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Toxicity of antiviral Remdesivir on human liver's ATP binding cassette subfamily D member 3 transporters

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Abstract

Background and objective: The World Health Organization advises against the use of the antiviral drug Remdesivir to treat severe COVID-19 infections due to potential toxicity. The molecular mechanism of this toxicity is not well established. ATP-binding cassette (ABC) transporters play an essential role in the transport of various drugs in many illnesses.

Objective: This study examines the possible role of ATP-binding cassette, subfamily D, member 3 (ABCD3) in Remdesivir toxicity.

Methods: Real-time PCR and MTT assays were used to demonstrate the toxicity of Remdesivir on *ABCD3* gene expression in the HepG2 cell line. Enzyme-linked immunosorbent assay was used to detect serum ABCD3 levels, Prestige24i was used to detect C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) in the serum. Nano-Checker710 was used to detect D-dimer in the serum of the patients.

Results: Remdesivir exhibits dose-dependent toxicity to the HepG2 cell line. The drug toxicity is significantly increased at three doses of 5, 10, and 20 µg/ml in virus-free hepatic cell lines. It suppressed *ABCD3* gene expression in both the HepG2 cell line and COVID-19 patients' sera. COVID-19 virus increases serum levels of CRP, ALT, AST and D-dimer. The drug lowers serum CRP, transiently lowers D-dimer, and increases ALT and AST levels.

Conclusion: Remdesivir suppressed ABCD3 gene expression and increased levels of inflammatory markers. Remdesivir contributes to hepatocyte damage independently of the COVID-19 virus.

Keywords: Remdesivir; Covid-19; Hepatotoxicity; Cell transporters; Expression.

Introduction

Remdesivir is a nucleotide analogue prodrug that inhibits viral RNA polymerases by metabolizing to an analogue of adenosine triphosphate. Remdesivir was created to treat severe viral diseases like Ebola. It has been discovered to have an impact on other Coronaviridae family RNA viruses, namely COVID-19, SARS-CoV, and MERS-CoV.[1] Remdesivir is not an effective treatment due to the high death rate (51.3%)in users.[2] Remdesivir was approved by the U.S. Food and Drug Administration (FDA) for the treatment of COVID-19 despite having side symptoms such as elevated liver enzymes, renal impairments, hypotension, diarrhea, rash, organ failure syndrome, and septic shock.[3]Despite the FDA's approval, the World Health Organization (WHO) continues to oppose the use of Remdesivir in COVID-19, particularly in severe cases.[4,5]

According to WHO there was no therapeutic benefit from the administration of Remdesivir in patients who were hospitalized with COVID-19, who needed

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ventilation devices, and had symptoms for more than 7 days. As a result, the use of Remdesivir for the treatment of COVID-19 is still debatable and under investigation. A few occurrences of acute respiratory distress syndrome, bacterial infection, and hepatatorenal syndrome have also been associated to Remdesivir.[6,7]

The most advantageous method for Remdesivir administration is intravenous, since it allows for 100% drug absorption.[8] Remdesivir is absorbed in the body after intravenous infusion during 30-120 minutes. Adults receive it by injection as follows: After a 200 mg intravenous loading dose on day one, the patient will receive 100 mg intravenously every day for the following 2-5 days, or up to 10 days.[9] In order to raise the concentration of Remdesivir triphosphate within cells, Remdesivir has been manufactured as a prodrug.[10] After a gradual infusion, the prodrug spreads over the cell membrane and undergoes hydrolysis processes in the cytoplasm. GS-441524, a nucleoside core, is hydrolyzed to produce a more water-soluble monophosphate that cannot leak out of the cells.[11]

The liver serves a critical function in protection from potentially dangerous drug assaults by converting lipophiles into more water-soluble compounds that can be efficiently eliminated from the body via the urine.[12,13] Hepatotoxicity, often known as liver toxicity, can occur as a result of medication exposure or contact with other non-pharmacological substances. There are many routes to support the liver's detoxification process and undo these consequences.[14] Among liver proteins that take part in liver detoxification of drugs are members of ATP-binding cassette (ABC) transporters. ABC transporters are a super family of membrane transporter proteins that effectively relocate a diverse range of molecules, including simple molecules such as nucleosides to complex organic compounds such as lipids, as well as xenobiotics including drugs and toxins across the plasma membrane and intracellular membranes.[15,16] ABC transporters are found in the plasma membrane of prokaryotes and eukaryotes. In eukaryotic cells, ABC transports are found on the membranes of many organelles such as peroxisomes, mitochondria, lysosomes, and endoplasmic reticulum.[15,17]

One of the most prevalent peroxisomal membrane proteins in hepatocytes is ABCD3, also known as CX3CL1, PMP70, PXMP1, C3Xkine, and fractalkine.[18,19] ABCD3 is the first peroxisomal ABC transporter to be identified, is involved in the transit of a number of fatty acids. It is required for the transportation of the peroxisomal membrane's substrates for peroxisomal metabolism. It has been established that ABCD3 has substrate specificity that overlaps with ABCD1 and ABCD2, but ABCD3 has been demonstrated to play roles in the transport of more hydrophilic substrates. Additionally, ABCD3 has the broadest substrate specificity because it is involved in the transport of branched-chain fatty acids like phytanic acid, dicarboxylic acids (DCAs), and C27 bile acid intermediates.[20, 21]

An ABCD3 deficit causes the condition known as congenital bile acid synthesis defect-5.Itis a very rare disorder that has only ever been identified in patients with severe liver disease. In this disease the final exon of the *ABCD3* gene is deleted, causing a loss of 24 amino acids at the protein's C-terminus and resulting in the ABCD3 insufficiency.[22] In some cases, the patient had progressive hepatosplenomegaly, which eventually caused death. Moreover, in comparison to the control, patient fibroblasts showed fewer expanded peroxisomes and less pristanic acid-oxidation.[23]

ABC transporters play important roles in the emergence of chemoresistance by controlling the flow of anticancer medications into cancer cells. Study findings indicate, fatty acid oxidation inhibition causes colorectal cancer cells

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to undergo apoptosis. ABCD3 is also implicated in the regulation of ABC transporters, transmembrane transport, fatty acid -oxidation, and ATP production after food catabolism, according to research using gene co-expression network analysis in CRC.[24] *ABCD3* down regulation has been linked to improved chemotherapy sensitivity and time to progression in ovarian cancer patients.[25]

Publications on the *ABCD3* cell signaling in hepatocytes exposed to the COVID-19 virus or Remdesivir are, nevertheless, scarce. Therefore, the goal of this study is to compare Remdesivir effects on liver function with and without COVID-19 infection, with an emphasis on liver ABCD3 transporters. Our hypothesis is that Remdesivir negatively affects ABCD3 expression, which then has an impact on how well patients respond to COVID-19 viral treatment.

Methods

Human liver cell line and blood samples from people infected with the COVID-19 virus were used to answer questions regarding the effects of Remdesivir on the human liver, in particular its effects on *ABCD3* gene expression and hepatotoxicity. This study also aimed to understand how the COVID-19 virus and Remdesivir affect the serum levels of ABCD3, ALT, AST, D-dimers, CRP and creatinine.

Cell culture

The HepG2 cell line was purchased from Cell Bank Pasteur Institute of Iran. The cells were cultured in Dulbecco's Modified Eagle Media (DMEM) medium powder high glucose (with L-glutamine, pyridoxine hydrochloride, 110 mg/L sodium pyruvate). The media contained 10% Fetal Bovine Serum, 1% Antibiotic solution and 0.1% Antifungal Amphotericin B. All cells were incubated at 37C with a CO2 level of 5%. Remdesivir drug used was purchased from BDR Pharmaceuticals Int'l Pvt.Ltd., India.

Human subject recruitment

Blood was drawn from the patients after consent from the patients and the Emirates Hospital administration in Erbil, Iraq and formal approval from the College of Sciences ethics committee at Salahaddin University-Erbil, Iraq, in order to ensure proper handling of human subjects. Patients with COVID-19 at the Emirates Hospital had their blood drawn. Total of 150 serum samples were collected, yet not all 150 samples were used for all analyses. 88 serum samples were used to analyze the ABCD3 protein, 96 were used to analyze the ALT and AST, 129 were used to analyze the CRP, 121 were used to analyze the D-Dimer, and 148 were used to analyze the creatinine. Patients were given the drug for 5 days, but rarely for 10 days if they were in critical condition. They were immediately given 200 mg of Remdesivir and then 100 mg by intravenous infusion mixed with normal saline for the next few days. The patients were divided into four groups, the control group who consisted of healthy individuals with no COVID-19 infection. The second group named 'No Remdesivir' group consisted of individuals who were PCR COVID-19 positive but they didn't take the drug by the time of blood collection. The third group; 'Remdesivir 1-2 days' group, the individuals of this group were PCR COVID-19 positive; they took the drug treatment of Remdesivir for one or two days. The fourth group, 'Remdesivir ≥ 3 days' group, received Remdesivir medication for three days or longer, occasionally up to ten days depending on the severity of the condition, and were tested by the hospital lab for PCR COVID-19 positivity.

MTT assay

Cell Proliferation Kit (MTT), (Merck, Germany) was used for non-radioscopic spectrophotometric quantification of cell proliferation and viability in cell populations in 96-well plate format. Cells were incubated overnight at 37°C with 5% CO2. The next day, after removing the medium

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the cells were treated with 100 µl of different concentration of Remdesivir 1µg/ml, 2.5 µg/ml, 5µg/ml, 10µg/ml and 20 µg/ml in DMEM serum free medium and incubated for 48h at 37°C with 5% CO2. After 48hr the media were aspirated and 100 µl tetrazolium reagent (10µl of 5 mg/ml 3-(4, 5-dimethyl-2-thiazolyl)- 2,5-diphenyltetrazolium bromide (MTT) was added, which was dissolved in 90µl of medium. The 96 well plate was incubated for 4 hr. at 37°C 5% CO2. After 4hr, the media were aspirated (kept the formazan precipitate) and added 100µl DMSO into the wells. Finally, the absorbance was read at 540 nm wavelength by a microplate reader. The percentage of cell viability was calculated.

ABCD3 gene expression

The subsequent step involved a molecular analysis of the *ABCD3* gene (using the GAPDH as a reference gene) to see whether the medication altered their expression. After treatment of the cells for 48 hours with (0, 1, 2.5, 5, 10, and 20µg/ml), total mRNA was extracted, cDNA for the genes of interest were amplified using real-time PCR. Changes in gene expressions were measured and compared to the control. The following kits were employed for gene expression measurements: Primers (Sinaclon,Iran), DENAzist Asia Total RNA isolation kit (Biotechnalogy Company, Iran), Easy TM cDNA Synthesis Kit (ParstousBiotechnalogyCompany,Iran), 2X Taq PCR Master Mix (ParstousBiotechnalogy Company, Iran), RealQ Plus 2x Master Mix Green Without ROX TM (AMPLICON,Denmark). The primers used were as follows:

ABCD3-F: 5-GCTGGTGTCTCGAACATATTGT-3,
ABCD3-R: 5-ATCTTTCCTGCTACGACCAATG-3,
GAPDH- F: 5-CCCCTTCATTGACCTCAACTAC-3,
GAPDH- R: 5-GATGACAAGCTTCCCGTTCTC-3

Serum biochemistry tests

Patients' serum were analyzed for detection of ABCD3, D-dimer, Creatinine, and C- reactive protein (CRP), AST, and ALT concentrations. Microplate reader (Awareness Technology, Inc. Stat Fax, USA) was used to read the results of ABCD3 protein, Prestige24i for CRP, AST, ALT AND Creatinine, Nano-checker710 for D-dimer. Human Fractalkine (chemokine C -X3 Motif Ligand 1) ELISA Kit (Al-shkairate establishment for medical supply, Jordon) for ABCD3, Nano-Check TM D.Dimer (Nano-Ditech Corporation, USA), CRP wide range BIOLIS KIT (CliniChem, Hungary), CREATININE ENZYMATIC BIOLIS KIT(CliniChem, Hungary), ALT (GPT) BIOLIS KIT (Clinichem, Hungary) and AST (GOT) BIOLIS KIT (Clinichem, Hungary) kits were used. The manufacturer's protocols were followed for protein concentration measurements.

Statistical analysis

Graph Pad Prism 8.0.1. was used for statistical analyses. In the case of non-parametric data results for ABCD3, D. dimer, CRP, AST, ALT and Creatinine proteins in serum, Kruskal-Wallis test was used. Dunn's multiple comparisons test was used as a Post hoc test to detect significant differences across groups. The results are expressed as median, 25th percentile and 75th percentile. In cell culture and PCR experiments, the data in the results were parametric. A standard one-way ANOVA was utilized, and Dunnet's multiple comparisons test was used as a post hoc test. The results are expressed as mean ± SEM. A *P*-value of ≤0.05 is considered statistically significant.

Results

Remdesivir decreases relative gene expression of ABCD3 gene in HepG2 human liver cell line

Figure 1 shows the effect of five different doses of Remdesivir (1, 2.5, 5, 10 and 20 µg/ml) on the relative gene expression of the *ABCD3* gene. With increasing drug concentration, the relative expression of *ABCD3* gene decreased. The mean expression of *ABCD3* in the control group that was not treated with the drug is 1 ± 0

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SEM. The mean gene expression of our examined gene was reduced in cells treated with 1 (µg/ml) Remdesivir, but the difference from the control was not statistically significant, with mean of 0.868 ± 0.068 SEM. The second dose 2.5 (µg/ml) decreased the expression of *ABCD3* gene to 0.555 ± 0.095 SEM, and a statistically significant difference was found compared to the control at a *P*-value of 0.011. Additionally, there was a reduction in our gene expression in cells treated with 5 (µg/ml) of our drug, mean expression was 0.324 ± 0.0399 SEM. In comparison to the control, the expression significantly decreased at *P* value of <0.001. Further decrease in *ABCD3* gene expression was found in cells treated with 10 (µg/ml), therefore, in comparison to the control, the expression reduced dramatically at *P*-value of <0.001, with mean expression of 0.296± 0.078 SEM. The highest decrease of gene expression was found in the last group of cells where they were treated with 20 (µg/ml) in comparison to the control, it was significantly different, *P*-value of <0.001, with a mean of 0.189 ± 0.033 SEM, Figure 1.

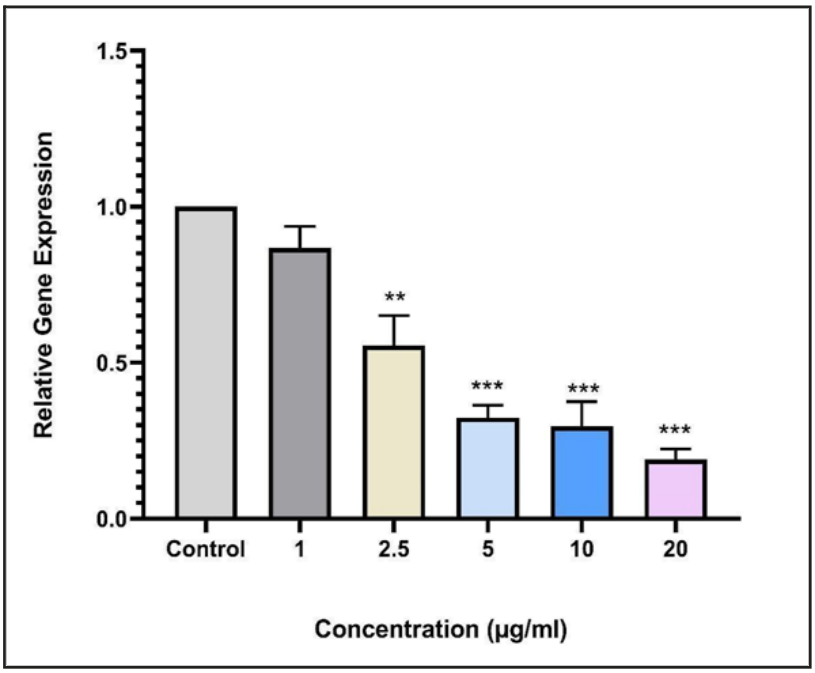

Figure 1 Effect of Remdesivir on *ABCD3* gene expression detected by Quantitative RT-PCR. The asterisks ** indicate statistical differences at *P* = 0.0011 or *P* <0.005, asterisks *** *P* < 0.001

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Remdesivir decreases cell viability in liver HepG2 cell line

Figure 2 represents the effect of five different doses of Remdesivir (0, 1, 2.5, 5, 10, 20µg/ml) on the viability of the HepG2 cell line. With increasing drug concentration, the viability of cells decreased. All treatment groups were compared to the control group (0µg/ml), with cell viability of 100%. In the second group, the dose was 1µg/ml the mean viability was 96.83% ± 2.191 SEM, the viability of cells was decreased but there was no statistically significant difference with the control group. In the third dose (2.5µg/ml) the mean viability of cells was 96.02% ±2.080 SEM, cell viability likewise decreased but was not statistically significant compared to the control group.

The fourth dose (5µg/ml) of Remdesivir decreased the mean viability of the cell to 82.73% ±3.482 SEM. Thus, there was a significant difference with the control group at p-value of 0.003. 10 µg/ml of Remdesivir also decreased mean cell viability to 84.23% ±3.068 SEM, and a significant difference was detected with the control group at a *P*-value of 0.009.

The last dose of Remdesivir (20 µg/ml) further decreased the mean of cell viability to 80.90 ± 2.236 SEM, hence the difference was statistically significant with control at a *P*-value of <0.001. The highest percentage of cell death detected in this group was approximately 20%.

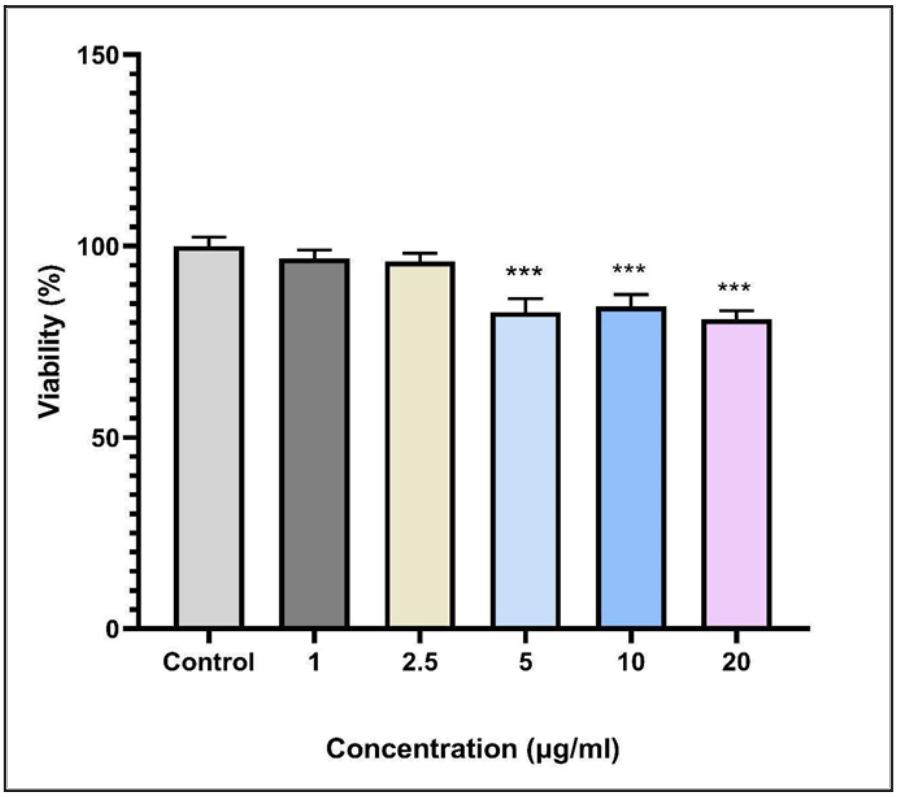

Figure 2. Cytotoxicity of Remdesivir on HepG2 cell lines. Cellular viability was analyzed by MTT assay. The asterisks *** indicate statistical differences at the *P*-value of 0.003, 0.009 and <0.001, respectively

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The COVID-19 virus boosts the levels of ABCD3, whereas Remdesivir suppresses them

Figure 3 illustrates differences in ABCD3 protein expression in sera of four different groups of patients. The lowest concentration of ABCD3 protein was seen in the first group, the control group, which was tested by PCR and verified to be free of COVID-19 virus and had not taken the drug Remdesivir. The median concentration detected was 681.6, Table 1. The second group of patients were documented by PCR to have COVID-19 virus infection but had not taken Remdesivir; the expression of ABCD3 protein was significantly increased in the second group in comparison to the control group, with the *P*-value of 0.037. The protein concentration in this group was the highest compared to all other groups. The median concentration detected was 1121, Table 1. COVID-19 patients in the third group were on their first and second days of Remdesivir medication when the sera were collected. This group had lower levels of ABCD3 protein than the second group, but the difference was not significant, with a median value of 833.3. The concentration of ABCD3 protein dropped even more from this group to the last, but the difference was not statistically significant, so there were no significant differences between this group with the control and the other groups. Patients who had taken Remdesivir for three days or more are in the fourth group of treatment there are no statistically significant differences between this group and the control. This group had the lowest concentration of ABCD3 protein when compared to the second and third groups with a median value of 535.8. This group differed significantly from the second (No Remdesivir) group. The ABCD3 protein concentration had dropped drastically, with a *P*-value of 0.001.

Table 1 The concentration of ABCD3 protein in sera of COVID-19 patients on different days after Remdesivir treatment

Treatment groups	Median (Interquartile range)
Control	681.6 (561.1- 944.1)
No Remdesivir	1121 (692.8-1439)
Remdesivir 1-2 days	833.3 (578.0-1041)
Remdesivir ≥ 3 days	535.8 (360.5-742.0)

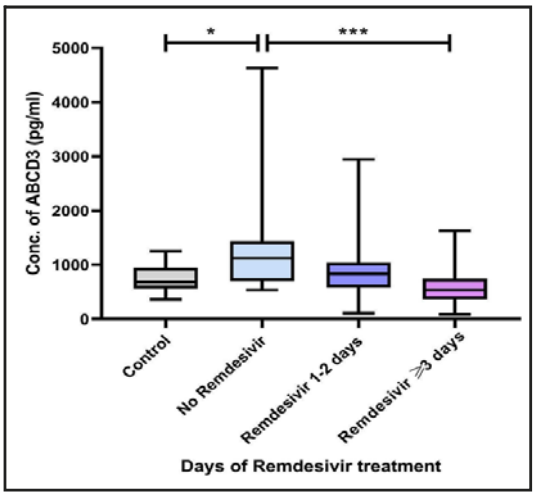

Figure 3 Effect of Remdesivir on ABCD3 protein expression in COVID-19 infected patients, detected by ELISA. The asterisks *** represents statistical differences at *P* <0.001, asterisks * statistical differences at *P* <0.037, N=88.

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Remdesivir elevates ALT levels significantly more than the COVID-19 virus

An ALT blood test is used to assess the health of the liver. This test can assist identify liver problems because ALT can escape into the blood if liver cells are damaged. This investigation tested three groups for ALT levels; control group (No virus, No medication), No Remdesivir group (patients with COVID-19 but no Remdesivir treatment), and Remdesivir treatment group (patients with COVID-19 and Remdesivir treatment). There was a significant difference between the control group and the No Remdesivir group (P = 0.006). Likewise, there were significant differences between the control and Remdesivir treatment group (P <0.001). Moreover, there is a significant difference between the No Remdesivir group and the Remdesivir treatment group with a P-value of 0.041. Our results show that COVID-19 infection alone can increase ALT levels significantly in comparison to the control group. Remdesivir treatment added to the liver cell damage indicated by a further increase in the level of ALT in serum of hospitalized COVID-19 patients, Figure 4, Table 2.

Table 2 The concentration of ALT enzyme in sera of COVID-19 patients before and after Remdesivir treatment

Treatment groups	Median (Interquartile range)
Control	26.10 (18.15-28.61)
No Remdesivir	44.0 (25.48-58.98)
Remdesivir Treatment	53.30 (41.60-92.0)

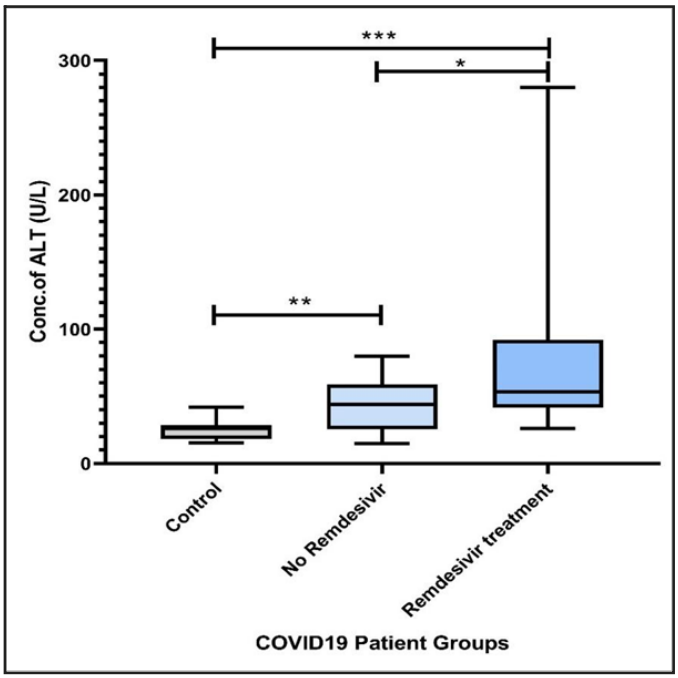

Figure 4 Effect of Remdesivir on ALT enzyme levels in the blood of COVID-19 patients detected by Prestige24i. The asterisks * represents statistical differences at P <0.05, asterisks** statistical differences at P = 0.006 and asterisks *** statistical differences at P <0.001, N=96.

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Remdesivir raises AST levels induced by COVID-19 viral infection

To determine how well the liver is functioning, AST levels in the blood are examined. An overabundance of this enzyme can be a sign of liver damage. Similar to ALT, this inquiry tested three groups for AST levels; control group (No virus, No medication), No Remdesivir group (patients with COVID-19 but no Remdesivir treatment), and Remdesivir treatment group (patients with COVID-19 and Remdesivir treatment). There was a significant increase in the AST levels in the No Remdesivir group and Remdesivir treatment group compared to the control group, with p-values of 0.035, and *P* <0.001, respectively. Besides, there is a significant difference between the No Remdesivir group and the Remdesivir treatment group with a *P*-value of 0.035. Our findings demonstrate that COVID-19 infection alone can considerably raise AST levels in contrast to the control group. Remdesivir medication increased the liver cell damage that was already present as seen by a further rise in the serum level of AST in the COVID-19 hospitalized patients, Figure 5, Table 3.

Table 3 AST enzyme levels in COVID-19 patients' sera are shown, together with their pre-and-post-Remdesivir therapy levels

Treatment groups	Median (Interquartile range)
Control	25.10 (18.55-30.60)
No Remdesivir	37.35(25.68-50.05)
Remdesivir Treatment	49 (35.05-69.65)

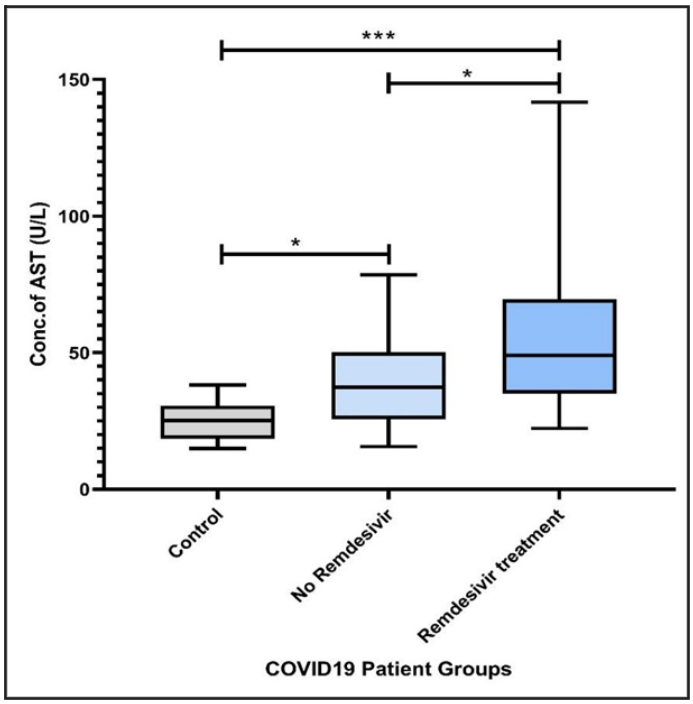

Figure 5 Effect of Remdesivir on AST enzyme levels in the blood of COVID-19 patients detected by Preatige24i. The asterisks * represent statistical differences at *P* <0.05 and asterisks*** statistical differences at *P* <0.001, N=96.

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Remdesivir reduces COVID-19 virus-induced CRP expression

A high CRP test result indicates acute inflammation. It could be triggered by a severe accident, prolonged illness, or infection. The CRP levels were measured in four groups; the control group (No virus, No medication), the No Remdesivir group (patients with COVID-19 but no Remdesivir treatment), and the Remdesivir 1-2 days group (patients with COVID-19 and 1-2 days Remdesivir treatment) and the Remdesivir ≥3 days group (patients with COVID-19 and ≥3 days Remdesivir treatment), Figure 6, Table 4. There were statistically significant differences between the COVID-19 infected groups and the control. Indicating that COVID-19 infection increases CRP levels. The *P*-values between the control, No Remdesivir, and Remdesivir 1-2 days were *P* <0.001, and *P* <0.001, respectively, while the *P*-value between the control and Remdesivir ≥3 days group was 0.007. CRP concentration was significantly dropped from Remdesivir 1-2 days' group to Remdesivir ≥ 3 days, with a *P*-value of 0.017. Thus the lowest concentration of CRP was seen in the last group that was taking drugs for more than three days in comparison to the other groups.

Table 4 C- reactive protein (CRP) concentration discrepancies in four separate COVID-19 patient groups

Treatment groups	Median (Interquartile range)
Control	0.90 (0.30-1.450)
No Remdesivir	5.60 (2.40-9.60)
Remdesivir 1-2 days	5.00 (4.00-7.50)
Remdesivir ≥ 3 days	2.20 (1.175-4.20)

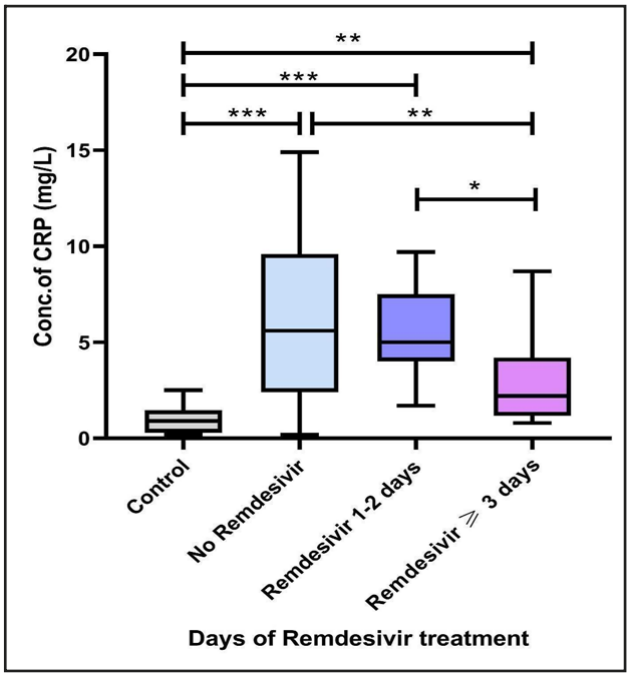

Figure 6 Effect of Remdesivir on C - reactive protein (CRP) concentration in COVID-19 patients detected by Prestige 24i. The asterisks *** indicates statistical difference at *P* <0.001, asterisks ** statistical difference at *P* = 0.007, ** also indicates *P* = 0.003, asterisks * statistical difference at *P* = 0.017. N=129.

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Remdesivir temporarily decreases the induction of D-dimer by the COVID-19 virus

The difference in D-dimer concentrations in four different patient groups is depicted in Figure7. The control group was significantly different from all other groups with a *P*-value of <0.001. Moreover, D-dimer in COVID-19 patients in the no Remdesivir treatments group, the concentration was significantly higher than in the control group. Similarly, D-dimer in COVID-19 patients who take Remdesivir is significantly higher than in the control group.

There was a statistically significant difference between the no Remdesivir and Remdesivir 1-2 days' treatment groups, with a *P*-value of 0.024, and D-dimer concentration was lowered from the second to the third group, after taking Remdesivir. Between the third and the fourth groups, there was an increase in D-dimer concentration but it was not significant.

There was no significant difference in D-dimer concentration between the no Remdesivir group and Remdesivir ≥ 3 days' groups, Figure 7, Table 5.

Table 5 The concentration of D-dimer protein in sera of COVID-19 patients on various days following therapy with Remdesivir

Treatment groups	Median (Interquartile range)
Control	202.0 (285.0-162.5)
No Remdesivir	2524 (1411-4882)
Remdesivir 1-2 days	1156 (752.8-2815)
Remdesivir ≥ 3 days	2415 (1216-4340)

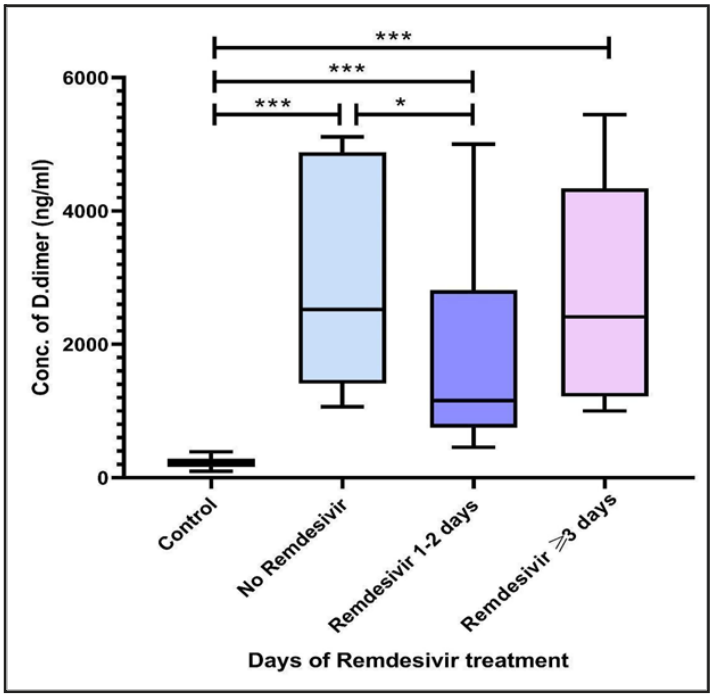

Figure 7 Effect of Remdesivir on D-dimer protein in patients with COVID-19, detected by Nano-checker 710. The asterisks *** indicate statistical difference at *P* <0.001, the asterisks * indicate statistical difference at *P* <0.242, N=121.

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Remdesivir or COVID-19 infection have no discernible effects on creatinine levels

Since there was no discernible difference between any of the groups, the tested medication has no impact on the levels of creatinine in the serum. Even yet, there was a modest rise in COVID-19 patients who did not take Remdesivir. Creatinine levels continued to rise over the first to two days of therapy with Remdesivir before declining again. However, none of the variations in creatinine concentration across the groups were statistically significant, as shown in Figure 8, and Table 6.

Table 6 Creatinine levels in sera of COVID-19 patients on different days after Remdesivir treatment

Treatment groups	Median (Interquartile range)
Control	0.81 (0.70-0.9850)
No Remdesivir	0.83 (0.60-1.080)
Remdesivir 1-2 days	0.91 (0.66-1.20)
Remdesivir ≥ 3 days	0.84 (0.68-1.015)

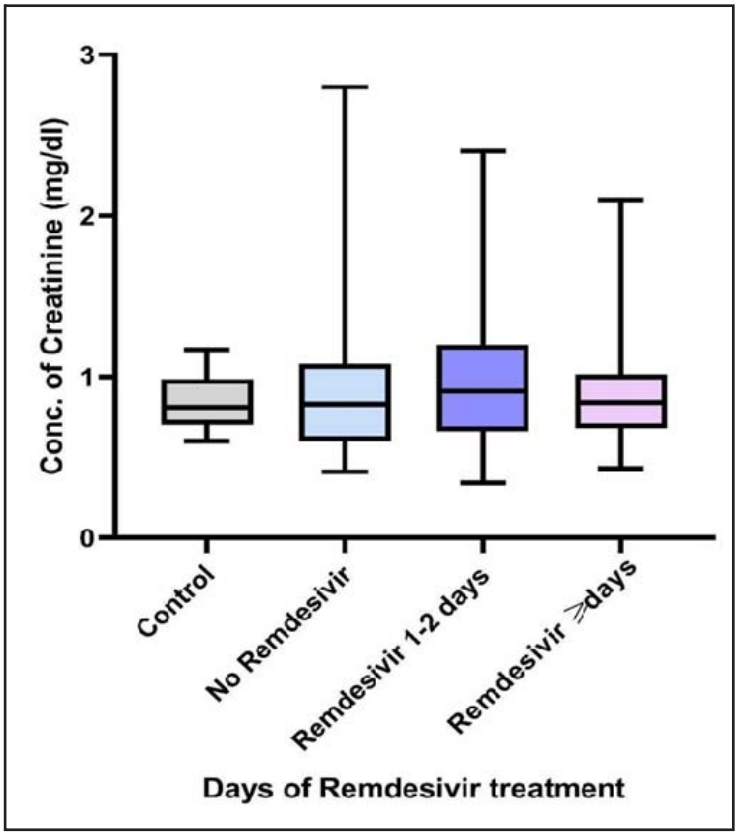

Figure 8 The effect of Remdesivir on Creatinine of COVID-19 patient detected by Prestige 24i, N=148.

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Discussion

The COVID-19 virus will boost the expression of the ABCD3 protein, as can be seen in the group that does not take Remdesivir and has the viral infection, confirmed by PCR. Yet, in patients taking Remdesivir therapy, the expression of the ABCD3 protein is markedly reduced. The findings show that Remdesivir toxicity on the HepG2 cell line is dose-dependent since not all doses cause significant toxicity; instead, the drug's toxicity is significantly raised at three dosages 5, 10, and 20 µg/ml, while it is negligibly low at lower doses of 1 and 2 µg/ml. The hepatotoxicity of Remdesivir was previously reported to occur at a dose of 10 µg/ml; our study shows the toxic dose to be even lower than those earlier reports, at 5µg/ml. Nonetheless, our results support other studies that found this antiviral medication to cause hepatocellular damage.[26-28]

Remdesivir caused significant liver and kidney damage in kidney organoids, which are 3D models of the kidney grown in vitro from stem cells, and liver spheroids, which are 3D culture systems that resemble the liver and allow for the lengthy culture of hepatocytes, at dosages comparable to doses at or below the dose necessary required to effectively stop the spread of COVID-19 virus proliferation.[29] Remdesivir shows liver toxicity, as determined by genetically engineered human tissue, at doses required to effectively control the COVID-19 virus infection. [30] Additionally, studies reported that EC50 (half-maximal effective concentration) for cytotoxicity for HepG2/3CA cells to be 1-10 µM for 2-3 days.[31] Remdesivir exhibits TC50 (Hazardous concentration that kills 50% of cells) values of > 2.0 µM against MRC-5 (normal human fibroblast lung cells). This indicates that Remdesivir is toxic to various cell lines in addition to liver cell lines; MRC-5, MT-4 and PHH cell lines. [32, 33] Yan et al. (2021) demonstrated that Remdesivir prevents the COVID-19 virus reproduction in Vero E6 cells; Remdesivir was able to reduce virus-induced CPE by 50% at 1 µg/ml and to cause 50% cytotoxicity at 100 µg/ml. [34]

Liver function tests, AST and ALT levels increased simultaneously, indicating an increase in liver tissue damage. Remdesivir may damage the liver by preventing the expression and activity of ABCD3 transporters, hence it is important to investigate this possibility even more. It is crucial to recognize that COVID-19 virus will increase the expression of ABCD3 protein in comparison to the healthy control group, as we can see in the group who do not take the medication and have the viral infection that has been confirmed by PCR. However, in groups that receive Remdesivir therapy, the expression of the ABCD3 protein is markedly decreased. Evidence that the virus can increase the expression of the ABCD3 protein without Remdesivir. Speculating that this protein may have a significant part in the body's reaction to a viral infection. In contrast, there are several studies speculating that the COVID-19 virus infection might be the cause behind the decreased expression of ABCD3 protein. Based on the logic that viral infection might reduce the number of peroxisomes that this protein resides on its membrane. Even though localization of this protein is not limited to peroxisomes in the cell. Cells infected with the dengue, West Nile, and Zika viruses have been found to have fewer peroxisomes.[35, 36]

Another study showed no change in the peroxisome compartment during viral infection. Consequently, COVID-19 virus infection of cells may have a more potent adverse effect on the structure of peroxisomes and their functionality than infection with other viruses.[37] Our results show that the drug Remdesivir can suppress *ABCD3* gene expression, contradicting these studies as we found when examining virus-free liver cell lines. Both the HepG2 cell line experiments and sera analysis for COVID-19 patients for ABCD3 expression measurements follow

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a similar pattern of reduced *ABCD3* gene and protein expressions after treatment with Remdesivir. Nevertheless, currently there are no studies on the effect of Remdesivir on the ABCD3 transporter in particular, but then it has been shown that Remdesivir is a mild inhibitor of other ABC transporters, ABCB1, ABCC1, and ABCG2. [4]

The COVID-19 virus increased serum D-dimer protein levels demonstrated by the strong distinction between the control group and the infected groups. The effect of Remdesivir on the D-dimer levels was opposing to that of the virus. In addition, Remdesivir decreases inflammation represented by CRP levels. Nonetheless, the creatinine level of the groups during the corona infection is not significantly affected by the COVID-19 virus or Remdesivir. Remdesivir plays a role in ALT and AST liver function test results during the corona infection, as evidenced by the fact more than 60% of individuals who took the drug had abnormal test values.

The data collected shows that Remdesivir contributed to the increase in ALT and AST levels in COVID-19 patients. The current research outcome pattern is consistent with other studies, ALT and AST levels were normal or slightly elevated at the beginning, but then increased after taking the drug.[2, 38] Zampino and his colleagues hypothesized that Remdesivir plays a direct role in hepatocellular tissue toxicity, and that COVID-19 infection alone can also increase aminotransferase levels. [38]

Furthermore, the enzymes ALT and AST have been found to be elevated in subjects treated with Remdesivir, which may indicate liver toxicity. [2, 39,40]The true culprit of the rise in AST and ALT after COVID-19 infection and Remdesivir therapy has not been identified in any other research.[41-45]adverse reactions to Remdesivir after treatment were reported to include nausea, vomiting, gastroparesis, or rectal bleeding. Additionally, elevated aminotransferase levels 1-5 days after starting treatment with Remdesivir was reported.[41] Unlike those who have a milder form of the disease, patients with severe COVID-19 appear to have evidence of liver dysfunction more frequently. Elevated levels of ALT, AST, and total bilirubin have been found in many ICU patients.[46] Given that 2-10% of COVID -19 patients have diarrhea and viral RNA has been found in both stool and blood samples, the likelihood of the presence of hepatitis viruses, and infection of liver cells with COVID-19 virus, cannot be completely ruled out.[47] In addition, it is likely that any immune-mediated inflammation, particularly cytokine storm, as well as pneumonia-induced hypoxia could cause liver damage in critically ill COVID-19 patients. [46]

To diagnose the presence of kidney disease, serum creatinine levels were measured. Our results show that neither Remdesivir nor COVID-19 had an effect on creatinine concentration during corona infection; since there is no statistically significant difference between the groups. Contrary to our results, several studies have documented an increase in creatinine levels in corona infections. Dodig et al. (2020) found that patients with the severe disease typically exhibit multiple organ imbalances, as evidenced by significantly higher levels of urea, creatinine, amylase, and D-dimer. [48] Patients using Remdesivir have shown increases in creatinine, which may indicate kidney damage.[30, 43] In contrast, our research shows no changes in renal function in response to Remdesivir in COVID-19 patients. The increased prevalence of kidney involvement at hospital admission may have been caused by certain COVID-19 patients with a history of chronic kidney disease. [49] In a cohort study of 799 COVID -19 patients, it was found that 113 non-survivors and 161 recovered patients had significantly higher levels of ALT, AST, creatinine, and D-dimer. Liver function also been recognized as an important predictor of mortality in COVID-19 patients.[50, 51]

High levels of D-dimer have been associated with fatal outcomes in patients

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with critical illnesses and a variety of serious diseases such as viral infections. In addition, numerous studies have shown that COVID-19 makes people more susceptible to thrombosis in their arteries and veins.[52-54] Elevated levels of D-dimer and many other parameters have been reported as indicative of coagulopathy in COVID-19 infection.[55] Our results show that COVID-19 disease significantly elevates D. dimer levels, as there was a significant difference between the control and no Remdesivir treated COVID-19 group. The effects of Remdesivir on D-dimer are variable. Increases in patients not taking Remdesivir. Then decreased on the 1st and 2nd days of treatment. The effect of Remdesivir on D-dimer decreased in patients who took Remdesivir for 3 or more days. Similar to our findings, one study found that after treating COVID-19 patients, levels of D-dimer decreased but then raised. The above study discovered a strong correlation between dynamic fluctuations in D-dimer levels and the prognosis of COVID-19. [56]

High levels of d-dimer may indicate increased blood coagulation in COVID-19 patients as a result of a systemic inflammatory response syndrome or as a direct result of the virus itself.[57] The coagulation process after acute and long-term lung injury is directly related to the D-dimer level in viral disorders of the lungs. Critically ill patients' significant lung injury caused the fibrinolytic system to activate excessively and break down more fibrin in the alveoli, resulting in higher D-dimer levels than in mild patients. Patients with pneumonia may experience a worsened sickness, increased in vivo inflammatory response, and a bad prognosis with the rise of D-dimer.[58] Prothrombin time (PT) and D-dimer levels were considerably increased in severe COVID-19 patients. This increase in D-dimer is indicative of DIC, which was frequently present in COVID-19 patients who passed away. Viral infections result in the formation of fibrin clots, which offer the virus defense. Therefore, it is possible that a severe COVID-19 infection could produce fibrinolysis that leads to DIC.[59, 60] D-dimer represents underlying pathophysiology contributing to adverse outcomes. D-dimer elevation may reflect underlying hypercoagulability, pathologic fibrinolysis, inflammatory processes, or may itself be pathogenic.[61]

Another protein tested in serum was acute phase pentameric CRP. Their levels increase in response to inflammation. Although other cell types including adipocytes, lymphocytes, endothelial cells, macrophages, and smooth muscle cells also produce CRP, hepatocytes are the primary site of production. In patients with COVID-19, CRP levels can accurately predict disease severity, adverse outcomes, prognosis, and mortality. [62]

Our results show that COVID-19 raises CRP concentration. There is a big disparity between the control group and the groups with COVID-19. Contrary to this, the drug we investigated decreased CRP concentration during the corona infection. Our observation that COVID-19 increases CRP and Remdesivir decreases are confirmed by Stoeckle et al., (2022) study, which found that corona patients' median CRP levels were significantly lower before and after treatment compared to pretreatment levels.[63] This is in line with several studies showing that COVID-19 patients treated with a 5 or 10 days' course of Remdesivir had a shorter time to recovery than those who received a placebo.[51,64] In patients with severe COVID-19 disease, tissue damage and excessive production of inflammatory cytokines were strongly associated with an increase in CRP levels.[65] Results demonstrated a substantial correlation between a collection of inflammatory indicators and several organ dysfunctions, including liver, renal, and cardiac damage.[66] It will be interesting to investigate how CRP levels and the inflammatory response are affected by the ABCD3 protein in future studies to find

a new drug target for inflammation management.

Conclusion

We conclude that Remdesivir enhances the risk of liver damage in patients with COVID -19. Down-regulation of the *ABCD3* gene and protein may be involved in Remdesivir toxicity, as the medication dose increases, the expression of ABCD3 declines and more liver cells die. Although this research supporting the use of Remdesivir to treat COVID-19, nonetheless, it may not be well tolerated in patients with liver dysfunction. COVID-19 along with Remdesivir treatment causes drug-induced liver damage, as elevated CRP, abnormal ALT, ASTs, and D-dimer levels are all signs of liver injury. It is also important to conduct further research on the combination of drugs used during COVID-19 infection on the expression of *the ABCD3* gene, for example, acetaminophen as well as other drugs that have a well-established association with liver injury.

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Competing interests

The authors declare that they have no competing interests.

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