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Evaluating The Antiviral Potential Of Fluoxetine, Sertraline, And Escitalopram Against SARS-CoV-2 In Vero Cells: A Drug Repurposing Study

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Abstract:
Background*: COVID-19, caused by SARS-CoV-2, remains a global health crisis with limited therapeutic options. Despite the availability of vaccines, the rapid evolution of viral variants necessitates the exploration of alternative treatments. Drug repositioning, the use of existing medications for new therapeutic indications, offers a promising approach. Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, sertraline, and escitalopram have shown potential antiviral properties due to their immunomodulatory effects. This study investigates the impact of these SSRIs on SARS-CoV-2-induced cytopathic effects (CPE) in Vero cells.*
Materials and Methods*: Vero cells were cultured and treated with varying concentrations of fluoxetine, sertraline, and escitalopram. Ivermectin, was used as a positive control. The cytotoxicity of these drugs was assessed using the Neutral Red assay. After viral infection of the cells, cytopathic effects were measured to determine the antiviral activity of the drugs. IC50 (half-maximal inhibitory concentration) values were calculated for each drug, and the results were compared using statistical analysis.*
Results*: Fluoxetine, sertraline, and escitalopram demonstrated dose-dependent inhibition of SARS-CoV-2-induced cytopathic effects, with escitalopram showing the highest efficacy (IC50 = 0.02557 μM, $p < 0.03$). The selectivity indices (SI) for fluoxetine, sertraline, and escitalopram were significantly higher than that of ivermectin, indicating their safety at higher doses. Escitalopram exhibited superior antiviral activity, with up to 38% inhibition of SARS-CoV-2-induced CPE, compared to ivermectin's 10%.*
Conclusion: *Fluoxetine, sertraline, and escitalopram, particularly escitalopram, are promising candidates for treating SARS-CoV-2 infections. These SSRIs exhibit significant inhibition of virus-induced cytopathic effects in Vero cells, suggesting their potential use as therapeutic agents against COVID-19. Further studies are required to confirm their clinical efficacy and safety in human subjects.*
Key Word*: Fluoxetine; Sertraline; Escitalopram; SARS-CoV-2; Cytopathic Effects; Drug repurposing.*

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I. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent responsible for coronavirus disease 2019 (COVID-19), which has inflicted a profound global impact on demographics and stands as the most significant health crisis since the 1918 influenza pandemic (CDC, 2018). With the global toll surpassing 7 million deaths and over half a billion confirmed cases (WHO, 2024), the pandemic has precipitated unprecedented disruptions to economic and societal activities through stringent preventive measures, including border closures and social distancing, implemented to mitigate its spread. Despite these efforts, the pandemic persists, emphasizing the need for effective therapeutic interventions.

COVID-19 manifests clinically as a spectrum of respiratory infections, ranging from mild symptoms akin to the common cold to severe viral pneumonia culminating in potentially fatal acute respiratory distress syndrome, alongside other complications such as stroke, cardiac injury, renal impairment, and mortality (Cascella et al., 2022).

Given the obligate intracellular parasitism of SARS-CoV-2, wherein the virus commandeers host cellular machinery for replication, the development of effective treatments poses considerable challenges. Moreover, the virus's propensity for rapid mutation exacerbates these challenges, necessitating innovative therapeutic approaches.

In response to the limitations of traditional drug discovery methods, which are resource-intensive and time-consuming with high failure rates, researchers have turned to drug repositioning as a pragmatic strategy. This approach leverages existing drugs with established safety profiles for novel therapeutic indications, bypassing the protracted process of lead identification and preclinical testing. Notably, advancements in molecular and computational biology have facilitated high-throughput screening of drug libraries against specific disease targets, enabling expedited identification of promising candidates for repurposing.

Unlike other classes of infectious agents, antiviral chemotherapy poses unique challenges due to its potential for off-target effects on host cellular pathways. Targeting key stages of the viral life cycle, including adhesion, entry, replication, and maturation, offers a promising approach to inhibiting viral propagation. Notably, several druggable targets within the SARS-CoV-2 proteome have been identified, offering opportunities for targeted intervention.

Justification for the Study

Even though there are vaccines against COVID-19, people still get the infection and therefore need to be treated. The conventional methods of drug discovery have proved extremely useful but it is unfortunately time demanding, capitally intensive and with low throughput. Consequently, the method cannot be relied on when it comes to novel infections like the SARS-CoV-2 because of the urgent needs for therapeutics. Therefore, to rapidly provide safe and effective therapies against SARS-CoV-2, drug repositioning is a timely, cheaper and rational alternative (Jourdan et al., 2020). Several clinical trials and observational studies have investigated the possibilities of repurposing various pharmacological agents against SARS-CoV-2, interestingly, the findings were mixed and sometimes even opposing to one another (Kim et al., 2020) this might be because most of the drugs investigated were based on clinical intuition. This lack of efficacy could also be due to the facts that the SARS-CoV-2 virus causes syndromic pathogenicity and therefore may require a compound with multi-target activity against the syndromic pathogenicity (Wang et al., 2020a). Remdesivir was the first drug that received an FDA emergency use authorization for severe COVID-19 treatment. Remdesivir has a very short half-life in the plasma of patients (approximately 20 min) and, moreover, requires activation through pro-drug enzymes such as carboxylesterases, cathepsin A and histidine triad nucleotide binding proteins which are preferentially expressed in the liver.

Previous research has investigated the repurposing of selective serotonin reuptake inhibitors (SSRIs) for the treatment of viral infections (Foletto et al., 2022) SSRIs, commonly used as antidepressant drugs, have shown potential antiviral properties. These medications work by selectively inhibiting the reuptake of serotonin, a neurotransmitter that regulates mood, sleep, and appetite. By increasing serotonin levels in the brain, SSRIs can alleviate symptoms of depression and anxiety.

In addition to their antidepressant effects, SSRIs have demonstrated immunomodulatory properties. Studies have highlighted that SSRIs can enhance the function of immune cells, such as T cells and natural killer cells, and increase the production of cytokines and chemokines involved in the immune response. This evidence suggests that SSRIs may possess antiviral properties and could be used to treat viral infections (Pashaei, 2021).

In this context, it is important to note that further research is needed to determine the safety and efficacy of SSRIs in the context of SARS-CoV-2 infection. While there is promising evidence for their potential use in the treatment of viral infections, challenges such as establishing optimal dosages and treatment durations need to be addressed. SSRIs may also have side effects, including nausea, diarrhea, and headaches, and their long-term use could potentially increase the risk of bleeding and cardiovascular events. Additionally, additional in vivo studies are necessary to confirm the findings from in-vitroand molecular docking studies and to investigate potential interactions between SSRIs and other drugs used to treat COVID-19 (Pashaei, 2021).

Meanwhile one of the selective serotonin reuptake inhibitors (Fluvoxamine) has been found effective against COVID-19 mainly due to its anti-inflammatory and immunomodulatory effects (Asadi et al., 2022; CDC, 2021).

Indeed, the pursuit of this strategy is of paramount significance, particularly in relation to the potential employment of off-patent selective serotonin reuptake inhibitor (SSRI) medications as a therapeutic approach. Given their ability to target specific protein pathways implicated in the disease, SSRI medications could offer a promising avenue for the amelioration of COVID-19 pathogenesis (Hoertelet al., 2021).

However, both Remdesivir and fluvoxamine are very expensive and not readily available in most African countries. Moreso, none of the randomized clinical trials on Fluvoxamine against COVID-19 were conducted in Africa (Asadi et al., 2022) and therefore its effectiveness in Africans with COVID-19 cannot be guaranteed. Therefore, COVID-19 continues to have a significant therapeutic gap in Africa.

In this study we intend to investigate toxicity profiles of fluoxetine, sertraline and escitalopram on Vero cells and their anti SARS-CoV-2 cytopathic effect on Vero cells..

II. Material And Methods

For cell culture studies, Vero cells ATCC CRL-1587 were utilized. Maintenance of cell cultures involved equipment such as a 5% CO2 Incubator, Thermo Scientific FC200 Fixed Centrifuge Machine, Molecular hybridization oven HybridGene Pro, AmScope IN200TB Inverted Trinocular Microscope, and Biosafety cabinet BioGuard Pro 2000. Culture media included Dulbecco's Modified Eagle Medium (DMEM), Fetal bovine serum (FBS), Penicillin/streptomycin, Trypsin-EDTA solution 0.25%, and Phosphate buffer saline (PBS). Additionally, a variety of materials ranging from molecular grade water to tissue culture flasks and pipette tips were used.

Chemicals and drugs for experimentation were sourced from MedChemExpress, including Fluoxetine, Escitalopram, Ivermectin, and DMSO (Dimethyl sulfoxide).

The study also involved the utilization of the SARS-CoV-2 clinical isolate, as well as the cell viability assay reagents Neutral red (from Beijing Solarbio Science & Technology Co., Ltd.) and glacial acetic acid. Various laboratory materials were employed, including freezers, refrigerators, liquid nitrogen, beakers, laboratory coats, boots, face masks, vortex mixers, weighing balances, water distillers, deionizers, stopwatches, and CO2 cylinders.

Vero cells Maintenance and Passaging

The maintenance and passaging of Vero cells involved thawing cryovials, decontamination with ethanol, centrifugation, and suspension in DMEM supplemented with FBS and penicillin-streptomycin. Cells were cultured in T25 flasks, monitored daily, and sub-cultured at 90% confluence. Cryopreserving involved trypsinization, centrifugation, resuspension of confluent monolayer of vero cells in freezing media, and storage at -80°C. Cell counting was conducted using the counting chamber method.

Passaging SARS-CoV-2 in Vero Cells

A confluent monolayer of Vero cells was trypsinized and re-suspended in DMEM (Modified Eagles Media). Using a Hemocytometer and trypan blue exclusion method, about 10 million Vero cells were counted and seeded in a T-75 flask and incubated for approximately 24 hours at 37°C in 5% CO_2. The flask was then viewed under an inverted microscope to confirm a confluence of 70 to 80%. Once the confluence monolayer was achieved, the culture medium was removed, leaving about 5 ml. Then, 500 µl of the filtered PCR positive clinical isolate of SARS-CoV-2 was added to the flask and incubated for 1 hour at 37°C in 5% CO_2. After that, 10 ml of DMEM containing 1% FBS was added to make a total volume of 15 ml. The flask was then incubated for 96 hours at 37°C in 5% CO_2, and cytopathic effects (CPE) were checked daily with an inverted microscope until significant CPE was observed. The supernatant was collected from the infected flask and centrifuged for 5 minutes at 500 × g, room temperature, to remove any cellular debris. The clarified supernatant was stored and labeled as passage 1 (P1) in 1.5ml screw-cap tubes at -80^{0}C in aliquots of 1 ml until use. P1 was repassage to obtain P2; the procedure was repeated to obtain lower passages (P3, P4…). It is worth noting that all experiments were conducted using viral passage 3 (P3) to maintain consistency (Coleman & Frieman, 2015).

Cell Viability Studies using Neutral Red Assay

Cytotoxicity assays are essential for evaluating a compound's potential toxicity on cells. This study used the neutral red uptake test to assess the cytotoxicity of selected drugs on Vero cells.

Procedure:

Cell Culture: Vero cells were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells (6x10^4 cells/mL, 200 µL per well) were seeded into a 96-well plate and incubated for 24 hours at 37°C with 5% CO2.

Drug Preparation: Stock solutions (100 mM) of selected drugs and ivermectin were prepared in DMSO and diluted in DMEM to concentrations of 1 µM, 10 µM, 100 µM, and 1000 µM. Control cells received DMSO.

Drug Treatment: Media was replaced with drug-containing media, and cells were incubated for 72 hours at 37°C with 5% CO2.

Neutral Red Assay: After incubation, media was replaced with neutral red dye solution (40 µg/mL in DMEM) and incubated for 3 hours at 37°C with 5% CO2. Cells were then washed with PBS, and dye was extracted using a destain solution. Absorbance was measured at 540 nm.

Analysis:

CPE Reduction Assay

Preparation of Vero Cells: 96-well plates were seeded with 6x10^4 cells/mL of Vero cells, with 200 µL per well, using DMEM containing 10% fetal bovine serum (FBS) and no antibiotics. The plates were incubated overnight at 37°C in a CO2 atmosphere. The following day, the plates were examined under an inverted microscope to confirm approximately 50% cell confluence.

Inoculation with SARS-CoV-2: Sixty minutes before drug treatment, the cell culture supernatant was removed from each well, and the wells were washed with 150 µL of phosphate buffer solution (PBS). Each well, except for the negative control wells where 50 µL of PBS was added, was infected with SARS-CoV-2 diluted in PBS at a multiplicity of infection (MOI) of 0.1. The plates were incubated for 1 hour at 37°C in a 5% CO2 atmosphere with intermittent shaking every 15 minutes to facilitate viral adsorption.

Drug Treatment

After viral adsorption, the infection supernatant was carefully aspirated from each well. Subsequently, 200 µL of the respective drugs diluted in DMEM were added to the respective wells. Three different doses of each drug, centered around the Cmax (ivermectin, concentrations of 10µM, 7.5µM, and 5µM were tested, while fluoxetine was tested at 30 nM, 20 nM, and 15 nM, and sertraline and escitalopram at 40 nM, 30 nM, and 20 nM), were tested for antiviral activity. Ivermectin, a known antiviral, served as the positive reference control. Other controls included wells with only Vero cells (positive control) and wells with Vero cells plus the virus (negative control). Each test was performed in triplicate. After 48 hours of treatment, the cells were examined under an inverted microscope for cytopathic effects (CPE), which were quantified using the Neutral Red assay.

Quantification of CPE Inhibition with Neutral Red (NR) Assay

A 40 µg/mL neutral red working solution was incubated overnight and filtered through a 2 µm membrane filter to remove precipitated dye crystals. The attached cells from the treatment groups were washed with 150 µL of PBS per well, and the washing solution was removed by gentle tapping. Next, 100 µL of the neutral red medium was added to each well, and the plates were incubated for 2 hours. After incubation, the plates were examined under an inverted microscope for any potential neutral red precipitation. The neutral red medium was then removed, and the cells were washed twice with 150 µL of PBS per well. Following this, 150 µL of neutral red destain solution was added to each well, and the plates were rapidly shaken on a microtiter plate shaker at 500 revolutions per minute for at least 10 minutes.

The absorbance of the neutral red extracted from each well was measured at 540 nm using a microtiter plate reader spectrophotometer, with blanks containing no cells as references. The percentage inhibition of CPE was calculated using the formula from Severson et al. (2007): ((Test substance - Virus control) / (Cell control - Virus control)) × 100.

Statistical Analysis

The data was presented in tables and graphs, and expressed as mean ± SEM. The half-maximal inhibitory concentration (IC_{50}) and half-maximal toxic concentrations (CC_{50}) were extrapolated from a sigmoidal dose-response curve. Statistical differences between the CPE reduction and enzyme inhibition from different compound groups were analyzed using a two-way ANOVA followed by Dunnett's multiple comparison tests. A p-value of < 0.05 was considered to be statistically significant. All analyses were performed using Graph Pad Prism version 5.01

III. Result

Cytotoxicity Evaluation of Ivermectin, Fluoxetine, Sertraline, and Escitalopram against Vero cells and their selectivity index

we conducted a cytotoxicity assay to assess the cytotoxic effects of Ivermectin, Fluoxetine, Sertraline, and Escitalopram, on Vero cells by culturing Vero cells in DMEM supplemented with fetal bovine serum and maintained in a humidified incubator at 37°C and 5% CO2 and treated with the respective drugs and a concentration range between 0.1 µM to 100 µM and incubated for 72 hours. The obtained absorbance values were used to calculate cell viability and determine the concentration-dependent cytotoxic effects of the drugs on Vero cells.

The concentration at which a substance exhibits 50% cytotoxicity (CC50), the concentration at which it inhibits 50% of the target (IC50), and the selectivity index (CC50/IC50) against SARS-CoV-2 were determined individually. The result shows concentration-dependent cytotoxic effects of Ivermectin, Fluoxetine, Sertraline, and Escitalopram on Vero cells with.The CC50 values for ivermectin, fluoxetine, sertraline and escitalopram on Vero-E6 cells were found to be 7.153 µM, 2.567 µM, 11 µM and 8.767 µM respectively see figure 1 (A, B, C and D) with selectivity index of 2 for ivermectin and >100 for , fluoxetine, sertraline and escitalopram respectively (table 1). The CC_{50} values were calculated using nonlinear regression analysis of GraphPad Prism software (version 5.01) by plotting log inhibitor versus normalized response (variable slope).

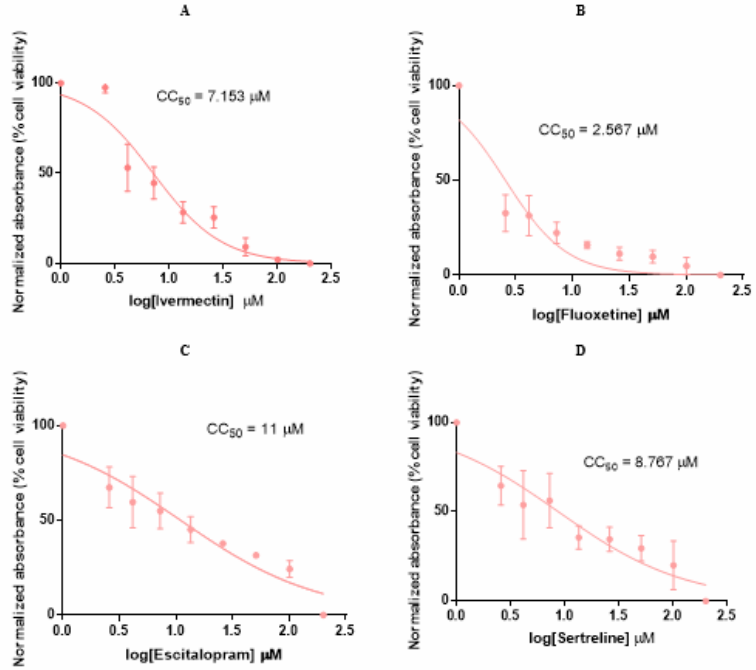

Figure 1 Determination of cytotoxic 50% (CC50) of ivermectin and selected antidepressants in Vero-E6 cells

Table 1 Selectivity Index (SI) of Ivermectin and the Selected SSRIs against Vero Cells

Drugs	CC_{50} (µM)	*IC_{50}(µM)	SI
Ivermectin	7.150	2.873	2.5
Fluoxetine	2.567	0.0181	141.5
Sertraline	8.767	0.0652	144.9
Escitalopram	11.000	0.0617	178.3

Qualitative Evaluation of CPE Inhibitory Effects of Ivermectin, Sertraline, Fluoxetine, and Escitalopram

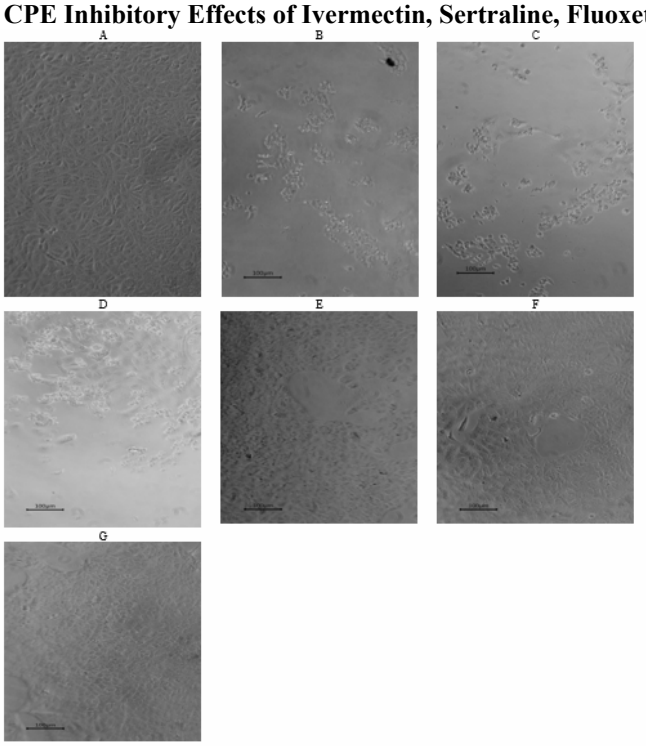

Figure 2: SARS-CoV-2 Infected Vero Cells Treated with Fluoxetine, Sertraline, Escitalopram, and Ivermectin

Inhibition by selected drugs of SARS-CoV-2 induced CPE in Vero cells (x10) (A) Vero cells. (B) SARS-CoV-2 infected Vero showing ≥ 80% Cytopathic effects (CPE) (C) SARS-CoV-2 infected Vero cells with 0.1% DMSO showing ≥ 80% CPE. (D) SARS-CoV-2 infected Vero cells with 5 µM Ivermectin in 0.1% DMSO showing ≥ 70% CPE. (E) SARS-CoV-2 infected Vero cells with 30 µM sertraline in 0.1% DMSO showing ≥ 60% CPE. (F) SARS-CoV-2 infected Vero cells with 30 µM Escitalopram in 0.1% DMSO showing ≥ 50% CPE. (G) SARS-CoV-2 infected Vero cells with 20 µM Fluoxetine in 0.1% DMSO showing ≥ 60% inhibition CPE

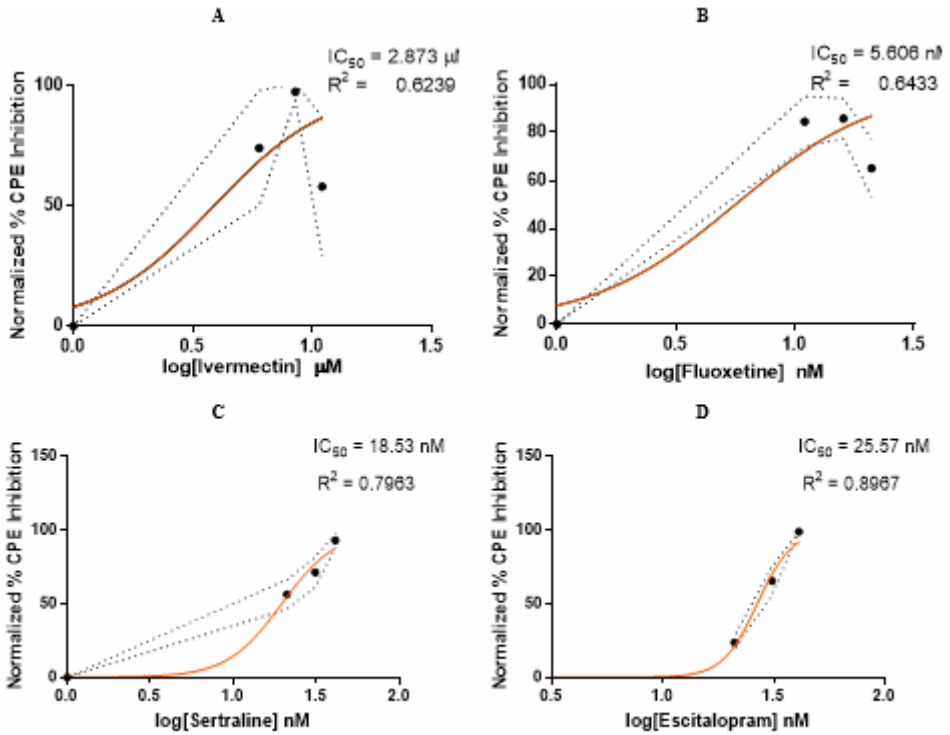

Figure 3: Determination of inhibitory concentration 50% (IC_{50}) of ivermectin and the selected antidepressants against SARS-CoV-2 in Vero cells

Quantitative Evaluation of SARSCoV-2 induced CPE Inhibitory effects of Ivermectin, Sertraline, Fluoxetine, and Escitalopram

The preliminary findings indicated that all tested doses (except for ivermectin) of the drugs were non-toxic to the Vero cells, used in the experiment. This suggests that the drugs themselves did not cause significant cellular damage or toxicity, ensuring the reliability of the subsequent analysis. The results further revealed that ivermectin, sertraline, and fluoxetine exhibited moderate inhibition of SARS-CoV-2-induced CPE, even though not statistically significant ($p>0.05$). The maximum inhibition achieved by these drugs was 10%, 6%, and 8%, respectively, at the tested dose. These findings suggest a potential trend of antiviral activity. In contrast, escitalopram, demonstrated more promising results. The CPE reduction assay showed a significant dose-dependent inhibition of SARS-CoV-2-induced cytopathic effect by escitalopram, with a maximum inhibition of 38% observed at a concentration of 20 nM ($p=0.03$). This finding indicates that escitalopram may possess potent antiviral properties against SARS-CoV-2 (table 5, figure 4).

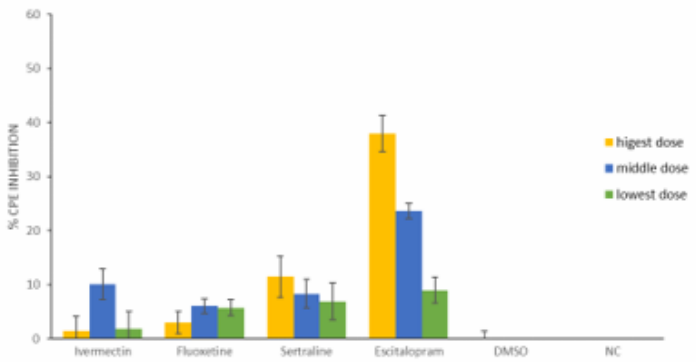

Figure 5: Inhibition of SARS-CoV-2 induced CPE in Vero cells

For Ivermectin Highest dose, Middle dose and Lowest dose respectively represent 10µM, 7.5µM and 5µM for Fluoxetine Highest dose, Middle dose and Lowest dose respectively represent 20 nM, 15 nM while for Sertraline and Escitalopram Highest dose, Middle dose and Lowest dose were 40 nM, 30 nM and 20 nM. The doses were selected based on achievable Cmax in human subjects at standard doses of the drugs. All concentrations of test drugs were found nontoxic to Vero cells. Analysis was performed with outliers because biological factors probably dictated differences.

Table 2: Inhibition of SARS-CoV-2 Cytopathic Effect

Drug	Maximal inhibition of CPE (%)	Dose at maximal observed* CPE inhibition of SARS-CoV-2 (μM)	IC_{50}(μM)	95% CI of IC_{50} (μM)	P
Ivermectin	10	7.5	2.873	1.368 to 4.652	>0.05
Fluoxetine	6	0.020 and 0.015	0.00561	0.0030 to 0.0093	>0.05
Sertraline	11.0	0.040	0.01853	0.0113 to 0.023	>0.05
Escitalopram	38.0	0.020	0.02557	0.023 to 0.028	<0.03

* 'Maximal observed' inhibition in this study is not the inherent maximum possible inhibition efficacy of the tested drug, but that observed within the dose range tested. These dose ranges were centred at achievable plasma concentration of the drugs at the current recommended doses except Ivermectin where ranges were derived from literature of concentrations previously found to inhibit SARS-CoV-2 proliferation. Ivermectin cause non-significant inhibition of CPE and then at an IC_{50} concentration not achievable in human plasma(800mcg/kg maximum single dose of ivermectin has C_{max} 108.1ng/ml or 0.124 μM/ml) Analysis were performed with outliers because biological difference probably dictated differences

IV. Discussion

The COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has presented an urgent need for effective therapeutics. Despite global efforts in developing small molecule drugs for COVID-19 treatment, an effective solution remains elusive. Although remdesivir is FDA-approved, high mortality rates persist despite its use, and recommendations from WHO and European Medical Association suggest its insufficiency. It is vital to prepare for emerging SARS-CoV-2 variants and unknown pathogenic viruses by establishing swift strategies for drug development alongside vaccines.

In order to validate our in silico predictions experimentally, we initiated a proof-of-concept study with the objective of assessing the potential efficacy of selected drugs against SARS-CoV-2, using a rapid qualitative cytopathic effect based assay. To accomplish this, we performed a single-dose experiment in three independent replicates, ensuring robustness and reliability.

The experimental procedure involved infecting Vero cells with SARS-CoV-2 at MOI of 0.1 and subsequently treating them with selected drugs, including ivermectin. The concentrations of drugs used were determined based on the maximum achievable plasma concentration (Cmax) of the selected drugs including ivermectin which was used as positive control to compare our result to previously published studies. By utilizing an inverted microscope, we were able to observe and evaluate the samples for the presence or absence of cytopathic effects (CPE), which are indicative of viral infection and replication. CPE refers to visible changes in cell appearance caused by viral infection, including cell rounding, detachment from the culture dish surface, and cell lysis. The spike protein variant of SARS-CoV-2 has been shown to exhibit greater cytopathic and fusogenic properties in Vero cells(Rocheleau et al., 2021). Cell damage and fusion can ultimately lead to cell death, which can be detected through the CPE-based assay. Hence, the CPE-based test was well-suited for detecting SARS-CoV-2 infection and monitoring the effectiveness of antiviral treatments.

Furthermore, Bello et al. (2023) have suggested that inhibiting virus-induced CPE, particularly in cases like COVID-19 where cellular death is a major aspect of the disease, is more effective and predictive of clinical success than solely targeting viral replication. Since our initial in silico selection was based on predicted activity against ten independent SARS-CoV-2 targets, any combination of which may be associated with inhibiting CPE, the CPE-based assay provided an insights into the potential effectiveness of the selected drugs.

While we acknowledge that the method employed in this study was subjective, it had been previously employed successfully by (Kudi & Myint, 1999) and (Malbariet al., 2019) to screen the antiviral activity of medicinal plant extracts and potential neuraminidase inhibitors against pandemic H1N1 respectively. With their research as a foundation, we proceeded to assess the antiviral potential of the selected drugs against SARS-CoV-2-induced CPE in Vero cells.

Upon analyzing the results, we found that all the drugs tested exhibited inhibitory effects on SARS-CoV-2-induced CPE in Vero cells. The percentage of inhibition ranged from 40% to 60% when compared to the SARS-CoV-2 infected, and DMSO treated control groups. These findings strongly suggest that the selected drugs, including ivermectin, possess promising antiviral properties against SARS-CoV-2.

In order to examine the inhibition of SARS-CoV-2-induced cytopathic effect CPE in Vero cells, we conducted a detailed quantitative assessment. To accomplish this, we employed a validated Neutral Red uptake assay, a widely recognized and accepted method for evaluating the effects of viral infections (Bello et al., 2022; Betancur-Galvis et al., 1999; Repetto et al., 2008; Selvam et al., 2006). This assay was specifically chosen due to its exceptional sensitivity and specificity enabling the identification and quantification of cellular damage caused by the SARS-CoV-2 virus (Repetto et al., 2008). The assay relies on the principle of quantifying the uptake of the dye Neutral Red, which is actively accumulated by viable cells. Therefore, any decrease in dye uptake indicates cellular damage caused by the virus. The relative viral infection was calculated by normalizing the average infection ratio of the mock control as 0% and the average infection ratio of negative control (0.1% dimethyl sulfoxide [DMSO]) as 100% in each assay plate. Recently, we used the same assay system to experimentally find erythromycin, retapamulin, and pyridoxine as drug candidates to inhibit SARS-CoV-2 induced CPE(Bello et al., 2022). To probe our result, we compared it with the results of the National Center for

Advancing Translational Sciences (NCATS) whom conducted an HTS study to detect SARS-CoV-2 antiviral candidates, using cytopathic effect (CPE) experiments on a large library of chemicals and drugs (NCATS, 2023). Our result was found to be similar for sertraline and ivermectin; this validates our strategy. On the other hand, fluoxetine and escitalopram were not tested by NCATS.

Accordingly, our studies demonstrated that fluoxetine, sertraline escitalopram and ivermectin dose dependently inhibit SARS-CoV-2 induced CPE in Vero cells statistically different from the vehicle control group. Interestingly, the maximum CPE inhibition observed for fluoxetine and sertraline was not statistically different from that of ivermectin even though, they show more than 50 folds more potent than ivermectin.

Accordingly, the results of our studies provide compelling evidence that fluoxetine, sertraline, escitalopram, and ivermectin exhibit dose-dependent inhibition of SARS-CoV-2-induced cytopathic effects (CPE) in Vero cells. This finding is particularly important as it demonstrates the potential of these drugs to combat COVID-19 infection.

Interestingly, when comparing the maximum CPE inhibition achieved by fluoxetine and sertraline with that of ivermectin, we made an interesting observation. Despite fluoxetine and sertraline being more than 50 times more potent than ivermectin, the difference in their maximum inhibitory effects was not statistically significant. With this, one may like to suggests that fluoxetine and sertraline, may not provide a substantial advantage over ivermectin in terms of CPE inhibition despite their superior potency. However, it is important to note that the maximal observed inhibition of cytopathic effects (CPE) does not necessarily represent the inherent maximum possible efficacy of the tested drugs. Instead, it reflects the inhibition observed within the dose range that was tested. These dose ranges were specifically centered around the achievable plasma concentrations of the drugs at their current recommended doses except for ivermectin were the dose ranges used in this study were derived from literature sources that reported concentrations previously found to inhibit SARS-CoV-2 proliferation. Furthermore, the IC50 of Ivermectin in this study was determined to be at a level (2.84 μM) similar to the value of 2.5 μM and 3 μM obtained by Caly and colleagues (2020) and Chable-Bessiaet al., (2022b) respectively against SARS-CoV-2. The levels of 2.5 and 5 μM of ivermectin correspond to plasma levels of 2190 and 4370 ng/mL, respectively. These concentrations are 50 to 100 times higher than the highest plasma concentration achieved when administering ivermectin at a dose of 200 μg/kg (the recommended dose by the US Food and Drug Administration for treating onchocerciasis) (Chaccouret al., 2017). Even when using a dosage ten times greater than this (i.e., 2000 μg/kg), only a peak plasma concentration of approximately 250 ng/mL has been observed (Guzzo et al., 2002). While on the other hand, the Cmax of fluoxetine, sertraline and escitalopram were 0.09 to 0.28 μM(Altamura et al., 1994); 20 to 50 μM(Murdoch & McTavish, 1992) and 20 to 64 ng/ml (Rao, 2007) respectively and these values are much higher than the respective IC50 values we obtained for fluoxetine, sertraline and escitalopram .

Ivermectin, a well-known drug with established antiparasitic properties, has gained considerable attention in the scientific community as a potential treatment option for SARS-CoV-2 due to its reported antiviral activity. Several studies and a systematic review by Heidary and Gharebaghi (2020) have explored the effects of ivermectin on COVID-19 and its associated cytopathic effects. They observed significant reductions in both the virus-induced cytopathic effect and viral replication, indicating the potential of ivermectin as a therapeutic agent against SARS-CoV-2.

In terms of inhibiting SARS-CoV-2-induced cytopathic effects, escitalopram has outperformed sertraline, fluoxetine, and ivermectin. The obtained results demonstrate that escitalopram exhibits higher potency, with an IC50 value of 0.02557 μM, compared to ivermectin's IC50 value of 2.873 μM. These findings suggest that escitalopram may be a more effective option in combating COVID-19 ($p<0.05$).

The SSRIs exhibit remarkable potential in treating various medical conditions, including improving oxygen saturation in severe COPD and providing anticoagulant benefits to COVID-19 patients with venous thrombosis (Folettoet al., 2022). Additionally, they possess proven antiviral properties against a range of viruses such as Ebola (Johansen et al., 2015), Coxsackievirus B4, dengue, hepatitis C, and HIV (Greeson et al., 2016). Notably, Paroxetine, sertraline, and fluvoxamine have demonstrated the ability to reduce viral replication and inflammation. Fluoxetine, for instance, has been shown to inhibit dengue and hepatitis C viruses while also increasing NK cell activity in HIV patients. Sertraline effectively reduces influenza-induced lung inflammation, and fluvoxamine acts as a protective agent against septic shock, reducing the inflammatory response in leukocytes.

Further more all the drugs except ivermectin demonstrated wide toxicity ranges with selectivity index (SI) of greater than hundred indicating their relative safety even with more higher dose. Even though there is no standard value for SI, many scientist favors SI of > 10 (Indrayantoet al., 2021b; Prayonget al., 2008). A selectivity index of < 3 may require reevaluation using another biosystem and if found consistently low may not be good for clinical usage. Consequently the SI of ivermectin found in this studies further demonstrate that ivermectin has narrow safety profile against Vero cells, similar low SI values of ivermectin were reported against equine peripheral blood mononuclear cells (PBMCs), Vero cells(Gupta et al., 2022), and human

pulmonary cell lines (Chable-Bessiaet al., 2022a). Consequently, further reducing the potential of ivermectin clinical application against SARS-CoV-2 infections due to impending cytotoxicity. Numerous scientific papers have documented the effectiveness of various compounds against SARS-CoV-2, but unfortunately, the majority of them fail to provide SI data, and consequently, the described publication had "very limited value" without the SI data of the reported compounds (Indrayanto et al., 2021b).

Despite the possible benefits of the selected SSRIs in significantly reducing SARS-CoV-2-induced CPE in Vero cells, it is important to recognize certain limitations within this study.

Firstly, the study had a limited range of doses, which hindered the exploration of the full spectrum of effects produced by the drugs. Nonetheless, this decision was deliberate, considering budget constraints and the objective of testing feasible concentrations in human plasma for off-label usage, which formed the theoretical basis of the study. Future research with a wider range of doses is necessary to thoroughly investigate the implications of expanded drug labeling and alternative delivery methods.

Secondly, the study involved three different laboratories, introducing the possibility of variability in laboratory standards. However, this potential concern was anticipated, and efforts were made to standardize the workflow across laboratories, thereby improving the data quality. No experiments conducted in one laboratory were replicated in another collaborating laboratory.

Thirdly, the study estimated the IC50; however, these estimations should be approached with caution due to the potential non-monotonic nature of the response, a recognized limitation of IC50/EC50 estimations. Although the 95% confidence interval estimates of the IC50 provide some level of confidence, it should be acknowledged that these estimates were derived by extrapolating unknown data from standard curves using accepted methodologies.

Despite these limitations, the study has several strengths. It utilized validated cell-based assays and can be easily carried out in well-equipped laboratories. Furthermore, the study successfully identified satisfactory levels of effects at therapeutic concentrations of the drugs.

V. Conclusion

The study has identified three antidepressants namely, fluoxetine, sertraline, and escitalopram as promising candidates for treating SARS-CoV-2 infection. These drugs exhibited significant inhibition of SARS-CoV-2-induced cytopathic effects in Vero cells.

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