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Original Research Article

SARS-COV2 ANTIVIRAL TESTING REPORT OF ACT 12 AND 13: SHORT COMMUNICATION

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ABSTRACT

Background: In the current scenario of COVID-19 to take a step towards nutraceuticals and herbal medicines as a potential antiviral against COVID-19, and to encourage the use of nutraceuticals and herbal therapies as preventive steps towards SARS-CoV-2 virus till the other specific drugs and vaccines are discovered. By considering the current need for an effective medicine against the Coronavirus, GP Life Healthcare Pvt. Ltd. has proposed a product with renowned Phytoconstituents to evaluate its antiviral potential against SARS-CoV-2 and developed formulation ACT 12 & ACT 13 a potential candidate in the treatment of COVID-19 infection. **Methods:** The test substances were screened to determine the cytotoxic and Antiviral activities. The assay was done in a 96-well plate format in 3 wells for each sample. The results were compared with the positive control (Remdesivir). The results were recorded at definite time intervals i.e. after 24hrs and 48 hrs. **Result:** In the present study, it was evident that the test substance demonstrates no cytotoxicity. The ACT 12 demonstrating favorable results in % inhibition of viral replication assay. **Conclusion:** It was found from the study that there was no toxicity observed related to the test substance. Thus it depicts the safety of test substances, as well as its potential in inhibition of viral replication in cell culture, demonstrates its efficacy.

Keywords: SARS-CoV-2, Remdesivir, Antiviral, Cytotoxicity

INTRODUCTION

COVID-19 is an infectious disease caused by the most recently discovered coronavirus SARS-CoV-2 virus. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019. COVID-19 is now a pandemic affecting many countries globally[1]. According to the WHO prevalence report, globally 84,474,195 confirmed cases of COVID-19, including 1,848,704 deaths till 5 January 2021, and in India, 10,356,844 confirmed cases of COVID-19 with 149,850 deaths are reported. **(1,2)**

Coronaviruses are enveloped positive-sense RNA viruses ranging from 60 nm to 140 nm in diameter with spike-like projections on their surface giving it a crown-like appearance under the electron microscope hence named coronavirus. The SARS-CoV-2 is a beta-coronavirus belonging to the family of Coronaviridae **(3)**.

The coronavirus genome is comprised of ~30000 nucleotides. It encodes four structural proteins, Nucleocapsid (N) protein, Membrane (M) protein, Spike (S) protein, Envelop (E) protein, and several non-structural proteins (NSP). The capsid is the protein shell, the core is nuclear capsid or N-protein which is bound to the virus single positive-strand RNA that allows the virus to hijack human cells and turn them into virus factories **(4)**.

The M- protein is the most prolific and central organizer in virus assembly. The protein is situated at the surface of the virus and it works on imitation of host-virus attachment by binding to receptors and

to a developed fusion between the viral and host to promote viral entry into the host cell **(5)**.

The E-protein is a small membrane protein composed of 76 to 109 amino-acid and a minor component of the virus particle, it plays an important role in virus assembly, membrane permeability of the host cell, and virus-host cell interaction **(6,7)**.

The present study evaluating the nutraceutical and herbal medicine's role as a potential antiviral against COVID-19, to encourage the use of nutraceuticals and herbal therapies as preventive steps towards the SARS-CoV-2 virus till the other specific drugs and vaccines discovered **(8)**. WHO also encourages traditional, complementary, and alternative medicine to have many benefits in covid-19 management. Medicinal herbs such as Ginger, Solanumnigrum (Kakmachi), GlycyrrhizinglabraLinn., Milk thistle (silymarin), Bacopamonnieri, and Medicago sativa Linn. Shown promising effects in the treatment of COVID-19.

By considering the current need for effective medicine against the coronavirus, GP Life Healthcare Pvt. Ltd. has proposed a product with renowned Phytoconstituents to evaluate its antiviral potential against SARS-CoV-2 and developed formulation Act 12 & 13 a potential candidate in the treatment of COVID-19 infection. It is a carefully designed product with the highest quality standards to improve immunity and support the body to combat the viral challenge in the situation of COVID-19.

MATERIALS AND METHODS

The study was conducted at the bioassay laboratory of the Department of Biotechnology's Faridabad-based Translational Health Science and Technology Institute (THSTI) will now function as an extension of the diagnostic facility of ESIC Medical College and Hospital, Faridabad for COVID-19 testing. DBT- THSTI's bioassay laboratory was established under the DBT-funded Translational Research Program of THSTI. It was set up for the clinical development of vaccines and biologicals. It is intended to meet the global standards in Good Clinical Laboratory Practice (GCLP) and will be applying for accreditation by National Accreditation Board for testing and calibration Laboratories (NABL) for vaccine development and testing.

Assessment of cytotoxicity:

Assay procedure:

The assay was done in a 96-well plate format in 3 wells for each sample. 1*10e4 cells were plated per well and incubated at 37-degree C overnight the monolayer formation. The next day, cells were incubated with the test substance (TS) at the indicated concentration with the final DMSO concentration. Being 0.5%. The control cells were incubated with 0.5%DMSO only. In intervals of time 24 and 48 hours, cells were stained with Hoechst 33342 and syntax orange dye. Images were taken at 10 X, 16 images per well, which covers 90% of the well area using image Xpress micro confocal (Molecular Devices). Hoechst 33342 nucleic acid stain is a popular cell permanent nuclear counterstain that emits blue fluorescence when bound to ds DNA. It contains all the live and dead cells. Sytox orange dye stains nucleic acids in cells with compromised membranes. This stain is an indicator of cell death. First, the software counts the total number of cells in the Hoechst image. In the sytox image, it counts the number of positive cells for sytox among the Hoechst positive cells.

Assessment of antiviral screening:

Assay procedure:

The assay was done in a 96-well plate format in 3 wells for each sample. 1*10e4 cells were plated per well and incubated at 37°C overnight the monolayer formation. The next day, cells were incubated with the test substance at the indicated concentration with the final DMSO concentration. Being 0.5%. The control cells were incubated with 0.5%DMSO only. The cells were infected with SARS-CoV-2 at an MOI of 0.01. 24 and 48 hours later, viral RNA was extracted from 100 µL culture supernatant and subjected to qRT-PCR (in duplicates) where Ct value for N and E gene sequence was determined. Inhibition of virus replication was determined based on the fold change in the Ct value in TS-treated cells compared to the control. Remdesivir was used as a positive control for viral inhibition.

RESULT

Assessment of cytotoxicity:

The test substance was evaluated for its cytotoxicity and when compared to positive control it was found that Remdesivir represents 99.23% cell variability after 24 hrs. and both the test substance ACT 12 and ACT 13 represents 109.79 and 106.77 respectively and after 48 hrs., Remdesivir represents 94.37% cell variability after 24 hrs., and both the test substances ACT 12 and ACT 13 represents 109.75% and 93.53% respectively which demonstrates the safety of test substances when compared to the positive control. From the present result, it can be concluded

that ACT 12 and 13 represent less cytotoxic nature than Remdesivir which ultimately proves it's safe to use for a longer duration. Results for the above parameter is depicted in Table no. 1

Table No.1: Assessment of Cytotoxicity:

Compound Name	Concentration	% cell Viability	
		24 Hrs.	48 Hrs.
Remdesivir	10 µM	99.23	94.37
ACT 12	1µl added from the ACT-12 solution in 200µl reaction	109.79	109.75
ACT 13	1µl added from the ACT-13 solution in 200µl reaction	106.77	93.53

Assessment of antiviral screening:

When test substances were evaluated for their antiviral activity towards SARS-CoV2, they represented a promising effect in % inhibition of viral replication. When positive control Remdesivir was compared with both the test substance after 24 hrs., the results found that ACT 12 demonstrates greater % inhibition of viral replication than Remdesivir and ACT 13.

When compare after 48 hrs., it was observed that the ACT12 and Remdesivir were showing comparable results. When compared between groups Remdesivir demonstrated better results than ACT 13. The results are depicted in Table no.2

Table No.2: Assessment of Antiviral Screening:

Compound Name	Concentration	% inhibition of virus replication			
		24 Hrs. post-infection		48 Hrs. post-infection	
		E	N	E	N
Remdesivir	10 µM	87.73	75.8	99.84	99.88
ACT 12	1µl added from the ACT-12 solution in 200µl reaction	96.54	96.5	73.06	71.33
ACT 13	1µl added from the ACT-13 solution in 200µl reaction	14.34	4.54	-31.66	-12.9

DISCUSSION

These products are subjected to many other scientific studies among which one most important study is the In-vitro Anti COVID study which was executed in ICMR approved lab RCB- Faridabad and outcomes are exceptional.

From the above data, it can be concluded that the test substance act on E gene/ proteins which are involved in process of viral assembly development, and also has ion channel activity which helps the virus to form validate assembly to attack the host cell system by blocking the activity of E gene produce a deficiency of E gen/protein which lead to disturbed viral titers, crippled viral maturation, or yield propagation incompetent progeny, which established its vital role in viral replication process **(9,10)**.

N terminal of N gene responsible for RNA binding the, but primary function of the N-protein is to protect the genomic RNA **(11)**.

N gene also enhances viral assembly development and transcription process by incorporating viral RNA into a host cell which increases its efficiency in disease progression **(12)**.

As the proposed test substance inhibits the N gene so it can be concluded that blocking N gene action directly affects its transcription process and stop infection at an early stage.

In this study, both the Products i.e. ACT 12 and ACT 13 are tested against the SARSCoV2 and cytotoxicity study. After 24 hrs., In ACT 12 group the % inhibition in E and N gene was found to be 96.10 and 96.54 which is considerably greater than the Remdesivir control group in which the % inhibition in E and N gene was found to be 87.73% and 75.79% respectively.

As test substances composed from herbal sources such as Ginger, Solanumnigrum (Kakmachi), Glycyrrhizin glabraLinn., Milk thistle (silymarin), Bacopamonnieri, and Medicago sativa Linn. The

above data of the Cytotoxicity assay of the test substances have proven its safety for oral use as per ICMR approved lab.

It was found from the study that there was no toxicity observe related to the test substance and thus it depicts the safety of test substances, as well as its potential in inhibition of viral replication in call culture, demonstrates its efficacy.

CONCLUSION

It was found from the study that there was no toxicity observe related to the test substance and thus it depicts the safety of test substances, as well as its potential in inhibition of viral replication in cell culture, demonstrates its efficacy. Remdesivir represents 94.37% cell variability after 24 hrs. and both the test substances ACT 12 and ACT 13 represents 109.75% and 93.53% respectively which demonstrates the safety of test substances when compared to the positive control. After 48 hrs. the result demonstrating ACT12 and Remdesivir showing comparable results. When compared between group Remdesivir demonstrate better results than ACT 13. So from the study, it can be concluded that ACT 12 and 13 represent less cytotoxic nature than Remdesivir which ultimately proven it safe to use for a longer duration. It can be concluded that ACT 12 and 13 have their preventive role against Covid1 19 infection as a frontline option. It is concluded that ACT 12 and 13 can develop an innate immune response in subjects before it uses subjects as host and sustained in the body by blocking possible pathways of infection, intervention stop an infection before it begins.

REFERENCES

1. Frequently asked questions about COVID-19 vaccination | CDC [internet] [cited Feb 16 2021]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/faq.html.
2. Middle East respiratory syndrome coronavirus (MERS-CoV) [internet] [cited Feb 16 2021]. Available from: https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers#tab=tab_1.
3. Singhal T. Review on COVID19 disease so far. Indian J Pediatr. 2020;87(Apr):281-6.
4. Sarma P, Shekhar N, Prajapat M, Avti P, Kaur H, Kumar S, Singh S, Kumar H, Prakash A, Dhibar DP, Medhi B. In-silico homology assisted identification of inhibitor of RNA binding against 2019-nCoV N-protein (N terminal domain). J Biomol Struct Dyn. 2020 May 16:1-9.
5. Kirchdoerfer RN, Cottrell CA, Wang N, Pallesen J, Yassine HM, Turner HL, Corbett KS, Graham BS, McLellan JS, Ward AB. Pre-fusion structure of a human coronavirus spike protein. Nature. 2016 Mar 3;531(7592):118-21. doi: 10.1038/nature17200, PMID 26935699, PMCID PMC4860016.
6. Gupta MK, Vemula S, Donde R, Gouda G, Behera L, Vadde R. In-silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. J Biomol Struct Dyn. 2020 Apr 13:1-.
7. Lissenberg A, Vrolijk MM, Van Vliet AL, Langereis MA, de Groot-Mijnes JD, Rottier PJ, de Groot RJ. Luxury at a cost? Recombinant mouse hepatitis viruses expressing the accessory hemagglutinin esterase protein display reduced fitness in vitro. J Virol. 2005 Dec 15;79(24):15054-63. doi: 10.1128/JVI.79.24.15054-15063.2005, PMID 16306576.
8. Panyod S, Ho CT, Sheen LY. Dietary therapy and herbal medicine for COVID-19 prevention: a review and perspective. J Trad Complement Med. 2020 May 30;10(4):420-7. doi: 10.1016/j.jtcme.2020.05.004, PMID 32691006.
9. Ruch TR, Machamer CE. The coronavirus E protein: assembly and beyond. Viruses. 2012 Mar;4(3):363-82. doi: 10.3390/v4030363, PMID 22590676.
10. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J. 2019 Dec;16(1):69. doi: 10.1186/s12985-019-1182-0, PMID 31133031.
11. Surjit M, Lal SK. The SARS-CoV nucleocapsid protein: a protein with multifarious activities. Infect Genet Evol. 2008 Jul 1;8(4):397-405. doi: 10.1016/j.meegid.2007.07.004, PMID 17881296.
12. McBride R, Van Zyl M, Fielding BC. The coronavirus nucleocapsid is a multifunctional protein. Viruses. 2014 Aug;6(8):2991-3018. doi: 10.3390/v6082991, PMID 25105276.

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