

The Mechanism of Anti-Viral Activity of a Novel, Hydroponically Selenium-Enriched Garlic Powder (SelenoForce[®]) Against SARS-CoV-2 Virus

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Abstract

Abstract: The pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is far from over as new strains are emerging all over the world. Selenium as a micronutrient is important for immunity and also has anti-viral activity.

Objective: The study evaluated the activity of a Selenium enriched garlic powder (SeGP or SelenoForce[®]) against SARS-CoV-2 viral replication in vitro and explored its possible mechanism of action.

Methods: The anti-SARS-CoV-2 activity assay was carried out in Vero E6 cells in vitro. Human lung carcinoma A549 cells were used to study the antioxidant activity, expression of angiotensin converting enzyme (ACE), transmembrane protease, serine 2 (TMPRSS2) and the activity of proprotein convertase, and furin. Anti-inflammatory activity was evaluated in lipopolysaccharide-activated RAW 264.7 cells.

Results: SeGP inhibited the replication of SARS-CoV-2 in Vero E6 cells with an IC₅₀ of 19.59 µg/ml. It exhibited significant antioxidant activity in vitro with IC₅₀ value determined as 43.45 µg/ml. The Selenium enriched product inhibited the expression of ACE and TMPRSS2 and also showed inhibition of furin protease activity. In the presence of SeGP, the secretion of nitric oxide, interleukin –6 and TNF-α were reduced in activated RAW 264.7 macrophages.

Conclusion: The results of the study suggest that Selenium enriched garlic powder could inhibit SARS-CoV-2 multiplication in vitro, reduce oxidative stress and inflammatory mediators suggesting that it could be developed as an effective supplement or adjunct therapy to combat viral infections.

Keywords

selenium, micronutrient, SARS-CoV-2, inflammation, oxidative stress

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Introduction

Infectious viral diseases pose a massive threat to public health and remain as one of the major causes of death all over the world. Viruses are called “acellular parasites of cellular hosts” as they utilize the host cellular mechanisms for propagation and replication which make it difficult to destroy them without affecting the host cell.¹ Immunization with effective

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vaccines has led to the eradication of few viral diseases like polio and smallpox and is effective to combat other viral diseases including, hepatitis virus, herpes simplex virus (HSV), respiratory syncytial virus (RSV), and influenza virus.² However, the emergence, resurgence and spreading of animal viruses have become serious public concern in the past few decades despite the tremendous progress in human medicine. The scenario got worsened when the world population was adversely affected by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the pandemic, Coronavirus disease-2019 (COVID-19). SARS-CoV-2 infection primarily induces pro-inflammatory cytokines (eg, IL-1 and IL-6) and lung inflammation. It ultimately affects the normal functioning of vital organs enhancing comorbidities and mortality.³ Till now, the SARS-CoV-2 virus has infected around 7 billion people and caused over 6 million deaths around the world (<https://www.worldometers.info/coronavirus>).

In addition to vaccines, drugs also become life-saver at the time of the pandemic. A treatment strategy combining the pharmaceutical drugs and herbal medicines as an adjunct therapy was found effective in managing the COVID-19 infection.⁴ Natural products, especially plants are a rich source of pharmacologically active substances and become excellent candidates for drug discovery due to their chemical diversity and safety. Several clinical studies provide evidence for the efficacy and safety of herbal extracts and phytochemicals as potential immune boosters to defend against SARS-CoV-2 infection.⁵⁻¹⁰ Additionally, novel formulations of natural products were found to be targeting multiple signaling pathways of SARS-CoV-2 infection and were suggested as supportive therapy to hasten recovery and reduce severe morbidity and mortality in patients.¹¹ An understanding of the molecular mechanisms of viral invasion and replication will enable us to design anti-viral drugs from natural products targeting the different stages of the viral replication cycle. Inhibitors of angiotensin-converting enzyme 2 (ACE2), transmembrane protease, serine 2 (TMPRSS2) and furin, the protease that activates the spike protein of SARS-CoV-2 virus were found to be effective in reducing viral load which make it a potent therapeutic approach for treatment of SARS-CoV-2 infection.¹² Further, antioxidant and anti-inflammatory activities are beneficial in preventing the secondary complication of the infection.^{13,14}

Micronutrients like vitamins and essential minerals are crucial in every stages of the immune response. Micronutrient deficiencies were found associated with immunosuppression and increased susceptibility to infections.¹⁵ Selenium (Se), an essential micronutrient has a long history of lowering the severity of viral infections.^{16,17} The source and chemical form of Selenium in food are important factors affecting its bioavailability in humans. Generally, organic Selenium compounds are more bioavailable than inorganic Selenium compounds and Selenium from plant sources shows better

bioavailability than that of animal sources.^{18,19} The physiological functions of Selenium are mainly executed by selenoproteins that are crucial for maintaining the redox balance in cells, and controlling other functions such as cell proliferation, differentiation, apoptosis, cytokine expression, and anti-viral activities.^{20,21} Selenium is known to lower the susceptibility and severity of viral infections and consequently, its deficiency impacts immune response to viruses, expression of viral proteins and antioxidant response.²²⁻²⁴ Selenium status was reported to have a positive correlation with the survival of patients with COVID-19 compared with non-survivors suggesting that Selenium supplementation may be helpful in reducing the comorbidities associated with COVID-19.²⁵⁻²⁷

Garlic or *Allium sativum* L. is popular as a functional food and a traditional medicine since ancient time. It contains organosulfur (allicin and alliin) compounds and flavonoids like quercetin which are found to be responsible for anti-viral activities via immune modulation.³ Selenium enriched garlic powder (SeGP) is manufactured using a proprietary soil-less culture technique containing a unique composition of organoSelenium compounds. In this process, garlic bulbs are naturally enriched with selenoamino acids, L-methyl selenocysteine (MSC) and L-selenomethionine (LSM) and dipeptides, γ -glutamyl Se-methyl-L-selenocysteine (GGMSC) and γ -glutamyl-selenomethionine (GGLSM). In the present study, we explored the anti-viral activity of SeGP (marketed as SelenoForce[®]), containing 1000 $\mu\text{g/g}$ of total Selenium in the form of GGLSM (65.2%), LSM (26.4%), MSC (4.6%) and GGMSC (3.8%). Apart from Selenium compounds, the garlic powder also contained Alliin at 399.2 $\mu\text{g/g}$.

Materials

SeGP (marketed as SelenoForce[®]) was obtained from Sami-Sabinsa Group Limited. Dulbecco's modified minimal essential medium (DMEM) from Life Technologies (CA, United States) and fetal bovine serum (FBS) from GIBCO/Invitrogen (Carlsbad, CA, United States), Trizol reagent[®] from Invitrogen (Carlsbad, CA, United States), Revert-aid First Strand cDNA synthesis kit from Thermo Fisher Scientific and ELISA kits from R&D Systems (Minneapolis, MN, United States) were used in the study. pERTKR-AMC (L-pyroglutamyl-Arg-Thr-Lys-Arg-7-amino-4-methylcoumarin) was purchased from R&D Systems (Minneapolis, MN, United States).

Methodology

Cell Culture

In vitro experiments in Vero E6 cells (derived from the kidney of African green monkey) were carried out at the Institute of Life Sciences, Bhubaneswar, an autonomous institute of the Department of Biotechnology, Govt. of India.

Human lung carcinoma A549 and RAW 264.7 macrophage cells were procured from the National Center for Cell Sciences (NCCS), Pune, India and maintained at 37°C in a 5% CO₂ atmosphere, in complete Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% of fetal bovine serum (FBS) and 40 µg/ml gentamycin.

Preparation of SeGP for in vitro Experiments

SeGP was dissolved in dimethyl sulphoxide (DMSO) at a concentration of 10 mg/mL, sonicated in a water bath sonicator at room temperature for 5 min and centrifuged at 2000 r/min for 5 min to remove the insoluble particles from garlic bulb. The stock was used for the preparation of different dilutions in culture media or buffers for in vitro experiments. The in vitro activities were studied at 50, 25, 12.5 and 6.2 µg/ml of SeGP which correspond to approximately 50, 25, 12.5 and 6.5 ng/mL of selenoamino acids respectively.

In vitro Anti-SARS-CoV-2 Activity

In vitro anti-SARS-CoV-2 activity assay was carried out in Vero E6 cells (derived from the kidney of an African green monkey) at the Institute of Life Sciences (ILS) Bhubaneswar, an autonomous institute of the Department of Biotechnology, Govt. of India, with established platforms for drug screening for viral diseases. SARS-CoV-2 strain ILS-03 (Accession no. EPI_ISL_1196305) successfully adapted and isolated in Vero cells at ILS, Bhubaneswar was used for this study. No animals/humans were used in the study.

Cytotoxicity Assay. Briefly, Vero E6 cells were seeded in the 96 well plate at 80% confluency and treated with various concentrations of SeGP. For toxicity determination, minimum of 3 concentrations were used. Cytotoxicity was determined 24 h post-treatment using (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay (MTT assay). 100 µl of MTT solution (5 mg/mL) was added to 96 wells and incubated for 2-3 h at 37°C. After incubation, 100 µL of DMSO was added to each well to solubilize the formazan crystals, under shaking conditions. The absorbance was recorded at 570 nm. Each concentration was assayed in triplicates and the percentage cell viability was calculated with respect to vehicle control.

Anti SARS-CoV-2 Activity Assay. Vero E6 cells seeded in 96-well plates at 80% confluency were infected with SARS-CoV-2 isolate at multiplicity of infection (MOI) of 0.1 for 2 h. Subsequently, the inoculum was aspirated and fresh media containing different concentrations of the SeGP was added to the cells. The supernatant of 24 h post-infection was subjected to viral RNA isolation followed by qRT-PCR for determining the SARS-CoV-2 viral load in the cells (cell associated) and culture supernatants (released virus particles).

The qRT-PCR was performed using primers specific for the viral spike, nucleocapsid and ORF1a for both released and cell-associated virus. Percentage reduction of viral loads in cells and culture supernatants was plotted in comparison to vehicle treated controls. Remdesivir was used as a positive control.

In vitro Assays in Human Lung Carcinoma A549 Cells and RAW 264.7 Cells

Cytotoxicity/Selection of Nontoxic Concentrations for Cell Assays. Human lung carcinoma A549 cells and RAW 264.7 cells (5×10^4 cells/well) were exposed to varying concentrations of SeGP for 24 h. Cytotoxicity/cell viability of SeGP was analyzed by using MTT colorimetric assay according to the standard method described previously.²⁸

H₂O₂ Induced Reactive Oxygen Species Scavenging Activity. Human lung carcinoma A549 cells were seeded at 5×10^4 cells/well in 96 well black plates and allowed to grow as a monolayer for 24 hours. Cells were exposed to 25 mM hydrogen peroxide (H₂O₂) with or without the test sample for 4 hours in DMEM supplemented with 2% FBS. Freshly prepared dichlorodihydrofluorescein diacetate (DCFH-DA) reagent was added to all the wells (1 µg/well). The plate was incubated at 37°C for 30 min and fluorescence was recorded at 485:520, Ex:Em wavelength in microplate reader (FLUOstar Optima, BMG Labtech, Germany).²⁹

Furin Inhibition Assay. Human lung carcinoma A549 cells were seeded at 5×10^4 cells/well in 96 well black plates and allowed to grow as a monolayer for 24 h. Cells were treated with samples for 24 h in DMEM containing 2% FBS. Wells with only DMEM +2% FBS media served as control. After 24 h of incubation, media was replaced with 25 µM fluorogenic peptide substrate, pERTKR-AMC (L-pyroglutamyl-Arg-Thr-Lys-Arg-7-amino-4-methylcoumarin) as substrate in 25 mM Tris buffer (pH-9, 1 mM CaCl₂, 0.5% (w/v) Brij-35, 0.1% BSA) for 1 h. The fluorescence was recorded at 380:460, Ex:Em wavelength in microplate reader (FLUOstar Optima, BMG Labtech, Germany). Furin activity was calculated in comparison to control.

ACE-2 and TMPRSS2 Inhibition Assay. The expression of Angiotensin converting enzyme (ACE) and Transmembrane protease, serine 2 (TMPRSS2) were studied in human lung carcinoma A549 cells. The cells were seeded at 4×10^5 cells per well in 6 well plates and allowed to grow as a monolayer for 24 h. Cells were treated with test samples for 24 h in DMEM 2% FBS. Wells with only DMEM 10% FBS media served as control. After 24 h of incubation, the supernatants were collected and the total cellular RNA was isolated using Trizol reagent[®] according to the manufacturer's instructions, followed by RNase-free DNase I treatment (Thermo Fisher Scientific) to remove any genomic DNA. Messenger RNA

quality and concentration were analyzed spectrophotometrically (NanoDrop Lite, Thermo Fisher Scientific). One microgram of total RNA was reverse transcribed into cDNA using Revert-aid First Strand cDNA synthesis kit (Thermo Fisher Scientific), according to the manufacturer's instructions, and stored at -80°C until use. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green qPCR master mix fluorescent dye using Light cycler 96 (Roche Life Science) according to the manufacturer's instructions (Thermo Scientific). The primer sequence is provided in Table 1.

Anti-Inflammatory Assay. RAW 264.7 cells (0.5×10^5 cells/well) were seeded into 96 well cell culture plates in growth media. The cells were stimulated with 100 ng/mL lipopolysaccharides (LPS) along with different concentrations of sample and incubated for 24 h at 37°C , in a CO_2 (5%) incubator. The wells without LPS (100 ng/mL) served as control. The supernatants were collected to detect the levels of inflammatory markers. The inducible nitric oxide (NO) level is detected based on the Griess reaction. Briefly, 50 μL of cell culture medium was mixed with 50 μL of Griess reagent I [1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid] and 50 μL Griess reagent II [0.1% (w/v) N-(1-Naphthyl) ethylenediamine dihydrochloride], incubated at room temperature for 10 min, and then, the absorbance was taken at 540 nm. The inflammatory markers like tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were quantified using ELISA kits as per the manufacturer's instruction (R & D Systems, Minneapolis, MN, United States).

Statistical Analyses

All data are presented as the mean \pm standard deviation (SD) of the triplicates. *P* values were determined by one-way analysis of variance (ANOVA) using GraphPad prism software version 10.0.2 (GraphPad Software, Inc, La Jolla, CA, USA). Significance was defined at 5% level.

Results

Selenium Compounds Present in SeGP

Garlic powder, naturally enriched with Selenium contained selenoamino acids and dipeptides as major Selenium compounds (Figure 1).

Table 1. List of Primer Sequences.

h- ACE2 F	AAACATACTGTGACCCCGCAT
h- ACE2 R	CCAAGCCTCAGCATATTGAACA
h-TMPRSS2 F	AATCGGTGTGTTTCGCCTCTAC
h-TMPRSS2 R	CGTAGTTCTCGTTCCAGTCGT
h-18SrRNA f	GGCGTTCCTAGTTGGTGGAGCG
h-18SrRNA r	CTGAACGCCACTTGTCCCTC

SeGP Showed Anti-SARS-CoV-2 Activity in vitro

The inhibitory efficacy of SeGP towards viral replication in living cells was investigated using Vero cell line. Initially, the maximum non-cytotoxic concentration of SeGP in Vero cells was detected by incubating the cells for 24 h followed by MTT assay. After treating with 25 $\mu\text{g}/\text{ml}$ of SeGP, 89% of the cells were viable and it was considered as the highest permissible non-cytotoxic concentration to be taken forward for assessing anti-SARS-CoV-2 efficacy in vitro. SeGP (10, 15, 20 and 25 $\mu\text{g}/\text{ml}$) treatment dose dependently reduced the viral load in Vero cells. SeGP at concentrations of 20 and 25 $\mu\text{g}/\text{ml}$ significantly reduced the viral load by 53 and 85% respectively whereas 10 and 15 $\mu\text{g}/\text{ml}$ did not show any detectable reduction in viral load (Table 2). The IC_{50} value of SeGP against SARS-CoV-2 was found to be 19.59 $\mu\text{g}/\text{ml}$. The cell viability experiments were performed simultaneously under the same conditions in the absence of virus which revealed no effects on cell viability at concentrations where the compounds showed anti-viral activity.

Cytotoxicity/Viability Assay in Human Lung Carcinoma A549 and RAW 264.7 Macrophages

Non-cytotoxic concentrations of SeGP towards different cell lines, human lung carcinoma A549 and RAW 264.7 macrophages were detected after 24 h of exposure followed by MTT assay. Both the cells showed significant cytotoxic response only at 100 $\mu\text{g}/\text{ml}$ of SeGP (Figure 2) and the non-cytotoxic concentrations of 12.5, 25 and 50 $\mu\text{g}/\text{ml}$ of SeGP were used for subsequent studies.

SeGP Showed Significant Inhibition of Furin Activity in Human Lung Carcinoma A549 Cells

Human furin inhibition activity of SeGP was measured using fluorogenic peptide pERTKR-AMC as substrate. Furin hydrolyzes the Arg-AMC amide bond and releases 7-amino-4-methylcoumarin (AMC), a highly fluorescent group detectable with the fluorescence reader. SeGP dose-dependently reduced the activity of furin compared to the normal lung carcinoma cells. At concentrations of 12.5, 25 and 50 $\mu\text{g}/\text{ml}$, SeGP, inhibited the activity of furin by 7 %, 10 % and 14% respectively compared to the normal cells and the changes were found statistically significant. The treatment with 6.25 $\mu\text{g}/\text{ml}$ of SeGP did not induce a significant change in furin activity (Figure 3).

SeGP Down Regulated the Expression of ACE2 and TMPRSS2 in Human Lung Carcinoma A549 Cells

SeGP treatment reduced the expression of ACE2 and TMPRSS2 in human lung carcinoma A549 cells. At a concentration of 12.5 $\mu\text{g}/\text{ml}$ the relative mRNA of ACE2 and

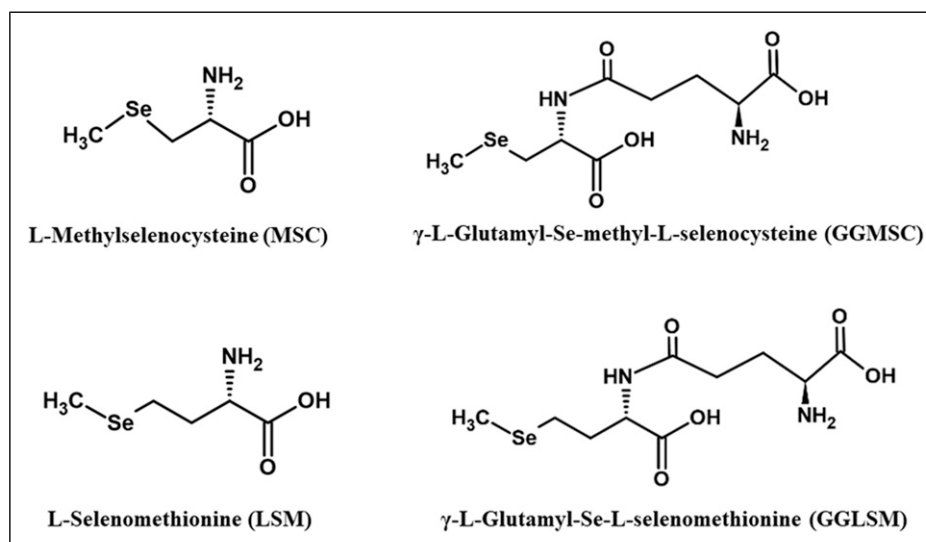


Figure 1. Selenoamino acids and dipeptides present in SeGP.

Table 2. Anti-SARS-CoV2 Activity of SeGP in Vero Cells.

Sample	Ct values	Viral Copy Number/MI	% Reduction
Control	25.625	37573.77	
SeGP (10 µg/ml)	25.25	48047.27	No reduction
SeGP (15 µg/ml)	25.332	45555.59	No reduction
SeGP (20 µg/ml)	26.803	17319.84	53
SeGP (25 µg/ml)	28.563	5445.427	85
Remdesivir (positive control)	32.096	533.69	98.5%

TMPRSS2 expression were reduced by 24.4% and 20.7% respectively compared to the untreated control (Figure 4).

SeGP Reduced Oxidative Stress in Human Lung Carcinoma A549 Cells

The antioxidant activity of SeGP was observed in H₂O₂-induced ROS in lung carcinoma A549 cells using fluorescent probe 2', 7'-dichlorodihydrofluorescein (DCFH). SeGP at concentrations of 12.5, 25 and 50 µg/ml reduced ROS levels to 72.7% ($P < 0.05$), 61.3% ($P < 0.005$) and 49.3% ($P < 0.0005$) respectively whereas treatment with 6.25 µg/ml did not show any significant reduction in ROS level (96%) compared to the lung cells induced with H₂O₂ (Figure 5). Thus, the sample showed a dose-dependent reduction in ROS levels with an IC₅₀ value of 43.45 µg/ml.

SeGP Showed Significant Anti-Inflammatory Activities in RAW 264.7 Macrophages

The inducible secretion of NO by the activated macrophages were detected using Griess reaction. The treatment with SeGP showed a significant reduction in the concentration of NO

dose dependently indicating its anti-inflammatory activity. Nitric oxide production was reduced from 26.48 µM to 21.5 µM (18.8% reduction) when LPS induced RAW 264.7 macrophages were treated with SeGP at a concentration of 50 µg/ml (Figure 6A).

The levels of inflammatory markers in the supernatant of RAW 264.7 macrophages activated with LPS were detected by ELISA. It was found that the secretions of inflammatory cytokines TNF- α and IL-6 were inhibited in a dose-dependent manner by SeGP treatment in LPS activated RAW 264.7 macrophage cells. Treatment with LPS induced the secretion of 24 476 \pm 1506 pg/mL of TNF- α . At the concentrations of 12.5, 25 and 50 µg/ml, SeGP reduced the TNF- α in culture supernatants to 20 722 \pm 355 pg/mL (84.6%), 20 231 \pm 48 pg/mL (82.7%) and 16 908 \pm 1147 pg/mL (69.1%) respectively (Figure 6B). The IL-6 levels were significantly reduced from 1995 \pm 225 pg/mL to 1146 \pm 288 pg/mL (57.4%), 911 \pm 11.5 pg/mL (45.6%) and 540 \pm 41.4 pg/mL (27.1%) at 12.5, 25 and 50 µg/ml respectively (Figure 6C).

Discussion

Selenium, an important micronutrient, is incorporated into selenoproteins, which are indispensable for the normal

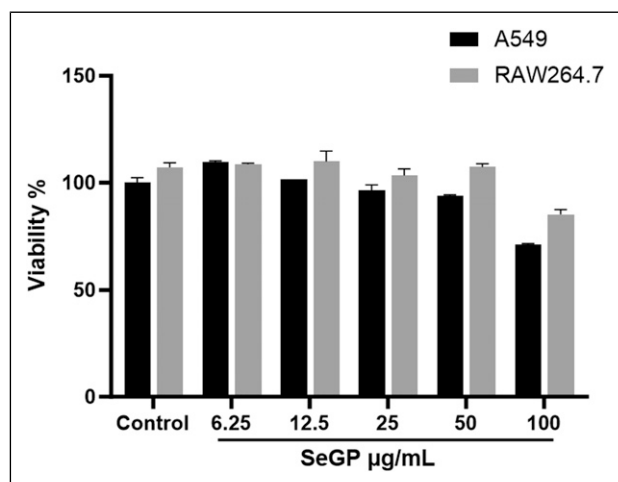


Figure 2. Cytotoxicity of SeGP in A549 and RAW264.7 cells by MTT assay.

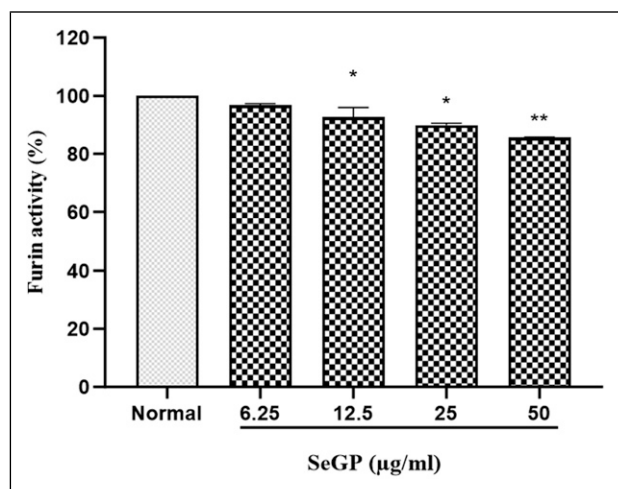


Figure 3. Effect of SeGP on furin activity. Data are expressed as mean \pm SD. * P < 0.05 and ** P < 0.005 vs control cells.

functioning of our body. Selenium is known to lower the susceptibility and severity of viral infections and consequently, its deficiency impacts immune response to viruses, expression of viral proteins and antioxidant response.²²⁻²⁴ Several preclinical and clinical studies suggested that selenoproteins are involved in reducing oxidative stress, inflammation, ferroptosis and endoplasmic reticulum (ER) stress associated with viral infections including COVID-19.^{26,27,30} Hence, Selenium supplementation is suggested to be beneficial in severely diseased and Selenium-deficient COVID-19 patients.³¹

However, excessive as well as deficient consumption of Selenium induces health risks. The World Health Organization (WHO) recommends a Selenium intake level of 55 µg/day for adults, with the tolerable upper limit set at 400 µg/day and plasma/blood levels in the general population lie around 100 µg Selenium/l.^{32,33} SeGP is a novel extract of

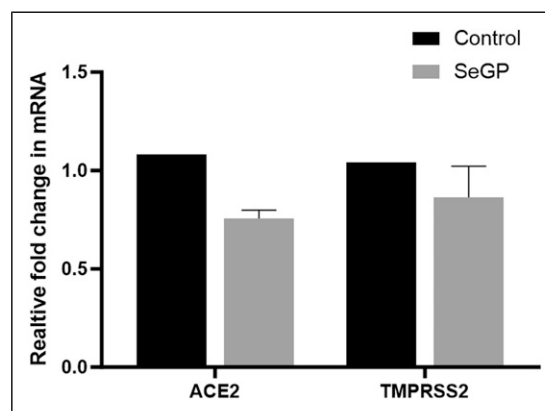


Figure 4. Relative expression of ACE2 and TMPRSS2 genes in SeGP (12.5 µg/ml) treated cells compared to untreated control.

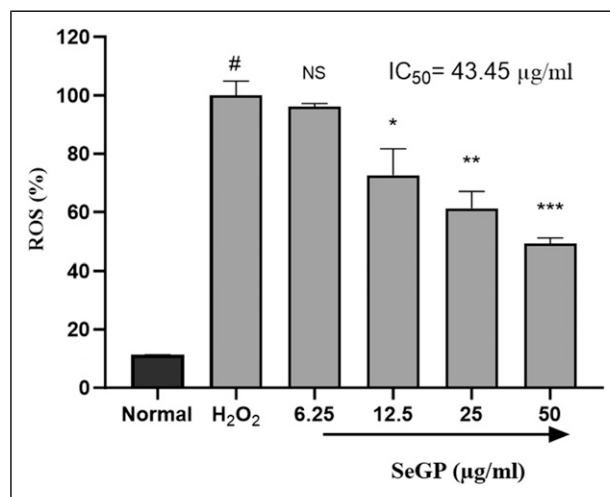


Figure 5. Effect of SeGP on H₂O₂-induced reactive oxygen species (ROS) generation in A549 cells. Data are expressed as mean \pm SD. # P < 0.0001 vs Control, and * P < 0.05, ** P < 0.005 and *** P < 0.0005 vs H₂O₂ treated cells.

organoselenium enriched extract of garlic, containing approximately 1000 µg of Selenium per gram of the extract. The dose of this supplement is fixed at not more than 100 mg/day equivalent to 100 µg of organoselenium. In the present study, we demonstrate the anti-viral, antioxidant and anti-inflammatory activities of SeGP in vitro.

As per the recent report by the WHO, there is a resurgence of SARS-CoV-2 infection globally, with a 52% increase in new cases during the 28-day period of 20 November to 17 December 2023 as compared to the previous 28-day period.³⁴ Hence, COVID-19 is still a concern due to its emerging variants, breakthrough infections and a general relaxation restrictions. Understanding the mechanisms of Coronavirus infections are crucial in developing new treatment strategies to attenuate the impact of the disease.

SARS-CoV-2 entry into the host cells is mediated by the binding of the viral spike protein to ACE2 and the activation

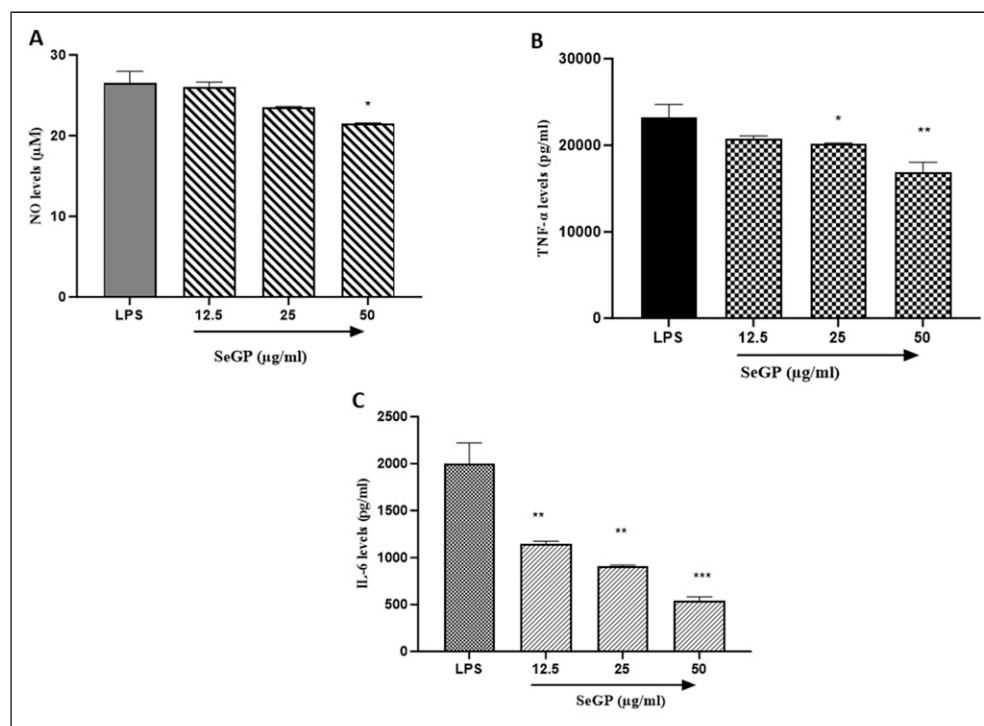


Figure 6. Effect of SeGP on inflammation. (A) NO, (B) TNF- α and (C) IL-6. Data are expressed as mean \pm SD. * $P < 0.05$, ** $P < 0.005$ and *** $P < 0.0005$ vs LPS induced cells.

of this spike protein by the proprotein convertases.³⁵ The capacity of SARS-CoV-2 to infect the cells is mediated by 2 indispensable cleaving steps of the viral spike protein. This protein is cleaved into S1 and S2 subunits, by the TMPRSS2 protease.³⁶ The S1 domain is responsible for receptor binding and the S2 domain initiates the membrane fusion.³⁷ Following endocytosis of the virus, lysosomal proteases further cleave the S2 subunit to release the hydrophobic fusion peptide to fuse with the cell membrane. Furin, is a proprotein convertase, located in the *trans*-Golgi network which cleaves precursor proteins with specific motifs (The first and fourth amino acids at the N terminus of the substrate cleavage site must be arginine “Arg-X-X-Arg ↓”) to produce mature proteins with biological activity.³⁸ One of the reasons for high infectivity of SARS-CoV-2 was proposed to be due to the presence of redundant furin cleavage site in its spike protein.³⁹ The reduced expressions of furin, ACE-2 and TMPRSS2 in lung cells and other tissues could be exploited by anti-viral compounds to ameliorate the spread of the virus. We observed that SeGP could inhibit the expression of all these proteins in vitro. These findings are consistent with earlier studies demonstrating the anti-viral properties of plant extracts and Selenium derivatives. Resveratrol, was reported to reduce the expression of nucleocapsid (N) protein essential for MERS-CoV replication in Vero cells.⁴⁰ Several phytochemicals have been shown to interfere with SARS-CoV-2 viral entry and replication proteins by docking studies.⁴¹ Ebselen, a synthetic organoSelenium drug molecule and its

derivative were shown to inhibit the SARS-CoV-2 main protease (Mpro), critical for viral replication.⁴²

SARS-CoV-2 infection involves complex interactions between the viruses and target cells where oxidative stress plays a crucial role. ROS induces tissue damage, thrombosis, and red blood cell dysfunction, contributing to the severity of SARS-CoV-2 infection.⁴³ SARS-CoV-2 infected patients have reported that excessive level of ROS is associated with severity of illness which promotes a cascade of biological events to drive host pathological responses.^{44,45} Increased oxidative stress significantly reduced anti-viral host responses and increased virus-induced inflammation leading to multi-organ damage.⁴⁶ Hence, the compounds with antioxidant activity are expected to be an active drug candidate in COVID-19 therapy. SeGP was effective in reducing oxidative stress in H₂O₂ induced A549 cells with an IC₅₀ value of 43.45 μ g/ml.

Although hyper-inflammation is a key characteristic of SARS-CoV-2 infection, no therapy has succeeded to reduce the exacerbated immune response associated with severe SARS-CoV-2 infection.^{47,48} Hence apart from preventing viral entry, attenuation of SARS-CoV-2 -induced hyper-inflammation also remains as a prime focus in the therapeutic modality of SARS-CoV-2 infection. The anti-inflammatory potential of SeGP was evaluated in activated macrophages in vitro. Nitric oxide (NO) serves as a pro-inflammatory mediator that induces several downstream inflammatory pathways⁴⁹ which was reduced significantly by

SeGP treatment. SeGP also significantly reduced the levels of TNF- α and IL-6 in the culture supernatant of activated macrophages. Several studies have suggested that elevated levels of IL-6 are associated with cytokine storm and longer hospital stays.⁵⁰ In a systematic review and meta-analyses, including 1798 patients, patients with COVID-19 were found to have elevated levels of IL-6 in serum.⁵¹ Thus, a reduction in IL-6 may have multiple benefits in patients with viral infection. Downregulation of these inflammatory components, consequently, reduces the severity and progression of the disease suggesting that SeGP could be a beneficial supplement to be used as an adjunct therapy for viral infections.

The molecular basis for this antiviral effect still needs to be completely elucidated. Further, the product also contained Alliin (S-allyl cysteine sulfoxide), at 0.04% w/w. Alliin is known to have antioxidant activity, which could have also influenced the overall activity of SeGP, which needs to be independently evaluated in future studies. However, it may be conceivable that the antiviral activity of SeGP may be mediated by multiple pathways viz inhibiting furin, ACE2 receptor and TMPRSS2. SeGP is also effective as an antioxidant and anti-inflammatory extract, which are beneficial in controlling the comorbidities associated with SARS-CoV-2 infection.

Conclusions

In conclusion, the results of this study demonstrate that Selenium enriched garlic powder inhibited SARS-CoV-2 multiplication in vitro, probably by interfering with the virus and host cell interactions. Further the Selenium product was also effective in reducing oxidative stress and inflammatory mediators, which will augment the effect of the product as an adjunct therapy for viral infection. Further studies are warranted to enumerate the detailed pathways of viral replication influenced by the product and to evaluate the efficacy in clinical settings.

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Author Contributions

Conceptualization, Muhammed Majeed and Kalyanam Nagabhushanam; Formal analysis, Lincy Lawrence and Lakshmi Mundkur; Investigation, Lincy Lawrence and Priji Prakashan; Methodology, Lincy Lawrence and Lakshmi Mundkur; Project administration, Kalyanam Nagabhushanam; Resources, Muhammed Majeed; Supervision, Lakshmi Mundkur; Writing – original draft, Lincy Lawrence and Priji Prakashan; Writing – review & editing,

Muhammed Majeed, Kalyanam Nagabhushanam, and Lakshmi Mundkur. All authors have read and agreed to the published version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: All the authors are employees of Sami-Sabinsa Group Limited or Sabinsa Corporation, which markets SelenoForce®.

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Patents

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Data Availability Statement

All data associated with the study are available in the manuscript. Any additional data may be provided on request to the corresponding author.

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