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Research Article

In-vitro Assessment of Antiviral Activity of the Herbo Mineral Capsule, Fema Sakthi™, against Human Coronavirus (HCoV) on VERO Cells

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A B S T R A C T

Introduction: With the spread of COVID-19 pandemic, healthcare workers and patients look for alternate medicines including Siddha, Ayurveda, Unani and other forms of traditional medicines as we still do not have promising antiviral drugs for COVID-19.

Objective: To evaluate the in-vitro antiviral activity of Fema Sakthi™ (FS) by eliciting the inhibition of cytopathic effect of Human Coronavirus (HCoV) on African Green Monkey kidney cells (VERO cells).

Materials and Method: The cytopathic effect (CPE) was performed on Vero cells with Human Coronavirus 229E, a type of Coronavirus associated with respiratory infections. The Median Tissue Culture Infectious Dose ($TCID_{50}$) was evaluated using Reed-Muench method. 100 $TCID_{50}$ of HCoV 229E viral suspensions were added to VERO cell culture to induce the cytopathic effect. Uninfected and untreated cells were used as control and five concentrations (62.5, 125, 250, 500 & 1000 µg/mL) of Fema Sakthi™ (FS) were used to study the anti-viral activity. After incubation for 72 hours, the cell viability was observed under the inverted microscope after staining with 0.1% crystal violet.

Results: Fema Sakthi™ (FS) was found to exhibit inhibition of cytopathic effect at lower concentrations (62.5, 125 and 250 µg/mL) but at higher concentrations (500 and 1000 µg/mL), the formulation itself was cytotoxic to the cells.

Conclusion: This preliminary study showed that FS has antiviral activity at lower concentrations 62.5, 125 and 250 µg/mL on the VERO cells. However, further specific studies have to be carried out to confirm the anti-viral activity and clinical efficacy using other preclinical and clinical models of Human Coronavirus (HCoV) including COVID-19.

Keywords: Antiviral Activity, VERO Cells, Human Coronavirus, Fema Sakthi™

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Introduction

Coronaviruses belong to the family of RNA viruses, responsible for various diseases ranging from mild upper respiratory infections to lethal and severe forms of diseases like Middle East Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS) and COVID-19.[1] Besides modern medicine, various other treatments have been used in treating respiratory infections including the virus-induced/ flu-like respiratory illnesses. Traditional plant-based preparations are an inherent part of the management of several infectious diseases including respiratory infections and viral infections in our country since ancient times.[2,3]

Presently, many of the pharmaceutical companies that deal with plant-based products come up with various herbal/ poly herbal/ herbo-mineral formulations for health-related ailments. The advantage of such finished herbal formulations is that the patients will have plant-based remedies readily available in the market. The government has also given detailed guidelines to ensure the quality of such marketed herbal products. With the spread of COVID-19 pandemic, healthcare workers and patients look for alternate medicines including Siddha, Ayurveda, Unani and other forms of traditional medicines as we still do not have promising antiviral drugs for COVID-19.

Fema Sakthi™ (FS) is a herbo mineral formulation available as capsules in the market. The ingredients present in Fema Sakthi™ (FS) capsule are extracts of *Mucuna pruriens*, *Hydrophila spinosa, Prunusa mygdalus*, *Orchis mascula* and crude mineral compounds obtained from Jathilingam (sulphide of mercury), muthu parpam (pearl parpam) and pavala parpam (coral basma). These components were reported to have anti-cancer,[4] anti-inflammatory,[5] and immune-modulatory properties.[6] The extracts of the plants used have documented evidence of detoxifying, antipyretic, anti-tussive, anti-asthmatic properties and found to be routinely used in treating respiratory infections caused by viruses in traditional medicine.[7-10]

Hence, the present study was undertaken to evaluate the antiviral activity of FS on African green monkey kidney cell (VERO cells) against Human Coronavirus.

Materials and Method

Test Substance

Fema Sakthi™ (FS) is a herbo mineral capsule with the extracts of *Mucuna pruriens*, *Hydrophila spinosa, Prunusa mygdalus*, *Orchis mascula*, Jathilingam (sulphide of mercury), muthu parpam (pearl parpam) and pavala parpam (coral basma) manufactured according to Good Manufacturing Practices (GMP) and marketed globally for respiratory illness and for boosting immunity.

Physical, Chemical and Botanical Properties

Fema Sakthi™ capsules contain extracts of four herbs that are obtained based on the Siddha system of traditional knowledge and processed along with basma of Pavalam (Corals), parpam of Pearls and Jathilingam (Sulphide of mercury). The raw materials used for the preparation of Fema Sakthi™ were detoxified as per standard pharmacopoeial procedures.

The presence of Active Ingredients (AI) was confirmed by testing the individual active ingredients of the capsules with their respective herbal standards and thereby validated. Further the results confirm the botanical authentication of the plant species used for the preparation of the herbal capsules. The amounts of various ingredients present in Fema Sakthi™ are tabulated in Table 1.

Table 1.Ingredients of FS and their Concentration

S. No.	Ingredient	Concentration (mg/100g)
1.	*Hygrophilaspinosa (seeds)*	312.12
2.	*Mucunapruriens (seeds)*	275.2
3.	*Orchismascula (roots)*	50.23
4.	*Prunusamygdalus (nuts)*	112.23
5.	Jathilingam (Sulphide of Mercury)	75.23
6.	PavalaParpam (Coral Basma)	102.23
7.	MuthuParpam (Pearl Parpam)	103.58

Table 2.Microbial Contamination in FS

S. No.	Type of Contaminant (PPM)	Results
1.	Total Aflatoxin	BQL (LOQ 0.001)
2.	Aflatoxin B1	BQL (LOQ 0.001)
3.	Aflatoxin B2	BQL (LOQ: 0.05)
4.	Aflatoxin G1	BQL (LOQ: 1.0)
5.	Aflatoxin G2	BQL (LOQ 0.001)

Microbial Contamination

Fema Sakthi capsules were tested for microbial contamination and the capsules do not have any microbial contamination, especially there is no Aflatoxin present in the capsules tested based on the Ayurvedic Pharmacopoeia. The microbial contamination data is given in Table 2.

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Viruses, Cell Lines and Chemicals

The human coronavirus (HCoV-229E) was obtained from American Type Culture Collection (ATCC; VR-740; Rockville, MD, USA) and African green monkey kidney cell (VERO E6) from National Centre for Cell Science (NCCS - Pune, India). Chemicals used in the experiment were DMEM (Dulbecco's Modified Eagle's Medium) with 10% foetal bovine serum (FBS), 1% of Antibiotics Antimycotic Solution (100 µg/mL streptomycin, 100 units/mL penicillin G, and 0.25 µg/mL amphotericin B) which were obtained from Sigma Aldrich.

Antiviral Activity Assay

The antiviral activity of FS was studied against Human coronavirus (HCoV-229E) using the inhibition of cytopathic effect (CPE).

Cytopathogenic effect is described as the structural changes that occur in the host cells after a viral invasion, which often results in cell death. Such morphological changes include swelling or shrinkage of cells, formation of syncytia, and the production of nuclear or cytoplasmic inclusions in the infected cells.[11]

$TCID_{50}$

The $TCID_{50}$ (Median Tissue Culture Infectious Dose) is the dose of virus that cause cytopathogenic effect or cell death in 50% of the wells of the Human Coronavirus. It was determined using Reed-Muench method.[12]

The VERO E6 cells (1×10^4 cells/well) were seeded in a 96-well plate one day prior to the experiment to form a confluence monolayer. After the incubation period, 100 $TCID_{50}$ of HCoV-229E virus suspensions were added to confluent cell monolayer and incubated at 37°C in 5% CO_2 for 3 hours to support virus adsorption. Then the entire solution was discarded and washed twice with DPBS (Dulbecco's phosphate-buffered saline) in order to remove unabsorbed virus particles from each well. After that, the cells were treated with the following concentrations of FS extract in DMEM: 62.5, 125, 250, 500 & 1000 µg/mL. Uninfected and untreated cells were used as cell control and infected cells were used as virus control and the plate was incubated for 72 hours at 37 °C in a 5% CO_2 humidified incubator. The cell viability was observed under an inverted microscope with 0.1% Crystal violet dye.

Results

The microscopic findings of the untreated cells, virus control and treated cells with different concentrations of the FS extract is shown in Figure 1. In comparison to the normal cell control, the untreated virus-infected cells showed a significant Cytopathic effect. The cells treated with 62.5, 125 and 250 µg/mL concentrations of FS showed cell growth indicating inhibition of cytopathic effect whereas at higher concentrations (500 & 1000 µg/mL), there was cytopathy observed in the infected cells.

The percentages of cell viability with different concentrations of the FS extract in the virus-infected cells, uninfected cells and untreated cells are shown in Table 3.

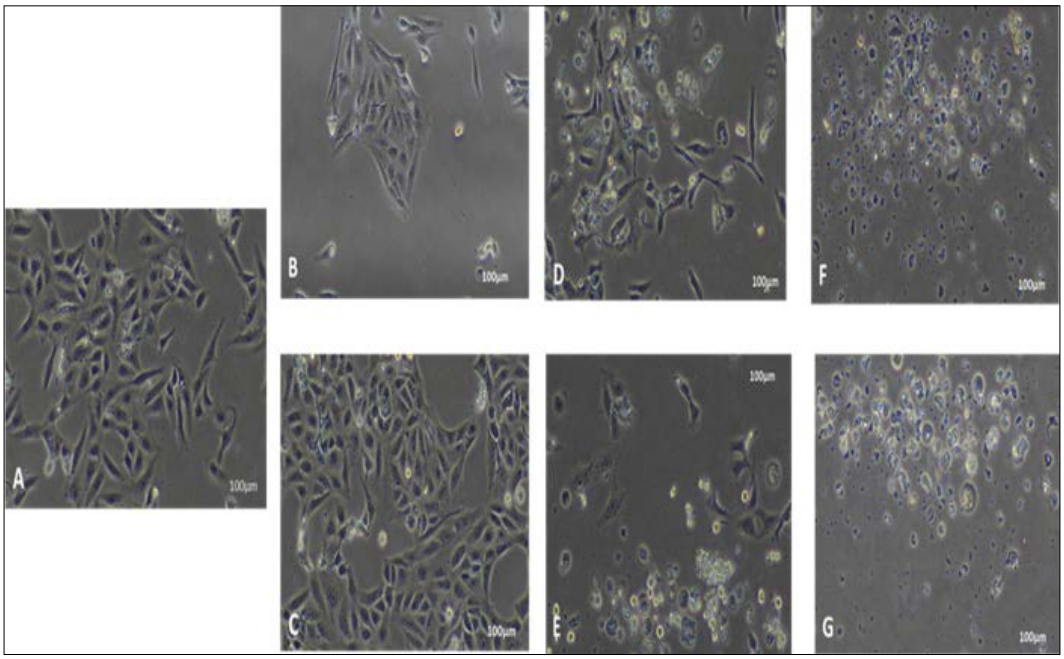

Figure 1.Cytopathic Effect of Herbo mineral FS Capsule on Post-infected VERO Cells by Human Coronavirus-229E at 100$TCID_{50}$

A: Cell control, B: Virus Control, C to G: Virus with five concentrations of Herbal extract (62.5, 125, 250, 500 & 1000 µg/ mL) respectively. (10X; Scale bars represent 100 µm)

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Table 3.Percentage Cell Viability

Sample	Concentration (µg/mL)	Cell Viability (%)
FS	1000	19.38
FS	500	42.61
FS	250	63.43
FS	125	90.33
FS	62.5	99.18
Cell control (Uninfected)	-	87.34
Positive control (Virus infected, untreated)	-	0.87

Discussion

For most of the RNA viruses causing respiratory infections including the SARS CoV-2 virus responsible for the current pandemic, there are no specific and successful antiviral drugs available. Hence, any treatment option including traditional medicines that shows antiviral activity could be further developed as a potential treatment option. In this preliminary in-vitro study, FS showed inhibition of cytopathic effect of Human coronavirus 229E (HCoV-229E) on VERO cells at 62.5, 125 and 250 µg/mL concentrations, but at higher concentrations (500 and 1000 µg/mL), the extract itself was cytotoxic to the cells. Hence, there is a possibility that FS at moderate doses could become a potential treatment option for Human Coronavirus (HCoV) infections. As the formulation is already approved and available in the market, there is no need for preclinical safety studies and only further pre-clinical efficacy and/or clinical studies in specific human corona viral infections including COVID-19 are needed to reaffirm its efficacy.

Conclusion

This preliminary study showed that FS has antiviral activity at lower concentrations 62.5, 125 and 250 µg/ mL on the VERO cells. However, further specific studies have to be carried out to confirm the anti-viral activity and clinical efficacy using other preclinical and clinical models.

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Conflict of Interest: None

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