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Effect of mineral nanoparticles obtained from thermal spring water on COVID-19

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Abstract

Background: Coronavirus disease 2019 (COVID-19) is presently a life-threatening condition, and despite of multiple attempts, no functional method has been introduced against this pandemic problem yet. In this study, for the first time, the possible antiviral aspects of mineral nanoparticles (MNPs) obtained from a natural source, thermal spring water, were evaluated.

Methods: At first, MNPs obtained from the Gishki thermal spring, Kerman province, Iran, were characterized by transmission electron microscope (TEM). Then, the presence of mineral elements in MNPs was identified by X-ray fluorescence (XRF), Energy-dispersive X-ray spectroscopy (EDX), inductively coupled plasma mass spectrometry (ICP-MS), and MTT assay. Finally, 17 cases suspected of COVID-19 were randomly selected, and their nasal swab samples were exposed to two concentrations of MNPs (50 and 100 u/mL).

Results: The results of real-time polymerase chain reaction (RT-PCR) test manifested that MNPs had a destructive effect on 4 (33%) COVID-19 cases.

Conclusion: Therefore, MNPs of thermal spring water may act as an obstacle against COVID-19.

Keywords: Hot springs, COVID-19, Minerals, Antiviral agents, X-Rays

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Introduction

Coronavirus disease 2019 (COVID-19), as a life-threatening disease, appeared in Wuhan, China, in December 2019, and quickly caused a public health alert (1-3). In severe and critical stages of the disease, pneumonia related to COVID-19 can give rise to acute respiratory distress syndrome, which in turn, may be the main reason for COVID-19 associated-death (2,4). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a causative factor for this pandemic viral condition and reflects some signs and symptoms, such as fatigue, cough, and fever (5). Because of the continual and rampant spread of the present contagious virus, characterizing and using an effective drug for the amelioration of COVID-19 is urgent. Despite several performed trials by scientists, no impressive treatment against the disease has been introduced yet (5,6). To design and develop new drugs for fighting SARS-CoV-2, the probable targets have a substantial role. Various studies have recognized several SARS-CoV-2 structural targets, like spike protein, main protease/3CL/M Pro, nonstructural protein 15 endoribonuclease, and RNA-dependent RNA polymerase (7). According to evidence, using nano-sized drugs have considerable advantages, like high dissolution rate and saturation solubility, better potential for adhesion to the cell membrane, high loading capacities, and capability to use at high concentrations (8,9). These nanomaterials are divided into two types, namely, organic and inorganic nanoparticles (NPs) (10). Organic NPs mean stable particles comprising organic components, like lipids, polymers, and micelles. Inorganic (mineral) NPs are classified according to non-carbon components and metals, like Au NPs, metal salts, quantum dots, etc (11,12). Among these, mineral nanoparticles (MNPs) have been shown to exert a function against the majority of these structural targets (13). MNPs are described as nano-size crystalline compounds that can be observed as a bulk formation and are frequent in natural resources, such as thermal spring water (14-17). Thermal spring water, as a part of health tourism, has acquired considerable popularity in different populations (18). After water seepage into the earth, and subsequently, water heating through the magma, thermal water is created and raised by pressure as thermal spring water (19). In the present study, the possible anti-viral impact of MNPs extracted from a thermal spring in Rabor county, Kerman province, Iran, against COVID-19 was evaluated through the real-time polymerase chain reaction (RT-PCR) test for the first time.

Materials and Methods

Sample collection and processing

Water samples (1000 cc) were collected from the Gishki thermal spring in September 2020. The mentioned spring, aquifer depth ≤ 1200 m, the temperature of groundwater > 42-45°C, and pH 7.7, is located in Kerman province, Iran. The mineral particles/NPs were settled on the container bottom using a centrifuge at 6000 rpm for 3 min. Afterwards, the samples were exposed to the air and left to dry at room temperature. Finally, the size of NPs was determined using a transmission electron microscope (TEM), also, the elements in the sediment were identified using inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence analysis (XRF), and energy dispersive X-ray spectroscopy (EDX).

Study population

Seventeen cases suspected to COVID-19 who were admitted at Golestan hospital, Kermanshah, Iran, between 12-17 October 2020, participated in the study. Inclusion criteria were some symptoms, such as fever, headache, shortness of breath, and cough, and exclusion criteria were compromised health conditions, like infectious diseases, malignancy, and autoimmunity. Also, data about the history of patients, manifestations at the time of administration, and risk factors were collected. Patients were tested using special swabs (Dacron tipped) from the nose, and then, swabs were placed in viral transport medium (VTM) solution, which was transferred to a virology laboratory. Next, samples were divided into three parts: two parts were used to treat the virus with NPs at concentrations of 50 and 100 µg/mL, and the third part was used as a control. The samples were incubated at 37°C for 24 hours.

Extraction of viral RNA from patient samples

According to the manufacturer's instructions, 150 µL of nasal swab samples were tested using the Invitrogen mini kit to extract RNA of coronaviruses in patient samples. The RT-PCR test (Thermo Fisher 96 light cycler) was performed to detect the presence of SARS-CoV-2 using the kits (KIA COV, Iran) by ABI Step One plus RT-PCR System. For each reaction, 10 µL R.T. enzyme mix, 3 µL primer and probe mix, and 3 µL DEPC water were added to a 5 µL RNA template. Cycling conditions for amplification of FAM(RdRP), HEX(E gene),Cy5(HRP) genes were 50°C for 15 minutes, 95°C for 3 min, followed by 45 cycles of 95°C for 10 seconds and 58°C for 40 seconds (time and temperature were modified). A cycle threshold value of < 38 Ct was defined as a positive test result. Viral RNAs were exposed to MNPs for 24 hours.

MTT assay

In this study, a human fibroblast cell line was used as a normal cell line. Also, the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) test was used to assess the cytotoxic potential of the MNPs on normal cells. The mentioned cell line was purchased from the Medical Biology Research Center of the Kermanshah University of Medical Sciences in Iran. The cell line was preserved and propagated in T-25 flasks (as a monolayer) in Dulbecco's Modified Eagle Medium (DMEM) supplemented with streptomycin (100 µg/mL), penicillin (100 U/mL), and 10% fetal bovine serum (FBS). The cells were grown in a humidified condition comprising 95% air and 5% CO_2 at 37°C overnight.

Statistical analysis

The obtained data were analyzed using SPSS software (version 16). Also, $P < 0.05$ was considered statistically significant.

Results

Inductively coupled plasma mass spectrometry results

Inductively coupled plasma mass spectrometry (ICP-MS) provides accurate measurements of most elements in the periodic table at milligram to nanogram levels (20). According to the ICP-MS results presented in Table 1, 45 elements in values less than 1000 ppm were observed. Meanwhile, according to this analysis, Sr, V, and Zr concentrations were higher than 100 ppm (Table 1).

X-ray fluorescence analysis results

The major elemental composition of the Earth material samples can be analyzed using XRF method (21). In this study, 16 different oxides of the Gishki thermal spring were identified by this method (Table 1). Accordingly, SiO_2, Al_2O_3, Fe_2O_3, and CaO had the highest percentages. The amount of loss on ignition (LOI) was calculated to determine chemical compounds (22).

Transmission electron microscope results

The TEM provides useful information about particle size, size distribution, and the morphology of NPs (23). In this investigation, TEM images of MNPs revealed the particles distributed in different shapes, such as spherical and oval shapes with a size range of < 50 nm (Figure 1).

Energy dispersive X-ray spectroscopy results

This method is known as an analytical approach for the chemical characterization of different materials (24). The EDX method demonstrates that silica, aluminum, calcium, and iron are the most common compounds in the Gishki thermal spring (Table 2 and Figure 2).

MTT assay results

The survival capability of fibroblast cells was evaluated

Table 1. The concentration and percentage of elements in the MNPs by inductively coupled plasma mass spectrometry and X-ray fluorescence

Element	Amount (ppm)	Element	Amount (ppm)	Element	Amount (ppm)
Sr	610.1	As	10.4	Sn	0.71
V	142	Nb	6.26	Ta	0.69
Zr	112	Pb	5.24	Tb	0.51
Zn	74	Pr	1.11	Te	0.45
Cr	51	Th	7.42	In	0.43
Cu	97	Hf	2.1	Ag	0.17
Li	58	Cs	3.31	Cd	0.38
Rb	38	Dy	3.24	Tm	0.23
Ce	26	Yb	2.47	Tl	0.19
Ni	29	Sm	2.51	W	<1
Y	22.9	Er	2.53	Tn	<0.5
Sc	19	En	2.37	Sb	<0.5
Co	17.5	Gd	2.26	Se	<0.5
La	14	Be	1.2	Bi	<0.1
Nd	16.9	U	1	Mo	<0.1

Element	SiO_2	Al_2O_3	LOI	CaO	Fe_2O_3	Na_2O	MgO	K_2O	TiO_2	SO_3	P_2O_5	MnO	BaO	Cr_2O_3	Cu	Pb	Zn
Amount (%)	54.2	16.07	8.52	4.73	5.88	4.62	2.35	1.82	0.80	0.64	0.11	0.12	0.04	0.01	<0.01	<0.01	<0.01

Table 2. Quantitative findings of energy-dispersive X-ray spectroscopy

Element	Line	Int	Error	K	Kr	Wt%
O	Ka	188.2	3.3070	0.3599	0.2024	51.78
Na	Ka	23.7	3.3070	0.0173	0.0098	2.13
Mg	Ka	28.5	3.3070	0.0194	0.0109	1.81
Al	Ka	132.4	3.3070	0.0887	0.0499	7.20
Si	Ka	446.9	3.3070	0.3133	0.1762	23.51
S	Ka	29.7	6.3981	0.0267	0.0150	1.98
K	Ka	22.0	0.8646	0.0266	0.0150	1.76
Ca	Ka	62.6	0.8646	0.0846	0.0476	5.44
Fe	Ka	19.0	0.4119	0.0632	0.0356	4.39
				1.0000	0.5624	100.00

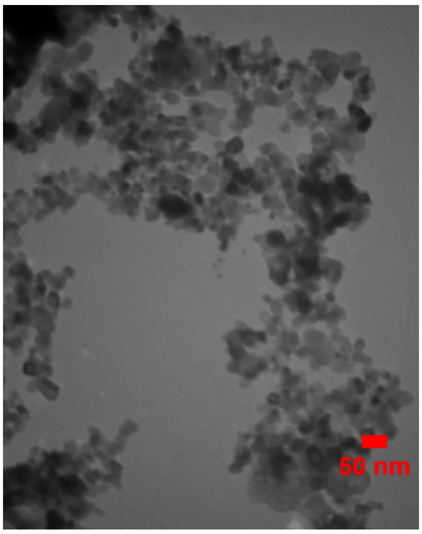

Figure 1. The image of transmission electron microscope related to mineral nanoparticles.

using different times of incubation (24 hours) and two concentrations of the MNPs (25-150 µg/mL). After 24 hours of incubation, MNPs reduced slightly the viability of normal cells at a concentration of 25-150 µg/m (Figure 3). However, a reduction in cell viability was found in the MNPs at the high concentration of 150 µg/mL.

Real time-PCR test results

Based on the findings of the RT-PCR test, out of 17 subjects suspected to COVID-19, 12 (70%) subjects have positive and 5 (30%) have negative results. Of 12 COVID-19 individuals, 8 cases were male (66%) and 4 (34%) were female. Also, the RT-PCR results indicated that MNPs at concentrations of 50 and 100 u/mL had a destructive effect on 4 (33%) patients infected with COVID-19. Also, it was observed that among patients, the male sex is affected more than the female sex by the MNPs (25% in women vs. 37% in men). However, the RT-PCR results of 8 samples with COVID-19 remained positive even after exposure to MNPs (Table 3). These findings suggest that MNPs can have destructive effects on COVID-19.

Discussion

COVID-19, as a pandemic disease, has involved a great population of subjects globally, and despite considerable efforts of researchers, there is no potent therapy against it. Fortunately, Nanotechnology-based remedies have attracted much interest in treating viral diseases (25). In this context, MNPs, which can be found in thermal spring water, have revealed antiviral potential (13,15). Hence, in this investigation, the possible therapeutic effects of these natural MNPs against COVID-19 were assessed. At first, the characterization of MNPs extracted

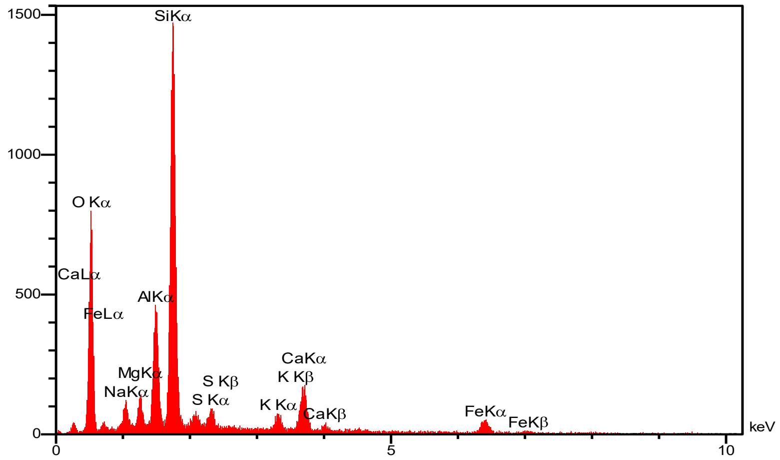

Figure 2. Graph of the spectrum of energy-dispersive X-ray spectroscopy

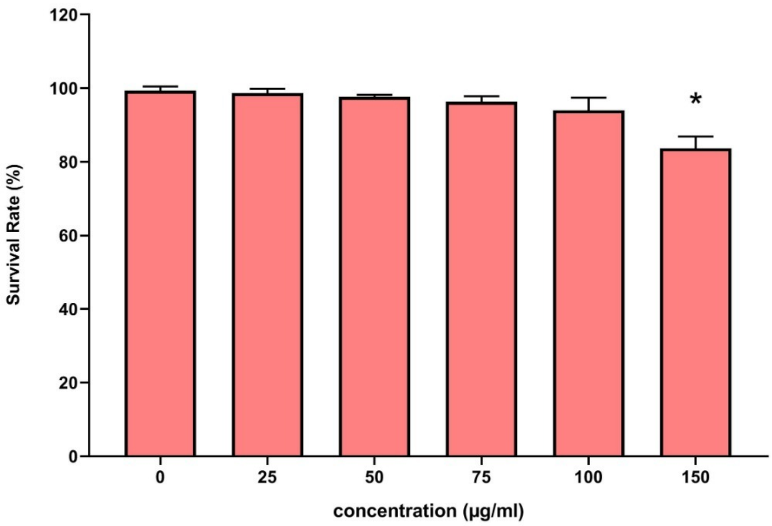

Figure 3. The survival rate of fibroblast cells incubated with mineral nanoparticles at different concentrations based on the MTT test.

from thermal spring water was carried out; then, mucus samples of COVID-19 cases were subjected to MNPs. Based on the RT-PCR test results, it was observed that 33% of patients whose samples were exposed to 50 or 100 u/mL MNPs were negative in terms of the virus presence. Coronaviruses (CVs) are classified as RNA and enveloped viruses divided into the α-CVs, β-CVs, γ-CVs, and δ-CVs genera, and SARS-CoV-2 is the main responsible for the occurrence of COVID-19 (5,26). One of the pathogenic tools of this virus is related to the spike protein function that acts as a fusion protein to promote viral entrance, and subsequently, the infection (27). According to new evidence, MNPs may have detrimental effects on this protein by interacting with the receptor-binding domain of the spike protein, which is involved in host cell binding, and subsequently, infection by this virus (13,28). In another viewpoint, electrostatic agents can affect protein behavior and structure, and biological function (29). Several studies have addressed that electrical charges are held by viruses, and the neutralization of virus charge within an electrical field may reduce viral load (30). Also, electromagnetic irradiation is capable of killing some viruses (5). On the other hand, the existence of some magnetic elements, like iron, nickel, chromium, and cobalt, has been approved in mineral particles (31-33). Moreover, NPs comprising zinc, silver, and titanium have been reported as effective components against influenza, HIV, herpes simplex, zika virus, smallpox, and RSV (34). These and other elements were also detected in our MNPs, and strontium, vanadium, zirconium, zinc, chromium, and copper elements had high concentrations. The antiviral capacity of some of these elements has been declared. For example, the antiviral features of copper comprise inhibition of single or double-stranded RNA or DNA viruses, annihilating of the viral genomes, and suppressing papain-like protease-2, a key enzyme for SARS-CoV-1 replication (35). A number of respiratory viruses and the human immunodeficiency virus can be

Table 3. The RT-PCR test in the presence and absence of mineral nanoparticles

No.	Gender	Age	Without nano	With 100 u/mL nano	With 50 u/mL nano
1	Male	44	Positive	Negative	Negative
2	Male	28	Positive	Negative	Negative
3	Male	22	Positive	Negative	Negative
4	Female	23	Positive	Positive	Positive
5	Male	40	Positive	Positive	Positive
6	Male	51	Positive	Positive	Positive
7	Female	45	Positive	Positive	Positive
8	Male	47	Positive	Positive	Positive
9	Male	50	Positive	Positive	Positive
10	Male	45	Negative	Negative	Negative
11	Female	45	Negative	Negative	Negative
12	Male	24	Negative	Negative	Negative
13	Male	34	Positive	Positive	Positive
14	Female	35	Positive	Positive	Positive
15	Female	30	Positive	Negative	Negative
16	Male	35	Negative	Negative	Negative
17	Male	32	Negative	Negative	Negative

treated with nanotechnology, including Lyme disease, influenza, and numerous respiratory viruses. Therefore, COVID-19 might be treated with nanotechnology. Fujimori et al. also demonstrated the antiviral effects of copper iodide NPs against the Influenza virus by increasing hydroxyl radical production, by which functional proteins of this virus, like HA and NA (glycoproteins), are degraded (36). Vanadium is another element that can exert its viricidal effects by inhibiting viral reverse transcriptase activity, which has a pivotal role in viral DNA integration into the genome of host cells (37). Reports highlighted the potential performance of vanadium element against SARS, HIV, and Influenza (37). In addition, zinc element can serve as an antiviral agent by influencing RNA-dependent RNA polymerase of RNA viruses, such as SARS-CoV-2 (38). However, the possible antiviral mechanisms of other elements have not been completely documented yet. On the whole, MNPs may affect COVID-19 through suppression of synthesis of viral RNA, T cell activation, inhibition of ATPase function of SARS-CoV-2 nsp13 and helix unwinding (34). Also, MNPs have revealed their capacity for suppressing SARS-CoV-2 proliferation and replication through interaction with the strands of the virus genome (13). This interaction can give rise to viral genomic DNA damage (39). The main mechanism of pathogenesis in SARS-2 coronavirus infection, such as SARS-1 coronavirus, is to induce a severe inflammatory response of innate, and then, acquired immunity, resulting in damages to the air sacs of the lungs, and in some cases, to intestinal enterocytes and other epithelial cells. In uncontrolled immune responses, the penetration of inflammatory macrophages, monocytes, neutrophils, and T cells into the lung alveoli increases with the overproduction of inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), IL-8, and interferon gamma (IFN-γ), etc. lead to tissue damages in the lungs and other organs (40).

The possible mechanism of nanoparticle suppression on COVID-19 is to reduce the severity of the systemic effects of cytokine release syndrome (CRS) by NPs. Previous studies have shown that NPs can absorb and neutralize proinflammatory cytokines. NPs have also been shown to reduce IL-6 and TNF-α. Because in COVID-19 patients with CRS, the two major proinflammatory cytokines, including IL-6 and TNF-α, are found at high levels. As a result, NPs may be considered as a therapeutic opportunity for the deadly CRS phenomenon (41). There is no 100% effective drug or vaccine. Thus, nanotechnology may provide a better therapeutic alternative for this pandemic disease (42).

Conclusion

It seems that using MNPs originated from thermal spring water may be effective in fighting COVID-19. However, more investigations are needed to investigated their curative capacity against COVID-19.

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Ethical issues

The present study was approved by the Institutional Ethics Committee of Kermanshah University of Medical Sciences (Ethical code: IR.UK.VETMED.REC.1401.010).

Competing interests

The authors declare that there is no conflict of interests.

Authors' contribution

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Funding acquisition: Maryam Ehteshamzadeh.
Investigation: Mehdi Firouzi.
Methodology: Mehdi Firouzi.
Project administration: Maryam Ehteshamzadeh.
Resources: Mehdi Firouzi.
Software: Mehdi Firouzi.
Supervision: Fakhredin Saba.
Validation: Gholam Reza Khayati.
Visualization: Mehdi Firouzi.

Writing – original draft: Mehdi Firouzi.
Writing – review & editing: Mehdi Firouzi.

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