# Effects of drugs commonly used in Sars-CoV-2 infection on renal tissue in rats

Azibe Yildiz[a,*], Onural Ozhan[b], Nigar Vardi[a], Mustafa Akyuz[a], Busra Bakar[c], Ahmet Ulu[c], Elif Taslidere[a], Zeynep Kucukakcali[d], Burhan Ates[c], Hakan Parlakpinar[b]

[a]Inonu University, Faculty of Medicine, Department of Histology and Embryology, Malatya, Türkiye
[b]Inonu University, Faculty of Medicine, Department of Pharmacology, Malatya, Türkiye
[c]Inonu University, Faculty of Arts and Sciences, Department of Chemistry, Malatya, Türkiye
[d]Inonu University, Faculty of Medicine, Department of Biostatistics and Medical Informatics, Malatya, Türkiye

## Abstract

**Aim:** In December 2019, the coronavirus disease was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Some drugs were repurposed for this disease treatment. Hydroxychloroquine (HCQ), favipiravir (FAV), molnupiravir (MOL), and dexamethasone (DEX) were widely used for the treatment of the disease. To increase the success of the treatment of coronavirus disease, there was used some of these drugs in combination. On the other hand, limited studies report these drugs' side effects. Therefore, this study was designed to evaluate the possible effects of these drugs and their combinations on the kidney tissues of rats without viral load.

**Materials and Methods:** Male Wistar albino rats were divided into control, HCQ, FAV, HCQ+FAV, HCQ+FAV+DEX, MOL, and MOL+DEX groups. At the end of the experiment, the serum kidney tissue samples were taken. Serum samples were analyzed for urea and creatinine. Kidney tissue samples were assessed as histopathological and immunohistochemical for heat shock protein 60 (HSP60), caspase-3, and receptor-interacting protein kinase-3 (RIPK3).

**Results:** Urea and creatinine levels were within the normal range in all groups. Histopathologically, all drugs and their combinations caused tubular degeneration. On the other hand, histopathological alterations were more prominent in the HCQ group. The oxidative stress marker HSP60 was significantly increased in FAV, MOL, and MOL+DEX groups, while it was similar to the control group in the HCQ groups. Apoptosis marker caspase-3 expression was found to be prominently higher in other drug groups except the FAV group. Expression of RIPK3, a marker of necroptosis, was significantly increased in all drug groups.

**Conclusion:** Taken together, the data of our study show that the administration of all drugs alone and in combination may cause structural damage to the kidney. Furthermore, our results indicate that HCQ can exhibit more damaging effects compared to other drugs.

## Introduction

The coronavirus disease, caused by SARS-CoV-2, emerged in December 2019 in China and has quickly spread to many countries and caused high morbidity and mortality worldwide. This disease affected more than 273 million people and has resulted in the death of over 5.3 million [1]. Although coronavirus disease appeared firstly as a lower respiratory tract infection transmitted via droplets, growing evidence documented multiorgan involvement in infected patients. This systemic involvement is postulated to be primarily related that the SARS-CoV-2 binds on angiotensin-converting enzyme 2 (ACE2) receptors on different cell-type membranes. The virus can directly damage these organs by binding to ACE2 receptors available in vascular endothelial cells, lungs, heart, brain, gut, liver, kidneys, and other tissues [2-4].

After the lungs, the kidneys are one of the major organs affected by coronavirus disease. Renal dysfunction and acute renal insufficiency are prevalent among patients admitted with SARS-CoV-2 infections. The mechanism and pathophysiology of renal involvement in SARS-CoV-2 infection are unclear but are presumably a multifactorial etiology. Still, some published literature has reported that direct

*Corresponding author:
*Email address:* azibe.yildiz@inonu.edu.tr (Azibe Yildiz)

viral effects through ACE2, which is highly expressed in the kidney, and indirect effects such as impaired perfusion, cytokine, and complement activation or nephrotoxic medications could play a role [4, 5].

Various vaccines were developed against the SARS-CoV-2 however, studies have shown that even vaccinated people were infected with novel coronavirus variants. For this reason, various drugs were used as candidate treatments for the management of coronavirus disease [6, 7].

HCQ is a medication that has been used for over a century. Although its most widespread use is for the treatment and prophylaxis of malaria, this drug also has anti-inflammatory and antiviral effects and is used in managing several chronic diseases such as rheumatoid arthritis and systemic lupus erythematosus [7, 8]. The antiviral activity of hydroxychloroquine has been the focus of the attention of researchers [9]. Several in vitro studies have demonstrated its action on SARS and it has been used in the treatment and prophylaxis of coronavirus disease. The most widespread adverse effects of HCQ, which is reported to be a relatively safe drug, include dermatological ailments, severe itching of the skin, and gastrointestinal disturbances that can be revealed in almost 10% of patients. The incidence of the most serious adverse effects such as cardiotoxicity, neuromyopathy, and irreversible retinopathy associated with the use of HCQ is low [8, 9].

FAV is one of the antiviral drugs used for COVID-19 treatment. FAV was approved in Japan in 2014 for novel epidemic influenza strains that had not responded to standard antiviral therapies. It selectively and effectively blocks the RNA-dependent RNA polymerase activity of RNA viruses [7, 10, 11]. It has been documented that the major side effects of FAV are teratogenicity and hyperuricemia [12].

MOL is a newer broad-spectrum antiviral drug that has lately taken on emergency use approval from the U.S. FDA. Structurally, MOL is the 5´-isopropyl ester prodrug of $\beta$-d-N4-hydroxycytidine, which is the active metabolite [13]. It has been reported that MOL exerts quite effective antiviral activity by inhibiting RNA-dependent RNA polymerase against RNA viruses, including SARS-CoV-2 [14]. It has been documented that the most common side effects of this drug were diarrhea, nausea, and dizziness [15].

DEX, approved by the FDA in 1958, is a synthetic corticosteroid medication having anti-inflammatory and immunosuppressive effects. Due to its effects, it was used in the treatment of coronavirus disease because it could limit SARS-CoV-2-mediated uncontrolled cytokine storm and severe acute respiratory distress syndrome [16]. However, DEX can disrupt organ functions and result in numerous clinical manifestations such as dyslipidemia, hypertension, and osteoporosis as well as it can furthermore increase the risk and severity of sequelae of coronavirus disease infection [17].

Drugs used in the treatment of coronavirus disease can also lead to a worse renal function profile in patients. A few cases of clinically apparent kidney injury were reported depending on the use of these medications [5, 15]. Therefore, to provide further evidence for kidney safety, we designed to investigate whether the effects of single and combined administrations of HCQ, FAV, MOL, and DEX used in the coronavirus disease treatment protocol without viral load could cause kidney damage by histochemically and immunohistochemically. In addition, creatinine and urea levels, which are important biomarkers for kidney failure detection, were estimated.

## Materials and Methods

*Animals and experimental procedure*

The experiment was performed on male Wistar albino rats aged 3 months, weighing 250-350 g obtained from the Inonu University Laboratory Animals Research Center. For rats' care and experimental procedures, the National Institutes of Health Animal Research Guidelines and ARRIVE Guidelines were followed [18]. The study protocol was approved by the Inonu University, Faculty of Medicine, Animal Research Ethics Committee (Protocol: 2021/1-6). The animals were housed under the standard conditions of 12 h light/12 h dark cycles with $60\pm5\%$ humidity, at a temperature of $22\pm2$ ºC, standard pellet diets, and tap water ad libitum. After one week of acclimation, the rats were randomly assigned into three groups as follows. The Random Allocation Rule technique was used for the randomization process. For randomization analysis, The Random Allocation Software was used [19]. The number of rats in each group was carried out using WSSPAS: Web-Based Sample Size and Power Analysis Software [20].

1. Control group (n:8): The rats were given 1 mL distilled water as vehicle solution orally for 5 days at 12-hour intervals.
2. FAV group (n=10): The rats were given FAV (Favimol® 200 mg film coated tab. Neutec Ilac Sanayi Ticaret A.S., Sakarya, Türkiye) orally at 12-hour intervals for a total of 5 days at a loading dose of 165.3 mg/kg on the first day and then at a maintenance dose of 62 mg/kg.
3. HCQ group (n:10): The rats were given 20.7 mg/kg HCQ (Plaquenil® 200 mg tab. SANOFİ Saglik Urunleri Ltd.Sti, İstanbul, Türkiye) orally for 5 days at 12-hour intervals.
4. HCQ+FAV group (n:10): The rats were given orally for 5 days with 12-hour intervals of 20.7 mg/kg HCQ and a maintenance dose of 62 mg/kg FAV followed by a loading dose of 165.3 mg/kg FAV on the first day of the experiment.
5. HCQ+FAV+DEX group (n:10): The rats were given orally for 5 days with 12-hour intervals of 20.7 mg/kg HCQ and a maintenance dose of 62 mg/kg FAV followed by a loading dose of 165.3 mg/kg FAV on the first day of the experiment. In addition, 0.62 mg/kg DEX (Dekort® 8 mg/2 mL i.m./i.v. injectable solution available in an ampoule, Deva Holding A.S., İstanbul, Türkiye) was administered intramuscularly to the rats in this group for 10 days.
6. Molnupiravir Group (n:10): The rats were given orally at 82.7 mg/kg MOL (Covunavir® 200 mg capsule, Abdi İbrahim Ilac Sanayi ve Tic A.S., İstanbul, Türkiye) at 12-hour intervals for 5 days.
7. MOL+DEX group (n:10): The rats were given 82.7 mg/kg MOL at 12-hour intervals for 5 days, and 0.62 mg/kg dexamethasone per day for 10 days.

The doses of the drugs to be administered to the experimental groups were adapted from the human treatment protocol, taking into account the formula and coefficients in the review published by Nair and Jacob in 2016 [21]. At the end of the experiment, all rats were euthanized with intraperitoneal overdose anesthesia (3.6 g/kg ethyl carbamate, Urethane®, Sigma-Aldrich, Inc., St. Louis, MO, United States), then, the kidneys were rapidly removed, washed in physiological saline, and immersed in 10% formalin for further histochemical and immunohistochemical studies.

### Creatinine and urea levels

Blood serum samples were collected from rats after the protocol, and the creatinine and urea levels were determined at the Inonu University Turgut Ozal Medical Centre (Malatya, Türkiye).

### Histopathological evaluations

Fixed kidney tissues were dehydrated in a series of increasing ethanol concentrations (70%, 80%, 96%), followed by absolute ethanol (100%), and finally embedded in paraffin. Then, paraffin-embedded tissue samples were sectioned and stained with H&E and PAS. The kidney sections were graded based on the presence and severity of histopathological changes in tubules The degree of tubular degeneration including necrotic changes, epithelial desquamation, and loss of brush border was assessed in ten randomly selected fields of the renal cortex at 200 magnification from each section using a semiquantitative scoring system (0: normal; 1: mild changes; 2: moderate changes; 3: severe changes) [22]. All assessments were carried out through a light microscope and image analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

### Immunohistochemical analysis

Immunohistochemical staining was carried out to demonstrate HSP60 as an oxidative stress marker, caspase 3 as an apoptosis marker, and RIPK3 as a necroptosis marker (HSP60, caspase 3, and RIPK3; Santa Cruz Biotechnology, Inc., Heidelberg, Germany).

The tissue sections were mounted on polylysine-coated slides. Xylene and a graded alcohol series were used for deparaffinization and rehydration. Antigen retrieval step was made in sodium citrate buffer (pH 6.0) in the pressure cooker for 20 min. Endogenous peroxidase activity was blocked by using 0.3% hydrogen peroxide for 15 min. Non-specific binding was blocked by using a protein block for 5 min. Then the slides were incubated for 60 min with primary antibodies (HSP60, caspase-3, and RIPK3). Then, the biotinylated-secondary antibody and streptavidin peroxidase were respectively applied for 20 min at room temperature to the slides. The immunoreactivity was visualized by using aminoethyl carbazole chromogen. The slides were washed with phosphate-buffered saline after each step. All slides were counter-stained with hematoxylin for 1 min, washed in tap water, and covered with coverslips.

For immunohistochemical evaluation, ten randomly selected areas in each slide were examined and the immunostaining was semi-quantitatively scored based on the prevalence of immunoreactivity (0: 0-25%, 1:26-50, 2:51-75%, 3:76-100%) and the intensity of immunoreactivity (0: none, 1: mild, 2: moderate, 3: severe). The total score was calculated as follows [23].

Total score= (immunostaining prevalence) X (immunostaining intensity).

All assessments were carried out through a light microscope and image analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

### Statistical analysis

Data were analyzed using the IBM SPSS software program for Windows, version 26.0 (SPSS Inc., Chicago, IL). The Shapiro-Wilk test was used to determine whether the data were distributed normally. Because the data is not normally distributed, the Kruskal-Wallis was used, and when significant differences were determined, pairwise comparisons were made using the Mann-Whitney U test with Bonferroni correction. $p < 0.05$ was considered a statistically significant difference. Data were expressed as median (minimum-maximum).

## Results

### Creatinine and urea levels

Table 2 shows the mean values of the kidney function indices (serum urea and creatinine) determined by the biochemical analysis of blood. Biochemical blood analysis showed significant differences ($p < 0.001$) in creatinine and

**Table 1.** The level of creatinine and urea of each group.

| Groups | Urea | Creatinin |
|---|---|---|
| Control | 59.0 (52-63) | 0.6 (0.5-0.7) |
| FAV | 57.0 (48-72) | 0.8 (0.5-1.0) |
| HCQ | 51.5 (43-57)[a] | 0.6 (0.4-0.6) |
| HCQ+FAV | 49.5 (45-63)[a] | 0.6 (0.5-0.7) |
| HCQ+FAV+DEX | 57.5 (49-66) | 0.5 (0.4-0.6)[a] |
| MOL | 43.0 (37-50)[a] | 0.5 (0.4-0.7)[a] |
| MOL+DEX | 51.0 (31-63)[a] | 0.5 (0.4-0.6)[a] |

Data are expressed as median (minimum-maximum).
[a]$p < 0.001$ significant increase compared to the control group.

**Table 2.** The results of the histopathological and immunohistochemical evaluation of each group.

| Groups | HSP60 | Caspase 3 | RIPK3 | Tubule degeneration |
|---|---|---|---|---|
| Control | 4 (2-6) | 0 (0-3) | 0 (0-2) | 0 (0-1) |
| FAV | 6 (2-9)[a] | 0 (0-4) | 0 (0-6)[b] | 1 (0-2)[c] |
| HCQ | 4 (2-6) | 4 (0-6)[a] | 4 (0-6)[a] | 2 (0-3)[a] |
| HCQ+FAV | 6 (2-6) | 3 (0-6)[a] | 3 (0-6)[a] | 1 (0-3)[a] |
| HCQ+FAV+DEX | 6 (3-6) | 2 (0-6)[a] | 3 (0-6)[a] | 1 (0-3)[a] |
| MOL | 6 (3-9)[a] | 3 (0-6)[a] | 2 (0-6)[a] | 1 (0-3)[c] |
| MOL+DEX | 6 (3-9)[a] | 2 (0-6)[a] | 2 (0-6)[a] | 1 (0-3)[c] |

Data are expressed as median (minimum-maximum).
[a]$p < 0.0001$ significant increase compared to the control group.
[b]$p < 0.05$ significant increase compared to the control group.
[c]$p < 0.01$ significant increase compared to the control group.
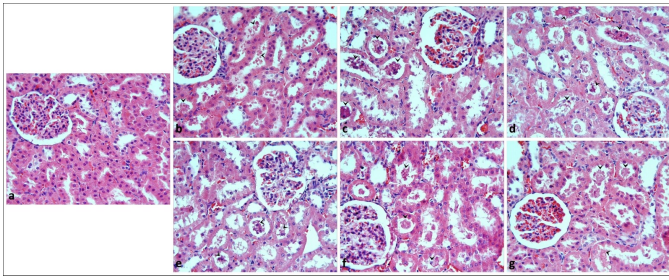
**Figure 1.** Representative photomicrographs of kidney stained with H&E. While the control group (a) is observed in normal appearance, FAV (b), HQL (c), HQL+FAV (d), HQL+FAV+DEX (e), MOL (f), and MOL+DEX (g) groups exhibit tubular desquamation (arrowheads). Magnification, 400x.
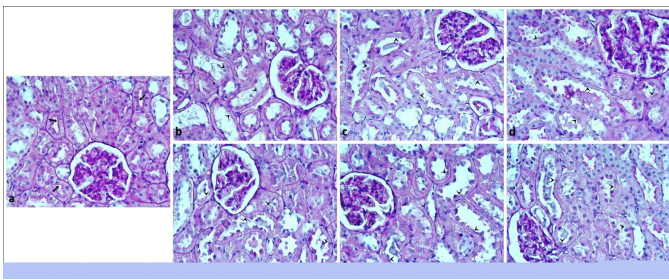


**Figure 2.** Representative photomicrographs of kidney stained with PAS. The control group (a) exhibits an intact brush border (arrows). FAV (b), HQL (c), HQL+FAV (d) groups show the loss of brush border (arrowheads). Magnification, 400x.
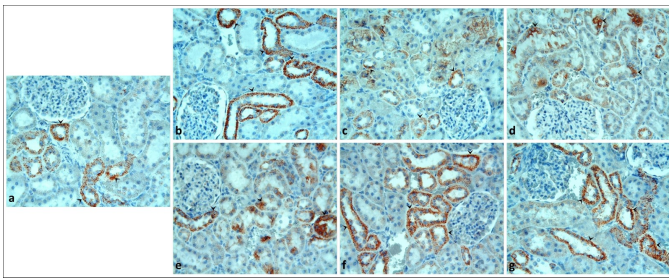


**Figure 3.** Representative photomicrographs of kidney tissues immunostained with HSP60 in the control (a), FAV (b), HQL (c), HQL+FAV (d), HQL+FAV+DEX (e), MOL (f), and MOL+DEX (g) groups. Arrowheads indicate immunoreactive cells. Magnification, 400x.

urea levels depending on different drug groups. Compared to the control group, in the groups exposed to MOL, MOL+DEX, and HCLQ+FAV+DEX drugs, creatinine levels decreased. However, the creatinine level was still within the normal range when compared with the literature since rat serum creatinine levels are usually in the range of 0.4–0.8 mg/dL. Another indicator of kidney function is the levels of urea. High urea levels suggest poor kidney function. The urea level range was 43-59 mg/dL. The urea level was estimated within the normal range for

all groups. The level of creatinine and urea of each group were summarized in Table 1.

*Histopathological findings*

In slides stained with H&E, tubular structures and glomeruli in the control group kidneys generally had a normal histologic appearance except rare and slight tubular epithelial desquamation (Figure 1). The brush border of proximal tubules in this group was observed as intact in the PAS-stained slides (Figure 2). Significant histopathological changes were observed in the tubules in the HCQ, FAV, and MOL groups and all combined groups (p<0.05). In tissue slides stained with H&E belonging to these groups, necrotic changes, and epithelial desquamation were observed in the renal tubules in certain areas (Figure 1). In addition, loss of brush border in proximal tubule epithelium was remarked in PAS-stained slides in these groups (Figure 2). Although no statistically significant difference was observed between these drug-administered groups in terms of histopathological changes, the HCQ group was the group with the most severe changes. The scores of histopathological changes in each group were summarized in Table 2.

*Immunohistochemical findings*

In immunohistochemical evaluations, HSP60 to determine oxidative stress, caspase 3 to determine apoptosis, and
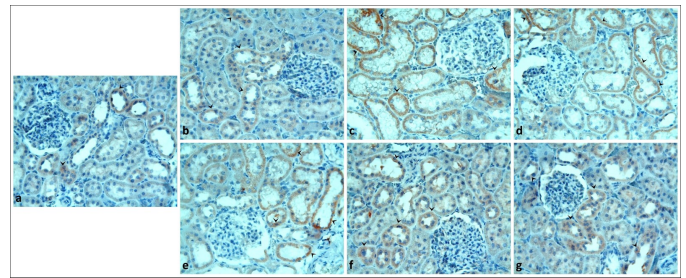


**Figure 4.** Representative photomicrographs of kidney tissues immunostained with caspase-3 in the control (a), HCQ (b), FAV (c), MOL (d), HCQ+FAV+DEX (e), MOL+DEX (f), and MOL+FAV (g) groups. Arrowheads indicate immunoreactive cells. Magnification, 400x.
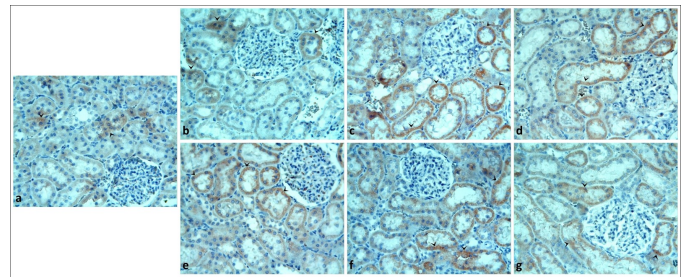


**Figure 5.** Representative photomicrographs of kidney tissues immunostained with RIPK3 in the control (a), FAV (b), HQL (c), HQL+FAV (d), HQL+FAV+DEX (e), MOL (f), and MOL+DEX (g) groups. Arrowheads indicate immunoreactive cells. Magnification, 400x.

RIPK3 to determine necroptosis were used. Although the immunoreactivity of HSP60 (Figure 3), caspase 3 (Figure 4), and RIPK3 (Figure 5) was observed as brown staining in the cytoplasm of renal tubule cells, there were no prominent immunoreactivity in the glomeruli.

The slight HSP60, caspase 3, and RIPK3 immunoreactivity were seen in the control group. Compared with the control group, the immunoreactivity of HSP60 was markedly increased in the FAV, MOL, MOL+DEX, and MOL+FAV groups (p<0.05). Interestingly, HSP60 immunoreactivity in HCQ group was similar to the control group. Although the HSP60 level was higher in the HCQ+FAV, and HCQ+FAV+DEX groups compared to the control group, this difference was not statistically significant. Caspase 3 immunoreactivity was similar to the control group in the favipiravir group, while it was significantly higher in all other groups compared to the control group (p<0.05). RIPK3 immunoreactivity was found to be significantly higher in all groups than in the control group. On the other hand, no statistically significant difference was observed between these drug-administered groups in terms of RIPK3 immunoreactivity. The results of the immuno-histochemical assessment of each group were summarized in Table 2.

## Discussion
SARS-CoV-2-mediated coronavirus disease occurred as one of the leading causes of death all around the world, and it became one of the most severe public health subjects in the past couple of years [1]. Because developing new drugs to manage coronavirus disease efficaciously is a compelling and time-consuming process the present drugs were used for the treatment of coronavirus disease after their effectiveness has been demonstrated after extensive studies. HCQ, FAV, MOL, and DEX are some of these drugs. Evidence from studies suggests that these drugs are effective drugs against SARS-CoV-2 [7, 24]. On the other hand, renal functions may be changed with the medications used for the treatment of COVID-19 [4, 5, 15]. But, there is limited data on the effects of these drugs on the kidney. In our study, rats were given orally these drugs and their combinations for 5 days adapted to the human treatment protocol, and the serum urea and creatinine levels were measured. In addition, histological changes in the kidney were examined, and HSP60, caspase 3, and RIPK3 expression levels were evaluated immunohistochemically in renal tissues.

In the current study, urea and creatinine are important biomarkers for kidney failure detection. Compared to the control group, in the groups exposed to different drugs, creatinine and urea levels changed depending on different drug groups. However, the levels were within the normal range [25].

In this study, the effects of HCQ, FAV, MOL, and their combination on oxidative status were evaluated by semi-quantitatively measuring the expression level of HSP60 by immunohistochemical pathways. HSPs, which are highly sensitive chaperones, are expressed at a low level under normal conditions, but HSP expression is rapidly induced in cells exposed to stress for a variety of reasons including chemicals, or pharmacological agents. Besides their involvement in cellular resuscitation, they are associated with cellular injury and are overexpressed against oxidative stress [26]. The results of our immunohistochemical analysis showed that FAV, MOL, and MOL+DEX treatment caused a significant increase in HSP60 expression in renal tissue. However, HSP60 immunoreactivity was found to be similar to the control group in HCQ, HCQ+FAV, and HCQ+FAV+DEX groups.

Increased HSP60 expression indicates that these drugs induce oxidative stress [26, 27]. Consistent with our results, several experimental studies have reported that FAV and MOL induce oxidative stress. FAV, given emergency use approval to treat COVID-19 in many countries, created organ damage associated with the induction of oxidative imbalance according to the results of recent studies. Günaydın-Akyildiz et al. [28] noted that FAV-mediated oxidative stress resulted in DNA damage in cardiomyoblast cells and fibroblasts in the skin. In addition, Doğan et al. [29] and Kara et al. [30] reported that FAV increased oxidative stress in rat liver and kidney tissue in their experimental studies. MOL, a new hope for the management of COVID-19, has also been shown in several studies to induce oxidative stress. In a recent study, researchers demonstrated that biomarkers of oxidative stress were increased in cultured cells treated with $\beta$-d-N4-hydroxycytidine, a MOL active metabolite [31]. Similarly, Kobayashi et al. [32] reported that in the isolated DNA, $\beta$-d-N4-hydroxycytidine induces ROS generation and subsequently oxidative DNA damage.

Oxidative stress, an important aspect of drug-induced toxicity evaluation research, is an imbalance between the formation of free radicals and reactive metabolites and their elimination by protective mechanisms called antioxidants or radical scavengers [33]. In this case, a series of pathological events may occur due to the formation of oxidative attacks [34]. Indeed, in the current study, it was observed that all drugs and their combination caused histological damage. FAV, MOL and MOL+DEX groups exhibited prominent necrotic changes, desquamation, and loss of brush border in renal tubule cells. Unlike the FAV group, necrotic changes were higher in the MOL groups.

It has been reported in previous animal studies that FAV treatment causes such histological changes in the kidney. Doğan et al. [29] and Kara et al. [30] observed degenerative changes such as tubular dilatation, tubular necrosis, and brush border loss in kidney tissue because of FAV administration. The elimination of FAV is through the kidneys (approximately 90%). Initially, FAV is metabolized in the liver by aldehyde oxidase and xanthine oxidase, resulting in T-705M1 which is an inactive metabolite. T-705M1 is then excreted by the kidneys. The possible renal toxicity of favipiravir may be related to the M1 pathway [35]. Disruption of kidney functions is one of the critical side effects of FAV frequently observed in clinical studies. These studies revealed an increase in serum urea, creatinine, and c-reactive protein concentrations with the administration of FAV in the results of serum function tests [5, 36]. It was concluded that the change in the renal functions caused by the FAV might lead to related toxicity.

Data related to the adverse-effect profile of MOL are limited to clinical trials. Although the most common side ef-

fects have not caused concern, some clinical cases including seizure, arrhythmia, cardiac arrest, cardiac failure, and renal impairment have emerged less frequently [36]. MOL is minimally excreted as an unchanged drug in the urine [24]. However, particularly noteworthy is the renal dysfunction reported in Hasan Esat Yücel's recent case report. In this case, it was documented that a 67-year-old patient developed severe kidney function deterioration with severe vomiting and diarrhea due to MOL [15].

Renal tubule cell death is a major cause of toxicity related to many drugs [37]. Cell death is an important process in the maintenance of tissue integrity through the elimination of excess or damaged cells in the development of an organism and throughout life. However, the uncontrolled progression of cell death may result in more serious pathological conditions [38]. Thus, we evaluated the expression levels of caspase-3 and RIPK3 proteins immunohistochemically to investigate these changes. Caspase-3 is one of the important apoptotic enzymes and responsible for the initiation of the apoptotic process [39]. RIPK3 is an important protein in the regulated cell death pathway that mimics features of apoptosis and necrosis called necroptosis [40].

In the current study, FAV treatment did not change the expression level of caspase 3. However, FAV led to a prominent increase in the expression levdose releeel of RIPK3 in the tubule cells. These findings are consistent with the other results of the present study. As mentioned above, morphological characteristics of necrotic cell death [38] such as decreased cytoplasmic eosinophilia, loss of the brush border, and cytoplasmic swelling were histopathologically observed in some slides.

MOL administration significantly increased caspase 3 and RIPK3 expression levels in the tubule cells compared to the control group. Histological data of our study revealed that MOL can cause necroptotic and apoptotic cell death-mediated renal tubular cell loss. Exposure to harmful stimuli mainly affects tubular epithelial cells. Growing evidence has documented that apoptotic or necroptotic cell death is the important reasons that caused renal tubular cell depletion and renal damage. Tubules are responsible for the reabsorption and secretion of several solutes, and damage to this nephron segment is one of the reasons for disruption in renal function [41, 42].

Interestingly, while the HCQ group was similar to the control group in terms of HSP60 immunoreactivity, which is a marker of oxidative stress, the increase in HSP60 immunoreactivity in the HCQ+FAV and HCQ+FAV+DEX groups were not statistically significant. HCQ, proposed as a treatment for COVID-19, has been examined for many decades, principally in the context of its use as an antimalarial medication, which kills the parasite by inducing the oxidative stress. However, there is evidence to suggest that HCQ not only causes oxidative stress on the parasite but also causes systemic oxidative stress [43]. The lower HSP60 immunoreactivity in HCQ groups can be explained by the fact that HCQ rapidly causes necrosis by affecting kidney tubule cells. Indeed, in our histopathological examinations, the most severe necrotic changes were observed in the HCQ groups. Consistent with these data, HCQ administration increased RIPK3 expression levels in the tubule cells compared to other all groups. In the current

study, HCQ administration also increased caspase 3 expression levels in the tubule cells. Significant renal tubular cell loss mediated by both necroptotic and apoptotic cell death will result in the downregulation of HSP60 expression as mentioned above. Consistent with our results, El Shishtawy et al. indicated that HCQ can cause focal necrosis in the kidney tubules of rats [44]. Ertugrul et al. also documented that exposure to HCQ increases can cause to initiate and spread apoptosis and mitochondrial oxidative stress in ARPE 19 cells [45]. Moreover, hydroxychloroquine, a lysosomotropic antimalarial drug, can result in significant damage to renal tissue due to its adverse effects such as increased lysosomal pH and inhibiting autophagy, a fundamental mechanism for the survival of injured cells [46].

The effects of DEX were also investigated in the study. It was determined that the HCQ+FAV+DEX group and MOL+DEX groups were not different from the groups in which only HCQ and MOL were administrated in terms of histopathological and immunohistochemical parameters. DEX therapy is a double-edged sword and there are conflicting data on the effects of DEX in the literature. DEX can cause oxidative stress in cells by consuming antioxidant molecules or inhibiting antioxidant enzymes. There are some research data resulted in the rising of lipid peroxidation and blocking of major antioxidant enzymes in response to high doses of DEX exposure [47, 48]. Conversely, some researchers reported that DEX alleviated lipopolysaccharide-induced acute kidney injury by showing anti-oxidative, anti-apoptotic, and anti-inflammatory effects [49, 50]. We are of the opinion that these conflicting results regarding DEX may be dose-related. As a matter of fact, the dose of DEX administrated in our study was high and was determined in accordance with the treatment protocol administrated in the clinic for the treatment of COVID-19.

## Conclusion

In our study, the effects of HCQ, FAV, MOL, DEX, and their combinations on kidney tissue without viral load were evaluated histologically and immunohistochemically. The data of our study revealed that all medications resulted in mild to moderate damaging effects on the renal tissue. Furthermore, HCQ caused more prominent damage than other drugs. However, according to the data of our study, these side effects of drugs seem to be tolerable in individuals without kidney disease. But, the side effects of these drugs on kidney tissue may be more clinically significant in patients with impaired renal function. Therefore, if these drugs are to be used in these patients, we recommend dose adjustments for these drugs.

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