVID-19 mostly affects the lungs, but it can directly/indirectly cause virus-induced liver injury, whose mechanisms are currently under investigation (27). The first study that reported abnormal liver tests in patients with COVID-19 was reported by Chen et al. (28). Liver injury is indicated by high bilirubin levels and abnormal alanine transaminase/aspartate transaminase (ALT/AST) levels (1).

The mechanisms underlying the link between severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and liver injury are complex and include direct cholangiocyte damage caused by SARS-CoV-2, immune overactivation and systemic inflammation, ischemia/reperfusion and hypoxia/reoxygenation injuries, and drug-induced liver injury (4,29). The majority of the data show that systemic inflammation is more likely to be the cause of hepatic injury during SARS-CoV-2 infection than to be triggered by a cytopathic effect that targets liver cells. In patients with COVID-19, viral RNA can be found in the liver tissue; however, infection of the liver cells has not yet been proven (2). Additionally, a number of drugs, biological agents,

Graphic 1. Comparative statistical graphs showing H-score analyses of immunostainings. (*) Statistically significant groups comparison to the sham control group, (♦) Statistically significant groups comparison to the acyclovir and hydroxychloroquine groups.

TNF-α: Tumour necrosis factor-alpha, IL-1β: Interleukin-1 beta.

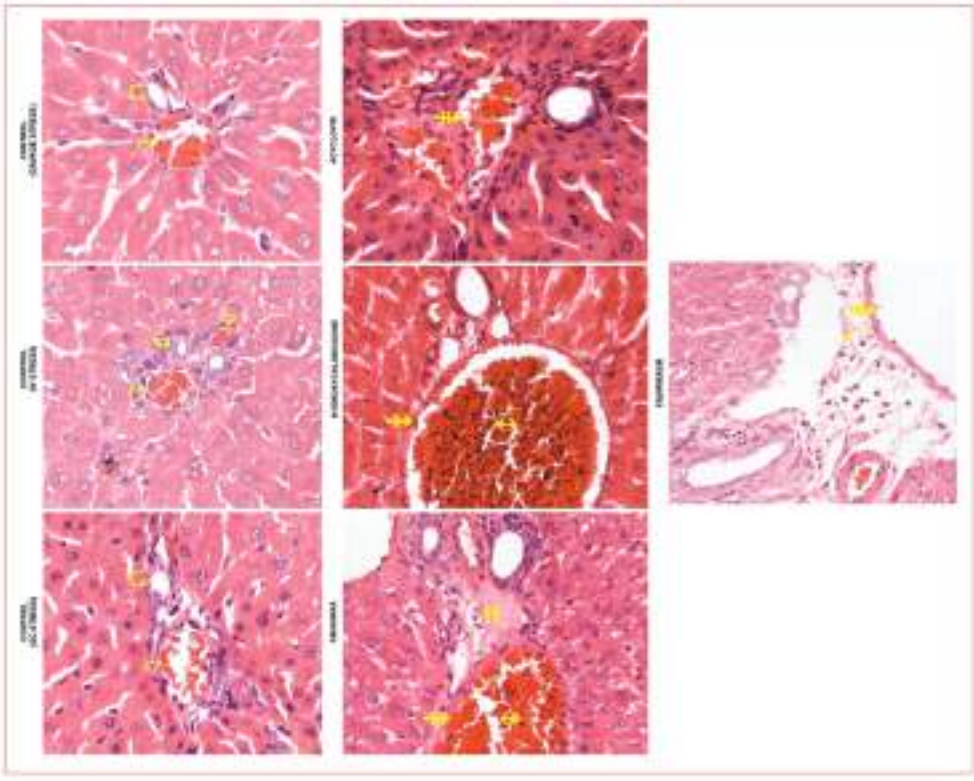

Figure 2. Liver sections (portal triad) for each group showed ↯: Hepatic artery, ↱: Portal vein, ↳: Bile duct, ‡: Dilatation in the portal vein, *: fibrosis (hematoxylin-eosin x400).

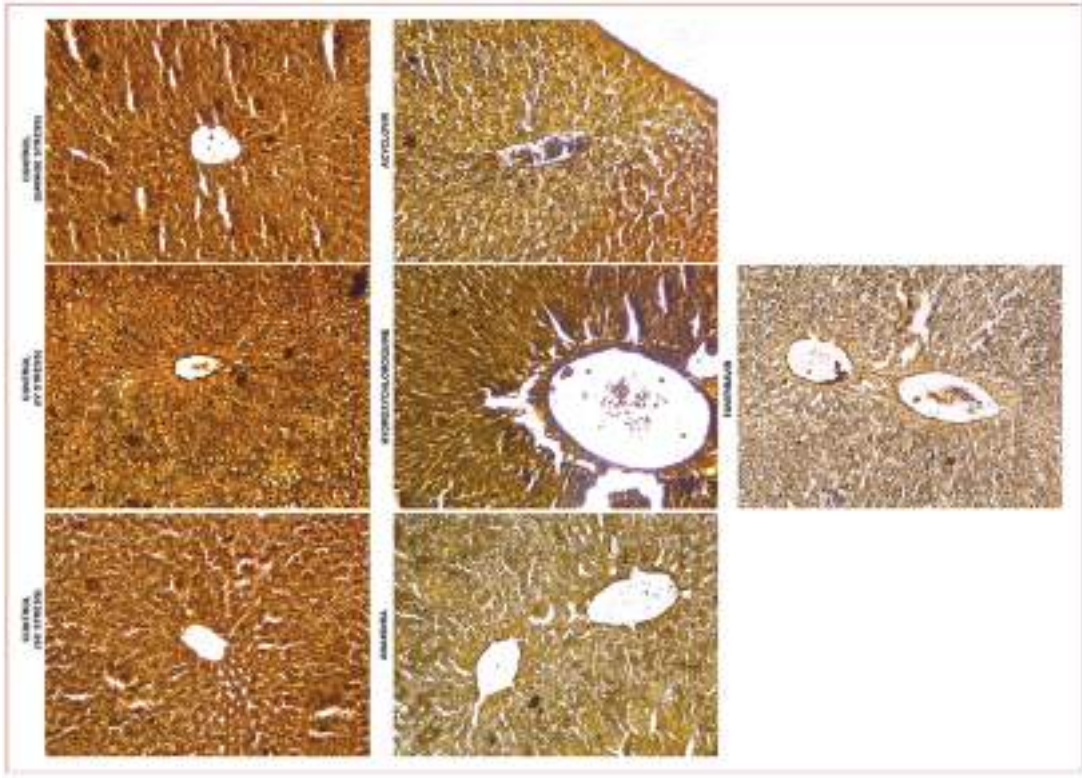

Figure 3. Liver sections for each group showed the reticular fibers, which were anastomosed in the tissue and stained black (silver Impregnation x200).

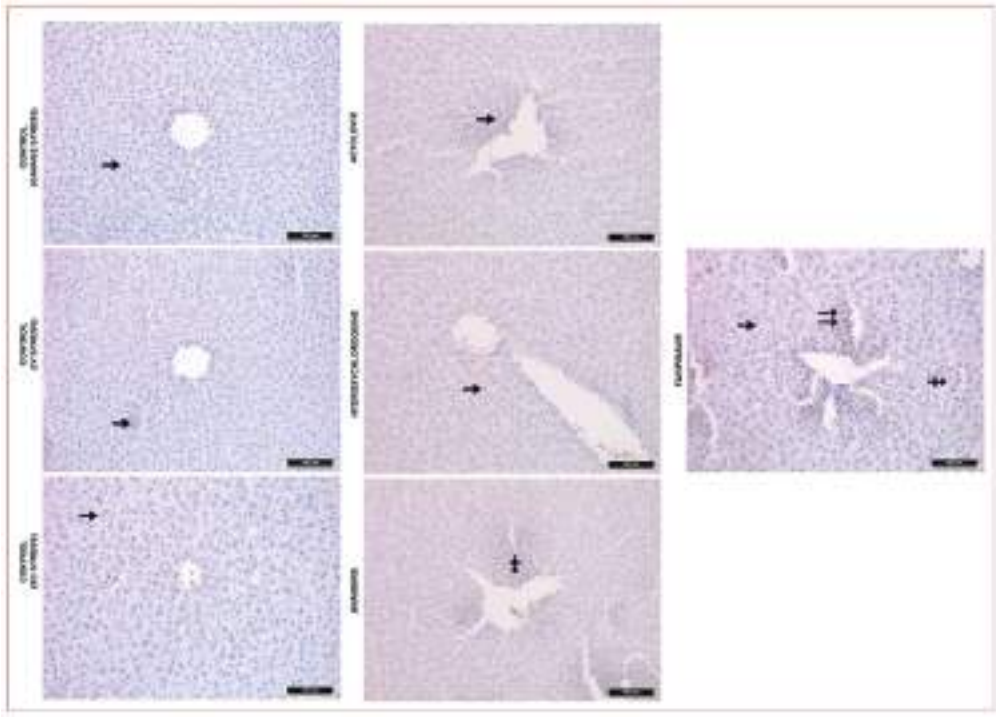

Figure 4. Liver sections of each group for TNF-α immuno-histochemistry staining showed ➙: Weak immuno-reactivity, ‡: Weak to moderate immuno-reactivity, ⇉: Moderate immuno-reactivity, ⇨: Moderate to strong immuno-reactivity, ➡: Strong immunoreactivity (immunperoxidase hematoxylin x200).
TNF-α: Tumour necrosis factor-alpha.

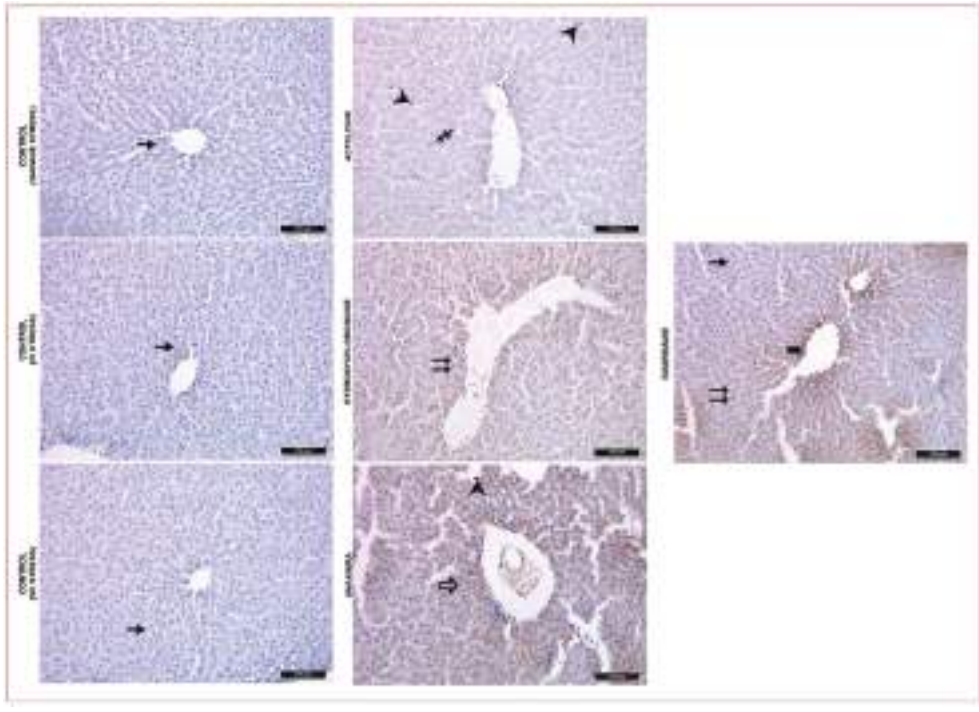

Figure 5. Liver sections of each group for IL-1β immuno-histochemistry staining showed ➙: Weak immuno-reactivity, ‡: Weak to moderate immuno-reactivity, ⇉: Moderate immuno-reactivity, ⇨: Moderate to strong immuno-reactivity, ➡: Strong immuno-reactivity, ➤: Strong immunoreactivity in Kupffer cells (immunperoxidase hematoxylin x200).
IL-1β: Interleukin-1 beta.

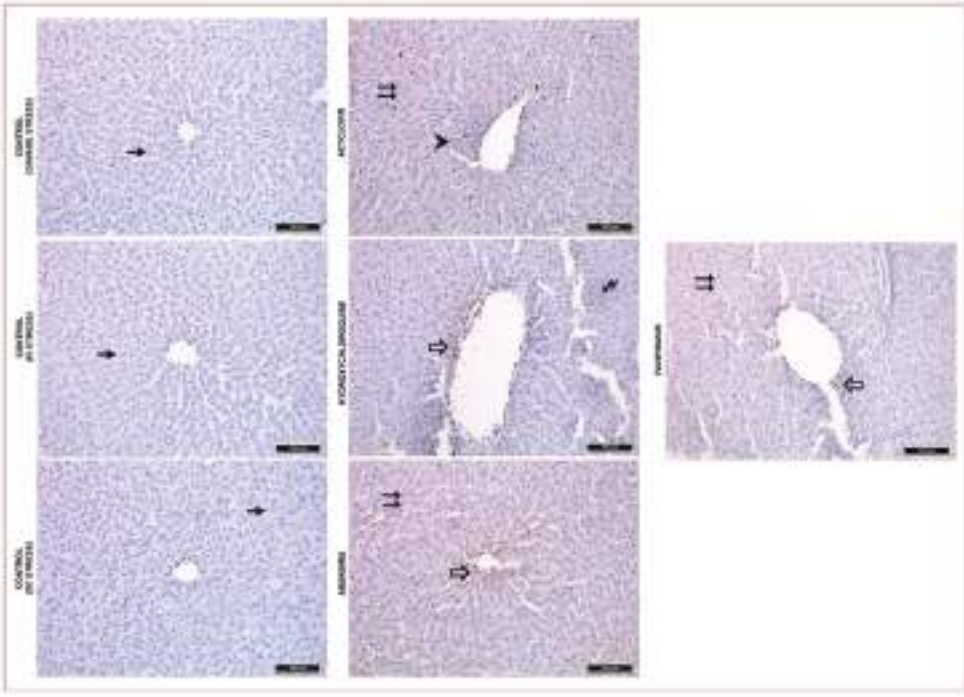

Figure 6. Liver sections of each group for IL-6 immunohistochemistry staining showed ➙: Weak immuno-reactivity, ⇞: Weak to moderate immuno-reactivity, ⇉: Moderate immuno-reactivity, ⇨: Moderate to strong immuno-reactivity, ➠: Strong immuno-reactivity, ➤: Strong immunoreactivity in Kupffer cells (immunperoxidase hematoxylin x200).

IL-6: Interleukin-6.

and new compounds, whose effectiveness in COVID-19 is under examination, have also been demonstrated to have the potential to lead to or worsen liver damage (27).

Drug-induced liver injury in hospitalized patients with SARS-CoV-2 infection was evaluated by Mihai et al. (25), and a significant increase in aminotransferase levels was observed during hospitalization, indicating drug-related hepatotoxicity. Indirect bilirubin increased and hepatocellular damage occurred, particularly ALT measurements were found to be higher than five times the upper limit of normal, and were both associated with the administration of potentially hepatotoxic medications (25). Other viewpoints in the study reviewed by Sodeifian et al. (23) claim that some research findings might indicate a direct role of drugs, whereas others could not. However, many studies found that medication administration may have caused liver damage, according to the same review (23).

Using literature reviews as our guide, we chose the most widely used antiviral drugs for treating COVID-19 patients as acyclovir, hydroxychloroquine, anakinra, and favipiravir in the present study.

If studies on liver damage associated with the administration of these drugs are to be investigated, Taylor et al. (21) indicated that the IL-1 receptor antagonist anakinra caused acute liver failure during the treatment of adult-onset Still's disease. The patient was administered 100 mg anakinra twice daily for 9 days by s.c. during the treatment. High bilirubin and ALT/AST levels were observed after administration (21). Khani et al. (12) used anakinra in COVID-19 treatment and finally reported that more patients had increased liver enzymes and thromboembolic events.

Similar to these studies describing anakinra-induced liver injury, in the current investigation, the anakinra-administered group was identified by vena centralis and sinusoids dilatation, infiltration, vacuolar degeneration, pyknotic nuclei, and karyolysis in the hepatocytes. In this group, the region of the portal triad also showed congestion and hyalinization of the connective tissue.

The first case of favipiravir-induced cholestatic liver injury was reported by Yamazaki et al. (30). The patient received 6000 mg/day of favipiravir on day 1 and 2400 mg/day on days 2-14. After the administrations, they found elevated alkaline phosphatase, ɣ-glutamyl transpeptidase, and total bilirubin levels, which suggested cholestatic liver injury. Finally, they concluded that this could happen when high doses of favipiravir were administered, especially in people with compromised liver function (30).

Kumar et al. (22) reported drug-induced liver injury in patients who used favipiravir for 2 weeks and 12 days during COVID-19 treatment. They evaluated a cholestatic liver biochemistry profile following laboratory results in a patient who used favipiravir for 2 weeks. They also noted moderate hepatocellular cholestasis and inflammation generated by lymphocytes and a few eosinophils in the portal area in the patient's percutaneous liver biopsies. In laboratory tests of another patient, they discovered markedly increased liver enzymes, and they reported favipiravir-induced acute hepatitis in this case (22).

Similar to the biopsy findings reported in this study, we also observed severe hepatocyte degeneration and a rise in connective tissue in the portal triad through our favipiravir-administered group.

Examining the expression of molecules linked to tissue degeneration is unquestionably one of the most important ways to determine the mechanism of liver damage.

The conventional view of hepatocyte cell death during liver injury is that it either occurred by programed cell death (apoptosis) or uncontrolled cell death (necrosis). Multiple cell death processes, such as necroptosis, proptosis, and autophagic cell death, can be induced after acute and chronic liver injury (31). Pyknotic nuclei associated with necrotic alterations were observed in drug-administered groups, and nuclei appearances compatible with karyolysis were differentiated in the anakinra and favipiravir groups according to the present investigation.

The inflammatory process starts with the production of TNF-α from macrophages, which is followed by an increase in IL-1, IL-6, and IL-8 expressions. IL-1β is also produced by macrophages, just like TNF-α. Therefore, all of these cytokines are referred to as proinflammatory cytokines because they are produced early on and initiate the inflammatory response (32-35). In keeping with this knowledge, we found that all three antibodies (TNF-α, IL-1β, and IL-6) increased in the drug-administered groups and that IL-1β was also significantly expressed in liver Kupffer cells in these groups.

TNF-α and IL-1β, the two main cytokines, contribute to the production of IL-6, and all three cytokines are linked to chronic inflammation. TNF-α increases the release of acute phase proteins from the liver together with IL-1 and IL-6 through hepatocytes, in addition to playing a role in the necrosis-induced death of tumors (36-38). The hallmarks of chronic liver disease are hepatocyte loss, inflammation, and liver fibrosis. TNF-α plays an essential role in acute and chronic liver inflammation that leads to liver fibrosis as well as apoptosis and proliferation (35). In the current investigation, drug-administered groups with elevated TNF-α expression, particularly in the anakinra and favipiravir groups, were observed to have an increase in connective tissue associated with fibrosis. We supported this finding using the silver impregnation method.

TNF-α and IL-1β both exert effects on the vascular endothelium, which results in thrombus/coagulation and edema by increasing cell permeability as a result of endothelial disruption. IL-1β alone can cause tissue damage, but the incidence of damage increases with TNF-α (36-38). In particular, in the hydroxychloroquine and anakinra groups, where significant expressions of IL-1β were found in the current investigation, endothelium degeneration surrounding the vena centralis, infiltration, and congestion due to potential endothelial injury in the portal vein were reported.

Yoshigai et al. (39) observed that IL-1β stimulation increased the TNF-α expression in rat hepatocyte cultures. In addition, organ failure and higher mortality rates are linked to TNF-α and IL-6 acting together (36-38). TNF-α, IL-1, and IL-6 suggest mediating hepatic damage in hepatitis through animal models (40,41). Rex et al. (34) reported that the action of hepatocytes during the liver inflammatory response is significantly affected by cytokines produced by macrophages. According to research, lipopolysaccharide stimulation causes the liver to release TNF-α and IL-1β, which then triggers the fas ligand-induced apoptotic pathway (34). The release of proinflammatory cytokines after myocardial infarction contributes to tissue repair and damage adaptation; however, over time, these cytokines cause permanent damage to the heart, according to a study by Turner et al. (42) This study also showed that 0.1-10 ng/mL TNF-α induction increased the mRNA levels of IL-1β and 6 in human cardiac fibroblasts.

Although numerous studies in the literature demonstrate drug-induced liver injury using laboratory tests under COVID-19 conditions, there is no research indicating the histological impact of antiviral drugs used in COVID-19 treatment on the liver. Taken together, liver damage was reported in the treatment of COVID-19 triggered by the drug applied, and among these medications, anakinra and favipiravir, and especially favipiravir, were more closely related to the degeneration according to the present study. It was concluded that TNF-α, IL-1β, and IL-6, but especially IL-1β, play an active role in these degeneration.

CONCLUSION

Consequently, this study represents the first histological study in this field. Our research was carried out on experimental animals because collecting postmortem tissues is problematic both in terms of ethics and time required. However, expanding the research on liver cell culture or liver organoids in vitro will make an essential contribution to the literature.

Ethics Committee Approval: Animal studies were conducted in accordance with ethical standards and were approved by the Institutional Animal Ethics Committee Guidelines of Gazi University (approval number: G.Ü.ET-22.038).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Author Contributions

Concept: C.M.S., G.T.K., Design: C.M.S., G.T.K., Data Collection or Processing: A.A.E.S., C.M.S., Analysis or Interpretation: A.A.E.S., C.M.S., G.T.K., Literature Search: A.A.E.S., C.M.S., Writing: C.M.S., Critical Review: C.M.S., G.T.K.

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