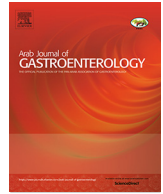




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## Original article

# Sofosbuvir/ledipasvir in combination or nitazoxanide alone are safe and efficient treatments for COVID-19 infection: A randomized controlled trial for repurposing antivirals

Mohammed A. Medhat<sup>a,#</sup>, Mohamed El-Kassas<sup>b,\*,#</sup>, Haidi Karam-Allah<sup>a</sup>, Ahmed Al Shafie<sup>b</sup>, Sherief Abd-El salam<sup>c</sup>, Ehab Moustafa<sup>a</sup>, Sahar M. Hassany<sup>a</sup>, Marwa Salama<sup>c</sup>, Mohamed S. Abd Elghafar<sup>d</sup>, Hamdy Sayed<sup>b</sup>, Mohamed Badr<sup>e</sup>, Dalia T. Kamal<sup>f</sup>, Ahmed Shamseldeen<sup>e</sup>, Ashima'a Ossimi<sup>e</sup>, Inas Moaz<sup>g</sup>, Hossam El-deen Esmael<sup>a</sup>, Azza M. Ezz Eldin<sup>f</sup>, Sameera Ezzat<sup>g</sup>, Hossam Abdelghaffar<sup>h</sup>, Khaled Abdelghaffar<sup>i</sup>

<sup>a</sup>Tropical Medicine and Gastroenterology Department, Faculty of Medicine, Assiut University, Assiut, Egypt

<sup>b</sup>Endemic Medicine Department, Faculty of Medicine, Helwan University, Cairo, Egypt

<sup>c</sup>Tropical Medicine and Infectious Diseases Department, Faculty of Medicine, Tanta University, Tanta, Egypt

<sup>d</sup>Anesthesia, Surgical Intensive Care and Pain Medicine Department, Faculty of Medicine, Tanta University, Tanta, Egypt

<sup>e</sup>Critical Care Medicine Department, Faculty of Medicine, Helwan University, Cairo, Egypt

<sup>f</sup>Clinical Pathology Department, Faculty of Medicine, Assiut University, Assiut, Egypt

<sup>g</sup>Epidemiology and Preventive Medicine Department, National Liver Institute, Menoufia University, Menoufia, Egypt

<sup>h</sup>Supreme Council of University Hospitals, Egypt

<sup>i</sup>Ministry of Higher Education and Scientific Research, Egypt

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## ABSTRACT

**Background and study aims:** Currently, there is no therapy approved for COVID-19. We evaluated the efficacy and safety of sofosbuvir/ledipasvir and nitazoxanide for the treatment of patients with COVID-19 infection.

**Patients and methods:** A multicenter, open-label randomized controlled trial included one hundred and ninety patients with non-severe COVID-19 infection. Patients were randomized into three groups. All groups received standard care treatment (SCT). In addition, group 1 received sofosbuvir/ledipasvir, and group 2 received nitazoxanide. Follow-up by reverse-transcriptase polymerase chain reaction (RT-PCR) was done at intervals of 5, 8, 11, and 14 days. The primary endpoint was viral clearance.

**Results:** Viral clearance was significantly higher in the sofosbuvir/ledipasvir and nitazoxanide groups compared to the SCT group in all follow-up intervals ( $p < 0.001$ ). In the sofosbuvir/ledipasvir arm, 36.9% showed early viral clearance by day 5. By day 14, 83.1% of the sofosbuvir/ledipasvir group, 39.7% of the nitazoxanide group, and 19.4% of the SCT group tested negative for SARS-CoV-2. Sofosbuvir/ledipasvir and nitazoxanide treatment were the only significant factors in Cox regression of negative RT-PCR with the highest OR (17.88, 95% CI: 6.66–47.98 and 2.59, 95% CI: 1.11–6.07, respectively). No mortality or serious adverse events were recorded.

**Conclusion:** The addition of sofosbuvir/ledipasvir or nitazoxanide to the SCT results in an early and high viral clearance rate in mild and moderate patients with COVID-19. These drugs represent a safe and affordable treatment for COVID-19.

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## Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) or Coronavirus disease of 2019 (COVID-19) is a novel coronavirus that belongs to the same family of SARS-CoV as Middle East respiratory syndrome coronavirus (MERS-CoV) [1]. SARS-CoV-2 mainly

\* Corresponding author at: Endemic Medicine Department, Faculty of Medicine, Helwan University, Ain Helwan, Postal code: 11795, Cairo, Egypt. Tel.: +20 111 445 5552.

E-mail address: [m\\_elkassas@hq.helwan.edu.eg](mailto:m_elkassas@hq.helwan.edu.eg) (M. El-Kassas).

# Contributed equally to the work

infects the respiratory system and is transmitted via respiratory droplets through close contact [2]. In early January 2020, it was identified as the cause of an epidemic of pneumonia in the Chinese city of Wuhan, which led to a subsequent global pandemic [2].

To date, there is no specific effective antiviral treatment for COVID-19. In most cases, the disease has a mild or moderate course, although up to 5%–10% of the patients develop a severe, potentially life-threatening disease. A global effort is being made to find an effective treatment against COVID-19. These efforts include trying lopinavir (LPV)/ritonavir (RTV), interferon (IFN)  $\beta$ -1a, hydroxychloroquine/chloroquine [3], and ivermectin [4], which have not proven to be successful.

Coronaviruses are large, positive-sense RNA viruses, including alpha, beta, delta, and gamma genera [5]. The coronavirus replication machinery is a large multi-subunit complex [6]. The most highly conserved protein in all known RNA viruses is the viral monomeric RNA-dependent RNA-polymerase (RdRp). There [6] are now several drugs, such as favipiravir (FVP) [7] and remdesivir [8], which bind to the RdRp active site and have been approved to treat other RNA viral diseases.

The available data about SARS-CoV-2 shows a viral genome replication similar to that of the hepatitis C virus (HCV) [9]. The virus enters the cell by endocytosis, is uncoated, and open reading frames 1a and 1b (ORF1a and ORF1b) of the positive-strand RNA is translated to produce nonstructural proteins, including cysteine and serine proteases, helicase, and RdRp. Recent data suggest that HCV direct-acting antiviral drugs (DAAs), which are used in HCV management, might be effective against the SARS-CoV-2 virus through RdRp targeting [10,11].

Sofosbuvir is a HCV-DAA with NS5B RdRp inhibitory activity. It has been used in combination with several NS5A protein inhibitors, such as daclatasvir or ledipasvir, with high efficacy for HCV treatment [12]. The introduction of DAAs was considered a milestone in HCV eradication in Egypt, with a sustained virological response rate of almost 100%, using certain DAA combinations [13].

Molecular docking was performed to test several HCV-DAAs, including sofosbuvir, against SARS-CoV-2 RdRp, and the results were promising. Therefore, DAAs might have inhibitory activity against the newly emerged coronavirus [14].

Nitazoxanide, a drug developed initially as an antiprotozoal agent, has a broad-spectrum antiviral activity and can inhibit the replication of a wide range of RNA and DNA viruses in cell-culture assays. It exhibits *in vitro* activity against MERS-CoV and several coronaviruses [15]. In humans, a Phase IIb/III clinical trial found that oral administration of nitazoxanide reduced the duration of clinical symptoms and viral shedding compared to placebo in individuals with influenza [16].

Currently, sufficient data are not available on the efficacy of DAAs or nitazoxanide in treating patients with COVID-19 infection. Therefore, we aimed to evaluate the efficacy and safety of sofosbuvir/ledipasvir and nitazoxanide to treat COVID-19 infection. Both drugs are inexpensive and available throughout the world.

## Patients and methods

All patients in this multicenter open-label randomized controlled trial were COVID-19 positive. COVID-19 diagnosis was based on positive reverse-transcriptase polymerase chain reaction (RT-PCR) testing for SARS-CoV-2 on nasopharyngeal swabs. Patients either attended, 1–2 days after diagnosis, to the outpatient clinic or were admitted to the quarantine hospitals of Helwan University Hospital, Al-Rajhy Liver University Hospital, and Tanta University Hospital, Egypt. Patients were included between July 2020 and October 2021 and were assigned randomly according to a randomization number generated by an independent statisti-

cian and provided in pre-sealed envelopes. Recruited patients received a fixed combination of sofosbuvir/ledipasvir (400 mg and 90 mg, orally) once a day for 14 days plus the standard care treatment (SCT) for patients with COVID-19 according to the Ministry of Health protocol (Group 1), nitazoxanide (500 mg, orally) four times per day for 14 days plus the SCT (Group 2), or SCT alone (Group 3) for 14 days. The treatment was started in groups 1 and 2 less than one week from the onset of symptoms. Nitazoxanide was used in a dose of 500 mg q.i.d. This dose assured the drug present in the plasma and lung, above the *in vitro* EC90 for most of the treatment period [17].

The SCT was given according to a standardized protocol issued by the Egyptian Supreme Council of University Hospitals and is composed of azithromycin for three days and vitamin C and zinc supplements for 14 days. Hydroxychloroquine was given to some patients who accepted to receive it. Oxygen therapy was used if SpO<sub>2</sub> is <92. Patients were indicated for steroids therapy and prophylactic anticoagulants once they required oxygen therapy according to the Egyptian protocol for COVID-19 management. The included patients were older than 18 years old, with a creatinine clearance of >30 mL/ml and without any malignancy. Cases with non-severe COVID-19 infection, according to WHO cases definition, were included [18]. Non-severe COVID-19 cases are defined by the absence of criteria for acute respiratory distress syndrome (ARDS), sepsis, septic shock, or other conditions requiring life-sustaining therapies such as mechanical ventilation (invasive or non-invasive) or vasopressor therapy. All included patients had oxygen saturation >90% on room air and did not have signs of pneumonia nor signs of severe respiratory distress [19].

Patients with severe or critical COVID-19 infection with signs of ARDS, sepsis, septic shock, and patients required invasive or non-invasive mechanical ventilation were excluded from the study. ARDS is diagnosed when the PaO<sub>2</sub>/FiO<sub>2</sub> ratio is < 200 [20]. Sepsis was considered when organ dysfunction developed as a result to dysregulated response to COVID-19 infection. Septic shock was diagnosed if patients with sepsis developed circulatory, cellular, and metabolic abnormalities, and presented as fluid-refractory hypotension requiring vasopressor therapy with associated tissue hypoperfusion (lactate > 2 mmol/L) [21]. According to the Egyptian protocol for COVID-19 management, non-invasive mechanical ventilation (NIV) is indicated for conscious patients with minimal secretions, with hypoxia SpO<sub>2</sub> < 90% on oxygen or PaCO<sub>2</sub> >40 mmHg provided pH 7.3 and above. Invasive mechanical ventilation is indicated in cases with failed NIV or not available or not practical, HACOR (heart rate, acidosis, consciousness, oxygenation, and respiratory rate) score > 6, PaO<sub>2</sub> < 60 mmHg despite oxygen supplementation, progressive hypercapnia, respiratory acidosis (PH <7.30), progressive or refractory septic shock, disturbed consciousness level (GCS  $\leq$ 8), or deterioration in consciousness level from baseline. Exclusion criteria also included pregnancy, renal impairment with a creatinine clearance of <30 mL/min, evidence of malignancies, and use of FVP or LPV/RTV therapy. Clinical data were recorded using the WHO COVID-19 case record form (<https://www.who.int/docs/default-source/coronavirus/who-ncov-crf>).

Our primary endpoint was to confirm viral clearance. After treatment initiation, the viral response was checked by RT-PCR testing of oropharyngeal swabs obtained at days 5, 8, 11, and 14. After having a negative RT-PCR, another confirmatory RT-PCR test was done to confirm the result, unless the patient refused. Quantitative PCR was not available in our centers. Baseline laboratory assessment was done by complete blood count, liver function tests, prothrombin time, international normalized ratio (INR), activated partial thromboplastin time, urea, creatinine, serum glucose level, erythrocytic sedimentation rate (ESR), C-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, and D-dimer. Radiological

assessment by chest CT was also done at baseline. The study's secondary endpoints were the mortality, requirement for admission to the intensive care unit (ICU), or development of serious adverse events. Written informed consent was obtained from all patients.

#### Randomization and masking

The participants were assigned randomly according to a randomization number generated by an independent statistician and provided in pre-sealed envelopes. Individuals involved in randomization and masking had no involvement in the rest of the trial.

#### Ethical considerations

The trial was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approvals were obtained from the relevant ethics committees in the participating hospitals. Before conduction, this study was registered on the [clinicaltrials.gov](https://clinicaltrials.gov) website, with registration number NCT04498936.

#### Statistical analysis

Data were collected and analyzed using Statistical Package for Social Science program for statistical analysis (version 13; Inc., Chicago, IL). The means, standard deviations, frequencies, and percentages were used as appropriate. Chi-square was used to measure the association between qualitative variables. One-way analysis of variance test was used to compare the three groups having normally distributed quantitative data, whereas the Kruskal-Wallis test was used when these data were not normally distributed. Kaplan-Meier survival analysis was utilized to explore the duration needed to get an RT-PCR negative event. If the patient had two consecutive negative RT-PCR, the time to event was considered the date of the first one. The Fisher exact test was then done to compare the three groups at each point of time. The Cox regression model gave an adjusted hazard ratio (odds ratio (OR)) and 95% confidence interval of the effect of the different risk factors for survival. A p-value of <0.05 was considered statistically significant.

#### Role of the funding source

The sponsor of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study, and the corresponding author had final responsibility for the decision to submit for publication.

**Table 1**  
Patient baseline demographics.

The studied variables	SOF/LED + SCT N = 70 n (%)	NTZ + SCT N = 77 n (%)	SCT N = 73 n (%)	p-value
Age (mean ± SD)	45.04 ± 8.14	44.32 ± 8.43	45.34 ± 5.59	0.695
<b>Gender</b>				
Female	38 (54.3)	50 (64.9)	32 (43.8)	0.035
Male	32 (45.7)	27 (35.1)	41 (56.2)	
<b>Comorbidities:</b>				
Hypertension	12 (17.1)	14 (18.2)	18 (24.7)	0.471
Diabetes Mellitus	12 (17.1)	12 (15.6)	17 (23.3)	0.445

SOF/LED, sofosbuvir/ledipasvir; NTZ, nitazoxanide; SCT, standard care treatment.

## Results

The study included one hundred and ninety patients in the three groups. Group 1 included 70 patients who received sofosbuvir/ledipasvir with SCT; Group 2 included 77 patients who received nitazoxanide with SCT; Group 3 included 73 patients who received SCT alone. As shown in [Table 1](#), there was no significant difference in the baseline demographic characteristics of the three groups except for gender ( $p = 0.035$ ). More than half were females in Group 1 (54.3 %) and Group II (64.9%), whereas males predominated in Group 3. The common comorbidities were hypertension and diabetes mellitus, with the highest frequency in Group III, but without significant difference ( $p = 0.471$  and  $0.445$ , respectively). Other comorbidities, such as obstructive lung disease, chronic liver or kidney diseases, and rheumatic heart disease, were of lower frequency ( $n = 9$ ).

The comparison between the studied groups regarding symptoms and signs is shown in [Table 2](#). No significant differences were found among groups regarding fever, heart rate, respiratory rate, blood pressure, and oxygen saturation, as shown in [Table 2](#). Oxygen therapy, steroids, and anticoagulants were used more frequently in Group 3, followed by Groups 2 then group 1, without statistical difference. Supportive treatment modality types received by the patients of each group is shown in [Table 3](#). The baseline laboratory data are summarized in [Table 4](#). Lymphocyte percentages were significantly lower in Group 2 ( $p = 0.049$ ), while direct bilirubin was significantly lower in Group 1 ( $p = 0.013$ ). Laboratory markers of infection, such as CRP, LDH, ferritin, and D-dimer, were not statistically different among all groups.

The cumulative incidence of negative RT-PCR following treatment, analyzed by the intention to treat method, reveals an overall significant difference among all groups at each follow-up interval ( $p < 0.001$ ) ([Fig. 1](#)). The highest percentage of negative RT-PCR at each follow-up was observed with group 1 (sofosbuvir/ledipasvir therapy), then group 2 (nitazoxanide), followed by group 3 (SCT). In group 1, 42.9% of the patients tested negative on day 5. By the end of the follow-up, at day 14, 85.7% of the patients who received sofosbuvir/ledipasvir, 35.1% of group 2, and 16.4% of group 3 tested negative by RT-PCR ([Fig. 1](#)). Similarly, the log-rank curve of the cumulative time to viral clearance ([Fig. 2](#)) shows that sofosbuvir/ledipasvir had a significantly higher treatment value compared with nitazoxanide and SCT ( $p < 0.001$ ). The pairwise log-rank curve for the cumulative incidence of negative RT-PCR showed that sofosbuvir/ledipasvir is significantly higher than nitazoxanide ( $p < 0.001$ ) and SCT ( $p < 0.001$ ) and that nitazoxanide is significantly higher than SCT ( $p = 0.01$ ). Nitazoxanide also had a higher curative rate than SCT ( $p = 0.01$ ) ([Fig. 2](#)). The Breslow test compared the survival rates among the three groups, which was statistically significant ( $p < 0.001$ ). Accordingly, sofosbuvir/ledipasvir therapy resulted in significantly earlier and higher rates of viral clearance than both nitazoxanide and SCT.

**Table 2**  
Clinical manifestations among the studied groups.

The studied variables	SOF/LED + SCT (N = 70) n (%)	NTZ + SCT (N = 77) n (%)	SCT (N = 73) n (%)	p-value
<b>Symptoms:</b>				
Fever	55 (78.6)	53 (68.8)	46 (63)	0.123
Dry Cough	41 (58.6)	44 (57.1)	44 (60.3)	0.927
Sputum	15 (21.4)	8 (10.4)	8 (11)	0.101
Hemoptysis	3 (4.3)	3 (3.9)	0 (0.0)	0.214
Sore Throat	29 (41.4)	35 (45.5)	32 (43.8)	0.885
Runny Nose	6 (8.6)	9 (11.7)	7 (9.6)	0.812
Anosmia	24 (34.3)	25 (32.5)	25 (34.2)	0.964
Ageusia	25 (35.7)	26 (33.8)	27 (37)	0.917
Wheezing	3 (4.3)	8 (10.4)	8 (11)	0.29
Dyspnea	19 (27.1)	15 (19.5)	20 (27.4)	0.44
Chest Pain	22 (31.4)	24 (31.2)	35 (47.9)	0.055
Muscle Pain	25 (35.7)	30 (39)	24 (32.9)	0.739
Joint Pain	26 (37.1)	26 (33.8)	21 (28.8)	0.563
Fatigue	45 (64.3)	48 (62.3)	41 (56.2)	0.579
Headache	31 (44.3)	48 (62.3)	40 (54.8)	0.089
Sweating	18 (25.7)	6 (7.8)	6 (8.2)	0.002
Abdominal Pain	6 (8.6)	12 (15.6)	8 (11)	0.405
Nausea	5 (7.1)	8 (10.4)	12 (16.4)	0.204
Vomiting	6 (8.6)	8 (10.4)	9 (12.3)	0.764
Diarrhea	16 (22.9)	12 (15.6)	5 (6.8)	0.027
<b>Signs: (mean ± SD)</b>				
Temperature	37.49 ± 0.72	37.29 ± 0.585	37.5 ± 0.772	0.08
Heart Rate	90.8 ± 12.76	92.58 ± 6.43	92.14 ± 6.79	0.467
Respiratory Rate	21.47 ± 1.64	22.01 ± 2.39	22.05 ± 2.74	0.245
Systolic Blood Pressure	116.93 ± 16.14	119.74 ± 11.27	122.4 ± 13.47	0.06
Diastolic Blood Pressure	76.07 ± 10.35	74.16 ± 7.41	75.62 ± 8.29	0.377
O2 Saturation	93.33 ± 1.65	93.61 ± 2.36	92.87 ± 1.93	0.083

SOF/LED, sofosbuvir/ledipasvir; NTZ, nitazoxanide; SCT, standard care treatment.

**Table 3**  
Supportive treatment modality types received by the patients of each group.

Treatment	SOF/LED + SCT (N = 70) n (%)	NTZ + SCT (N = 77) n (%)	SCT (N = 73) n (%)	p-value
Hydroxychloroquine	5 (7.1)	4 (5.2)	7 (9.6)	0.584
Oxygen Therapy	12 (17.1)	17 (22.1)	20 (27.4)	0.337
Steroids	32 (45.7)	28 (36.4)	38 (52.1)	0.15
Anticoagulants	29 (44.6)	33 (42.9)	39 (53.4)	0.284

SOF/LED, sofosbuvir/ledipasvir; NTZ, nitazoxanide; SCT, standard care treatment.

The Cox regression analysis (Table 5) shows that sofosbuvir/ledipasvir and nitazoxanide were the only significant factors with the highest HR (11.137, 95% CI: 5.928–20923, and 2.248 % CI: 1.133 – 4.461, respectively). No mortality or progression to severe COVID-19 or ICU admission was recorded in any included patients. A mild gastric upset was recorded as an adverse effect in four patients in the sofosbuvir/ledipasvir and ten patients in the nitazoxanide group, whereas headaches occurred in two patients sofosbuvir/ledipasvir group.

## Discussion

Several antiviral drugs are under evaluation for the treatment of COVID-19 infection. The SARS-CoV-2 replication and transcription cycle depends on several essential enzymes, notably RdRp, main protease (Mpro), and helicase. Sofosbuvir and remdesivir have chemical similarities, including the molecular weight and predicted COVID-19 RdRp binding strength; however, sofosbuvir is better absorbed orally [22]. The efficacy of remdesivir was tested in randomized trials, but the results are contradictory [23,24]. SARS-CoV-2 has an exonuclease-based proofreader to maintain viral genome integrity. Any antiviral drug targeting SARS-CoV-2 RdRp must display a certain resistance level to this proofreading

activity. Sofosbuvir terminated RNA resists removal by the exonuclease to a substantially higher level than RNA terminated by remdesivir [25].

Our study indicates a positive effect of sofosbuvir/ledipasvir as a treatment for COVID-19. Approximately 85.7% of patients who received sofosbuvir/ledipasvir tested negative by the end of the study (day 14). The cumulative time to negative RT-PCR was significantly shorter in the sofosbuvir/ledipasvir group than in SCT ( $p < 0.001$ ). No mortality or need for ICU admission was recorded in our cohort. Our study corroborates a smaller study that reported that the addition of sofosbuvir/ledipasvir to SCT accelerated clinical response [26].

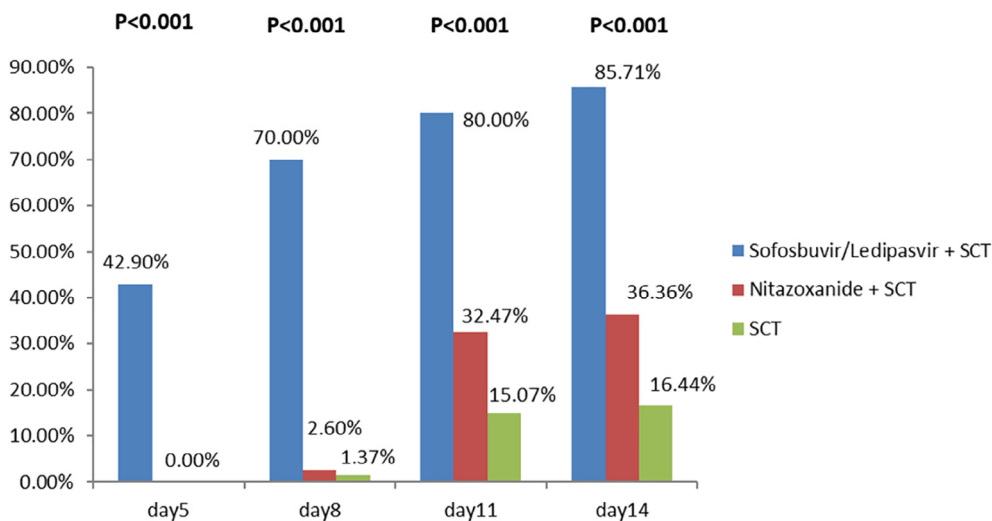
Daclatasvir is an NS5A protein inhibitor, similar to ledipasvir, and is used in combination with sofosbuvir with high efficacy in HCV management. Recently, the Roozbeh *et al.* study on sofosbuvir/daclatasvir in mild cases of COVID-19 reported no significant alleviation of symptoms after seven days of therapy compared with the control. Fewer hospitalizations were observed in the sofosbuvir/daclatasvir group but without statistical significance [27].

Sofosbuvir/daclatasvir treatment of patients with severe COVID-19 was reported to significantly lower hospitalization time, time in ICU, and mortality rate compared with ribavirin treatment

**Table 4**  
Baseline laboratory data of the studied groups.

Studied variables	SOF/LED + SCT (Mean ± SD)	NTZ + SCT (Mean ± SD)	SCT (Mean ± SD)	p-value
Hb (g/dl)	13.01 ± 1.6	12.48 ± 3.17	11.93 ± 4.3	0.136
HCT %	32.32 ± 16.95	37.1 ± 9.23	35.39 ± 12.35	0.086
PLT (×1000/mm <sup>3</sup> )	234.03 ± 65.23	224.77 ± 83.66	212.26 ± 100.75	0.305
WBCs (×1000/mm <sup>3</sup> )	6.73 ± 2.89	7.34 ± 3.29	6.57 ± 3.21	0.284
Lymphocytes %	28.94 ± 12.15	23.96 ± 12.11	26.85 ± 12.63	0.049
Neutrophils %	66.17 ± 12.83	64.73 ± 20.02	59.19 ± 24.09	0.08
Total Bilirubin (mg/dl)	0.55 ± 0.34	0.62 ± 0.17	0.63 ± 0.33	0.207
Direct bilirubin (mg/dl)	0.15 ± 0.11	0.18 ± 0.78	0.2 ± 0.13	0.013
Albumin (g/dl)	3.89 ± 1.44	3.88 ± 0.35	3.56 ± 1.2	0.12
AST (IU/L)	24.94 ± 12.61	32.43 ± 25.61	32.64 ± 27.04	0.077
ALT (IU/L)	30.64 ± 20.5	32.94 ± 23.84	28.30 ± 22.14	0.445
ALP (IU/L)	78.16 ± 44.51	71.43 ± 29.99	75.93 ± 26.83	0.48
Prothrombin Time	13.42 ± 4.55	14.06 ± 3.15	14.75 ± 1.87	0.062
Prothrombin Concentration	85.04 ± 24.49	84.69 ± 12.88	81.78 ± 11.26	0.449
INR	1.15 ± 0.33	1.14 ± 0.15	1.2 ± 0.12	0.238
APTT	34.36 ± 17.68	29.41 ± 9.43	30.95 ± 8.84	0.053
Urea (mg/dl)	26.57 ± 13.18	34.1 ± 22.89	33.9 ± 29.29	0.083
Creatinine (mg/dl)	0.79 ± 0.27	0.99 ± 0.45	1.02 ± 1.21	0.125
Glucose (mg/dl)	115.81 ± 57.21	107.66 ± 25.61	111.47 ± 29.60	0.46
ESR	27.67 ± 21.79	25.05 ± 21.5	29.64 ± 21.34	0.425
CRP (mg/l)	33.41 ± 55.35	45.54 ± 74.87	47.66 ± 53.29	0.339
LDH (U/l)	284.41 ± 148.49	290.17 ± 157.67	308.11 ± 174.45	0.653
Ferritin (ng/ml)	418.63 ± 335.08	450.49 ± 427.6	414.92 ± 396.81	0.828
D-dimer (ng/ml)	778.47 ± 664.7	788.74 ± 837.37	878.15 ± 782.68	0.69

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; APTT: Activated partial thromboplastin time; AST: Aspartate aminotransferase; CRP: C-reactive protein; ESR: Erythrocytic sedimentation rate; Hb: Hemoglobin; HCT: Hematocrit; INR: International normalized ratio; LDH: Lactate dehydrogenase; NTZ, nitazoxanide; PLT: Platelets; SCT, standard care treatment; SOF/LED, sofosbuvir/ledipasvir; WBCs: White blood cells.



**Fig. 1.** Cumulative incidence of negative RT-PCR test at each time point after treatment initiation.

[28]. Likewise, Sadegh *et al.* concluded that the addition of sofosbuvir and daclatasvir to SCT for 14 days in moderate or severe cases of COVID-19 significantly reduced the duration of hospital stay, resulting in a lower mortality rate compared with SCT alone [29].

In the current study, we also evaluated the efficacy of nitazoxanide in the treatment of COVID-19. Negative RT-PCR was observed in 36.4% of the nitazoxanide group compared with 16.4% of the SCT group (p = 0.01). The broad-spectrum antiviral activity of tizoxanide, the active metabolite of nitazoxanide, is attributed to interference with host-regulated pathways involved in viral replication rather than a virus-targeted mechanism. These pathways might include IFN or mammalian target of rapamycin complex 1 (mTORC1) signaling [30,31]. Nitazoxanide also upregulates host defense mechanisms that viruses target to bypass host cellular defenses [15]. In addition, nitazoxanide inhibits the pro-

duction of cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-2, IL-4, I-5, IL-6, IL-8, and IL-10 in peripheral blood mononuclear cells [15].

Similar to the current study, Rocco *et al.* evaluated nitazoxanide in patients with mild COVID-19, finding that symptom resolution did not differ between nitazoxanide administered as 500 mg three times a day and the placebo group after five days of therapy. However, early nitazoxanide therapy was safe and reduced the viral load significantly. Swabs collected were negative in 29.9% of the patients in the nitazoxanide arm versus 18.2% in the placebo arm (p = 0.009) [32]. Another study showed that early usage of nitazoxanide in combination with ribavirin, ivermectin, and zinc supplements was associated with a significantly shorter duration for SARS-COV2 clearance from the nasopharynx than symptomatic therapy [33].

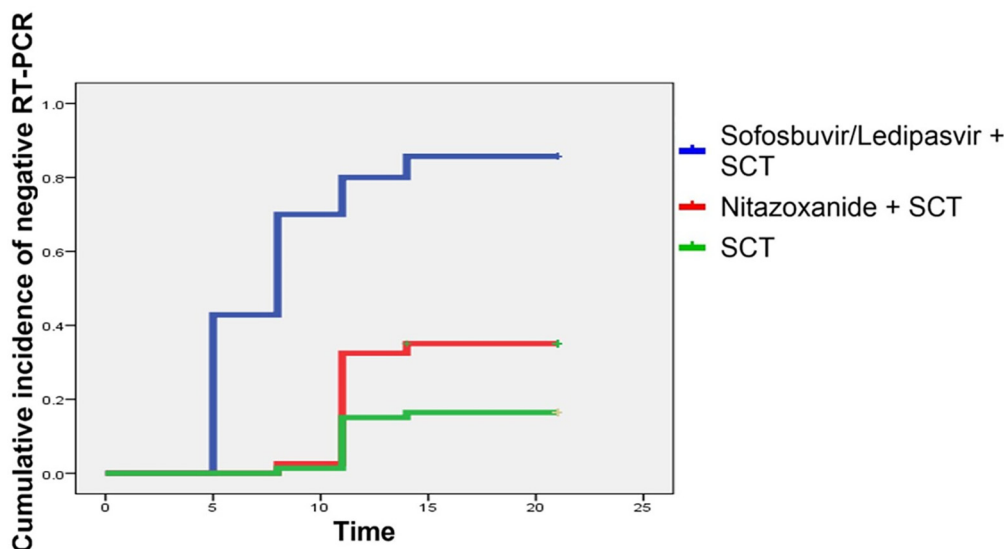


Fig. 2. Log-rank curve of the cumulative time to negative RT-PCR in all groups.

Table 5

Cox regression analysis of viral clearance (negative RT-PCR test).

Variables	p-value	HR (95 % CI)
Sofosbuvir/ledipasvir	< 0.001	11.137 (5.928–20.923)
Nitazoxanide	0.021	2.248 (1.133–4.461)
Sex	0.593	0.897 (0.602–1.337)

Antiviral therapy for COVID-19 infection shows variable viral clearance results among different drugs. A double-blinded randomized controlled trial (RCT) revealed no superiority of Remdesivir over placebo in time to clinical recovery, 28-day mortality, or viral clearance [20]. In the efficacy of the LPV/RTV and arbidol against novel coronavirus infection (ELACOI) trial with a single-blinded RCT, including 44 patients with mild-to-moderate COVID-19, no differences in the time to negative RT-PCR test among the LPV/RTV, umifenovir, and control groups (8.5, 7, and 4 days, respectively) were reported [34].

The rate of RT-PCR negative conversion in hospitalized patients with COVID-19 who received LPV/RTV was similar to those who received the SCT at different time points [35]. Although the use of FVP in SARS-CoV-2 infected patients was associated with significant clinical and radiological improvement, it was not superior to the SCT regarding viral clearance [36]. However, in a multicenter randomized Phase II/III clinical trial, FVP enabled SARS-CoV-2 viral clearance was observed in 62.5% of patients within four days versus 30% in the SCT group [37]. However, the combined use of FVP with inhaled IFN- $\alpha$  showed viral clearance at a significantly shorter duration when compared with combined LPV/RTV and inhaled IFN- $\alpha$  (4 days vs. 11 days) [38]. In a recently published study, adding sofosbuvir/daclatasvir to the SCT for COVID-19 was associated with faster PCR negativity and shorter hospital stay [39]. Molnupiravir is an antiviral drug which was initially used as a possible treatment of influenza viruses and viral encephalitis caused by alphaviruses like Venezuelan, Eastern and Western equine encephalitic viruses [40,41]. Some *in vitro* and *in vivo* studies showed ability of molnupiravir to inhibit replication, reduce the viral load of SARS-CoV-2 virus, in addition to improvement the pulmonary function [42,43]. A recently published systematic review about the use molnupiravir in COVID-19 showed that it is beneficial in reducing hospitalization or death in mild COVID-19, but its role in moderate to severe COVID-19 is debatable [44]. A novel

SARS-COV-2 protease inhibitor (PAXLOVID™) produced by Pfizer Inc. showed that it can lead to a 89% reduction in COVID-19-related hospitalization or death compared to placebo [45].

In the current study, sofosbuvir/ledipasvir resulted in earlier viral clearance than nitazoxanide and SCT. Importantly, this could shorten the duration of infectivity and the risk of viral transmission from infected patients, reducing the rapid viral spread observed in this pandemic.

Although there is a significant difference among the groups regarding gender, this factor had no significant impact on the viral clearance in the Cox regression analysis. Notably, both drugs in the regression analysis were the only significant factors affecting the viral clearance, and sofosbuvir/ledipasvir had much higher odds of obtaining viral clearance than nitazoxanide. This could result from the targeting of two viral proteins by the combination of sofosbuvir/ledipasvir, thus enhancing its antiviral activity and reducing the ability of the virus to develop resistance.

Reporting the clinical improvement as a marker for treatment efficacy might not be accurate because non-severe cases of COVID-19 have good clinical outcomes and recovery, even when using SCT regimens alone. Our primary endpoint was viral clearance by serial RT-PCR testing on nasopharyngeal swabs. Our primary endpoint enabled us to conclude that sofosbuvir/ledipasvir resulted in faster COVID-19 virus elimination. However, the small sample size of this study is a limitation that should be stated. Further multicenter studies are needed to evaluate the efficiency of sofosbuvir/ledipasvir and nitazoxanide to treat severe COVID-19 cases. Also, comparing the efficacy of different types of sofosbuvir-based DAAs therapy, such as sofosbuvir/daclatasvir, in the management of COVID-19 is recommended.

In conclusion, the addition of sofosbuvir/ledipasvir or nitazoxanide (to a lesser extent) to SCT results in an earlier and higher viral clearance rate in mild and moderate COVID-19 compared with SCT alone. Sofosbuvir/ledipasvir shows the highest efficacy in promoting viral clearance. These drugs are well tolerated for the duration of therapy. Thus, they represent a promising, safe, and affordable treatment in the management of COVID-19.

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## Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

- [1] Ashour HM, Elkhatib WF, Rahman MM, Elshabrawy HA. Insights into the recent 2019 novel coronavirus (SARS-CoV-2) in light of past human coronavirus outbreaks. *Pathogens* 2020;9:186.
- [2] Shi Y, Wang G, Cai X-p, Deng J-w, Zheng L, Zhu H-h, et al. An overview of COVID-19. *J Zhejiang Univ Sci B* 2020:1.
- [3] WHO. Global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments 2020.
- [4] Hosseini FS, Malektojari A, Ghazizadeh S, Hassaniyazad M, Davoodian P, Dadvand H, et al. The efficacy and safety of Ivermectin in patients with mild and moderate COVID-19: a structured summary of a study protocol for a randomized controlled trial. *Trials* 2021;22:1–3.
- [5] Paules CI, Marston HD, Fauci AS. Coronavirus infections—more than just the common cold. *JAMA* 2020;323:707–8.
- [6] Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat Commun* 2019;10:1–9.
- [7] Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. *Proc Japan Acad B* 2017;93:449–63.
- [8] Warren TK, Jordan R, Lo MK, Ray AS, Mackman RL, Soloveva V, et al. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature* 2016;531:381–5.
- [9] Ju J, Kumar S, Li X, Jockusch S, Russo JJ. Nucleotide analogues as inhibitors of viral polymerases. *bioRxiv* 2020:2020.2001.2030.927574.
- [10] Chen Y, Yiu C, Wong K. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CLpro) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates [version 1; peer review: 3 approved]. *F1000Research* 2020;9.
- [11] Elfiky AA. Ribavirin, remdesivir, sofosbuvir, galidesivir, and tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): a molecular docking study. *Life Sci* 2020;253:117592.
- [12] Aghemo A, De Francesco R. New horizons in hepatitis C antiviral therapy with direct-acting antivirals. *Hepatology* 2013;58:428–38.
- [13] Omran D, Alborai M, Zayed RA, Wafi M-N, Naguib M, Eltabbakh M, et al. Towards hepatitis C virus elimination: Egyptian experience, achievements and limitations. *World J Gastroenterol* 2018;24:4330–40.
- [14] Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B* 2020;10:766–88.
- [15] Rossignol J-F. Nitazoxanide, a new drug candidate for the treatment of Middle East respiratory syndrome coronavirus. *J Infect Public Health* 2016;9:227–30.
- [16] Haffizulla J, Hartman A, Hoppers M, Resnick H, Samudrala S, Ginocchio C, et al. A randomized, double-blind, placebo controlled clinical trial of nitazoxanide in adults and adolescents with acute uncomplicated influenza. *Lancet Infect Dis* 2014;14:609–18.
- [17] Rajoli RK, Pertinez H, Arshad U, Box H, Tatham L, Curley P, et al. Dose prediction for repurposing nitazoxanide in SARS-CoV-2 treatment or chemoprophylaxis. *medRxiv* 2020.
- [18] Organization WH. Therapeutics and COVID-19: living guideline, 24 September 2021: World Health Organization; 2021.
- [19] Organization WH. Therapeutics and COVID-19: living guideline. 31 March 2021: World Health Organization; 2021.
- [20] Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Resp Crit Care Med* 1994;149:818–824.
- [21] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016;315:801–10.
- [22] Ahmad M, Dwivedy A, Mariadasse R, Tiwari S, Kar D, Jayakanthan J, et al. Prediction of small molecule inhibitors targeting the severe acute respiratory syndrome coronavirus-2 RNA-dependent RNA polymerase. *ACS Omega* 2020;5:18356–66.
- [23] Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19—preliminary report. *New Engl J Med* 2020.
- [24] Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020.
- [25] Jockusch S, Tao C, Li X, Chien M, Kumar S, Morozova I, et al. Sofosbuvir terminated RNA is more resistant to SARS-CoV-2 proofreader than RNA terminated by Remdesivir. *Sci Rep* 2020;10:1–9.
- [26] Nourian A, Khalili H, Ahmadinejad Z, Kouchak HE, Jafari S, Manshadi SAD, et al. Efficacy and safety of sofosbuvir/ledipasvir in treatment of patients with COVID-19: a randomized clinical trial. *Acta Bio Medica: Atenei Parmensis* 2020;91.
- [27] Roozbeh F, Saeedi M, Alizadeh-Navaei R, Hedayatzadeh-Omran A, Merat S, Wentzel H, et al. Sofosbuvir and daclatasvir for the treatment of COVID-19 outpatients: a double-blind, randomized controlled trial. *J Antimicrob Chemother* 2020.
- [28] Eslami G, Mousaviasl S, Radmanesh E, Jelvey S, Bitaraf S, Simmons B, et al. The impact of sofosbuvir/daclatasvir or ribavirin in patients with severe COVID-19. *J Antimicrob Chemother* 2020;75:3366–3372.
- [29] Sadeghi A, Ali Asgari A, Norouzi A, Kheiri Z, Anushirvani A, Montazeri M, et al. Sofosbuvir and daclatasvir compared with standard of care in the treatment of patients admitted to hospital with moderate or severe coronavirus infection (COVID-19): a randomized controlled trial. *J Antimicrob Chemother* 2020;75:3379–3385.
- [30] Rossignol J-F. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. *Antiviral Res* 2014;110:94–103.
- [31] Lam KKY, Zheng X, Forestieri R, Balgi AD, Nodwell M, Vollett S, et al. Nitazoxanide stimulates autophagy and inhibits mTORC1 signaling and intracellular proliferation of Mycobacterium tuberculosis. *PLoS Pathog* 2012;8.
- [32] Rocco PR, Silva PL, Cruz FF, Junior MAC, Tierno PF, Moura MA, et al. Early use of nitazoxanide in mild Covid-19 disease: randomised, placebo-controlled trial. *Eur Respir J* 2020.
- [33] Elalfy H, Besheer T, El-Mesery A, El-Gilany A-H, Soliman M-A, Alhawarey A, et al. Effect of a combination of nitazoxanide, ribavirin, and ivermectin plus zinc supplement (MANS.NRIZ study) on the clearance of mild COVID-19. *J Med Virol* 2021;93:3176–83.
- [34] Li Y, Xie Z, Lin W, Cai W, Wen C, Guan Y, et al. Efficacy and safety of lopinavir/ritonavir or arbidol in adult patients with mild/moderate COVID-19: an exploratory randomized controlled trial. *Med* 2020;1:105–113.e4.
- [35] Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe covid-19. *N Engl J Med* 2020.
- [36] Shrestha DB, Budhathoki P, Khadka S, Shah PB, Pokharel N, Rashmi P. Favipiravir versus other antiviral or standard of care for COVID-19 treatment: a rapid systematic review and meta-analysis. *Virology* 2020;17:141.
- [37] Ivashchenko AA, Dmitriev KA, Vostokova NV, Azarova VN, Blinow AA, Egorova AN, et al. AVIFAVIR for Treatment of patients with moderate Coronavirus Disease 2019 (COVID-19): interim results of a phase II/III multicenter randomized clinical trial. *Clin Infect Dis* 2020.
- [38] Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental treatment with favipiravir for COVID-19: an open-label control study. *Engineering* 2020;6:1192–8.
- [39] El-Bendary M, Abd-Elsalam S, Elbaz T, El-Akel W, Cordie A, Elhadidy T, et al. Efficacy of combined Sofosbuvir and Daclatasvir in the treatment of COVID-19 patients with pneumonia: a multicenter Egyptian study. *Exp Rev Anti-infective Ther* 2022;20:291–5.
- [40] Crotty S, Cameron CE, Andino R. RNA virus error catastrophe: direct molecular test by using ribavirin. *Proc Natl Acad Sci* 2001;98:6895–900.
- [41] Agostini ML, Pruijssers AJ, Chappell JD, Gribble J, Lu X, Andres EL, et al. Small-molecule antiviral  $\beta$ -d-N 4-hydroxycytidine inhibits a proofreading-intact coronavirus with a high genetic barrier to resistance. *J Virol* 2019;93:e01348–01319.
- [42] Wahl A, Gralinski LE, Johnson CE, Yao W, Kovarova M, Dinnon KH, et al. SARS-CoV-2 infection is effectively treated and prevented by EIDD-2801. *Nature* 2021;591:451–7.
- [43] Sheahan TP, Sims AC, Zhou S, Graham RL, Pruijssers AJ, Agostini ML, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Sci Transl Med* 2020;12:eabb5883.
- [44] Singh AK, Singh A, Singh R, Misra A. Molnupiravir in COVID-19: a systematic review of literature. *Diab Metabol Syndrome: Clin Res Rev* 2021;15:102329.
- [45] Pfizer. Pfizer's novel COVID-19 oral antiviral treatment candidate reduced risk of hospitalization or death by 89% in interim analysis of phase 2/3 EPIC-HR study.