ISSN: 2577-8455

Research Article

Archives of Infectious Diseases & Therapy

D.SAP, an Apple-Based Formulation Can Treat SARS-CoV-2 Infection, and Reduce Associated Inflammatory Responses in COVID-19 Infected Mice Model

Hossein Dezhakam[1, 2], Amin Dezhakam[1, 2], Ani Dezhakam[1, 2], Arvin Haghighatfard[1*]

[1]*Khwarizmi Institute of science and technology, Qeshm Island, Hormozgan, Iran*

[2]*Congress 60 Non-governmental organization*

[*]Corresponding author

Arvin Haghighatfard, Khwarizmi Institute of science and technology, Qeshm Island, Hormozgan, Iran.

Submitted: 09 Aug 2022; **Accepted:** 22 Aug 2022; **Published:** 30 Aug 2022.

Citation: *Dezhakam, H., Amin Dezhakam, A., Dezhakam, A., Arvin Haghighatfard, A. (2022). D.SAP, an Apple-Based Formulation Can Treat SARS-CoV-2 Infection, and Reduce Associated Inflammatory Responses in COVID-19 Infected Mice Model. Archives of Infect Diseases & Therapy, 6(2), 183-188.*

Abstract

Introduction: *The SARS-CoV-2 virus is a virus that may cause severe respiratory disorders with an unclear rate of death. Natural vinegar may have an immune-boosting quality that effectively fights influenza-like respiratory infections. We aimed to evaluate the role of a specially formulated Iranian apple vinegar called Dezhakam sap (D.SAP) in the mice model of covid-19 in the treatment of infection and reduction of covid19-associated lung inflammation.*

Methods: *We designed covid-19-positive Inbred BALB/c mice by viral exposure. Then all mice were examined for active SARS-CoV-2 infection. Mice with positive covid-19 infection were separated into different groups to study the effect of treatment by different concentrations of D.SAP smoke for seven days. Expression assessments for viral RNA on covid-19 affected mice models for three SARS-CoV-2 genes (RDRP, N, and E) and three inflammation markers (IL6, IL1b, and TNF) in lung tissue of all mice were assessed using Real-time PCR.*

Results: *Results showed a significant decrease in viral load in the RDRP gene, N gene, and E gene in all infected rats who were treated with D.SAP compared with off-treatment mice. In addition, all three inflammation factor genes in infected mice that were treated with D.SAP were significantly reduced compared to the off-treatment infected mice.*

Conclusion: *Findings showed the effectiveness of specially formulated apple vinegar D.SAP to attack new coronavirus. Also, results may suggest the effectiveness of D.SAP to reduce the lethal inflammation in COVID-19 infection with low side effects. Anti-inflammatory agents block certain substances in the body that cause inflammation. D.SAP may consider a potential natural anti-inflammatory that may increase the survival rate of COVID-19 infection. It seems these effects could be related to the antimicrobial and antioxidant effects of D.SAP due to its polyphenolic compounds.*

Keywords: COVID-19, Apple Vinegar, D.SAP, IL6, IL1b, TNF-α

Introduction

The SARS-CoV-2 virus is an RNA virus from the Coronavirus family, which cause severe and potentially lethal respiratory disorder. COVID-19 patients, which required intensive care, show higher levels of the pro-inflammatory factors, granulocyte colony-stimulating factor, macrophage inflammatory protein, and tumor necrosis [1]. The world community is facing an unexpected pandemic of novel coronavirus disease 2019 (COVID-19), which is caused by a newly mutated virus of the Coronavirus family called Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2). The lethal rate of COVID-19 is still unclear and statistics are extremely controversial. It seems that the development of sustainable methods to combat current and potentially future mutated types of Coronaviruses is a grave health concern worldwide. Coronaviruses are highly transmittable and can infect different persons of different ages, gender, and physiological situations [2]. So the treatment strategy for the Coronavirus should be applicable and cost-effective for all types of Coronavirus family and with minimum side effects for different individuals with different physiology, background diseases, and genetic diversities [3].

The Coronavirus family members including SARS-CoV-2 are positive-sense RNA viruses, which typically infect the upper and lower respiratory tracks and cause disease by severe cytotoxic effects and the induction of host cytokines disease. The life cycle of SARS-CoV-2 is mostly similar to other members of the coronaviruses family such as the virus that causes SARS-

CoV-1 detected in 2003. In the beginning, the virus enters the cell (mostly lung epithelial cells) by attaching to the angiotensin-converting enzyme-2 (ACE-2) receptors that act as the gateway for the entry of the virus inside the body [4].

Previous studies have shown that apple cider vinegar has antimicrobial properties and can kill bacteria, yeast, and fungal infections. According to the Environmental Protection Agency (EPA) of the United States, a disinfectant material should be able to kill about 99.9 percent of disease-causing bacteria and viruses [5]. Vinegar only works against some germs, like E. coli and Salmonella. Apple cider vinegar has also significant antioxidant effects and huge amounts of these effects are due to its polyphenolic compounds. Polyphenolics are active chemical compounds in plants, including fruits and vegetables. While other types of fruit vinegar, like grape vinegar, also contain polyphenolic compounds; white vinegar does not contain these compounds. Antioxidant effects of polyphenolic compounds act may prevent the formation of free radicals, which can damage tissue. Potential treatment effects of polyphenols on some cancers, coronary heart disease, and various inflammatory disorders have been reported [6]. Certain types of unfiltered apple cider vinegar include the "mother," which is a colony of beneficial probiotic bacteria, especially some of the lactobacillus serotypes that have a major role in the improvement of the gut and immune system functions [7].

Dezhakam sap of apple (D.SAP) is a specially formulated and unfiltered Apple vinegar that producing with a traditional method in Iran. Because of the selection process of apples and especially the traditional production of D.SAP, it is full of antioxidants, probiotic compounds, micronutrients, and cellulose fiber. Taken together the contents of D.SAP may have a potentially positive effect on the treatment of acute respiratory disorders such as influenza and SARS-CoV-2 virus regarding its anti-inflammation effects.

In SARS-COV2 infection, overwhelming inflammation especially in lung tissue may lead to dangerous side effects or even death. Several therapeutic strategies have progressed to modulate harmful inflammation in COVID-19. Some of these approaches are not effective and some of them which target the immune system may reduce the ability of the body to fight against the virus. Abnormalities in immune cells and dysregulation of inflammatory markers have been reported in patients with severe respiratory disease including COVID-19 that in turn will lead to cytokine storm, hyperinflammation, and multi-organ failure [8].

IL6, IL1b, and TNF are three major inflammatory markers that may use to detect inflammation patterns in patients with acute respiratory diseases. Proinflammatory cytokines especially IL-1, IL-6, and TNF-α can significantly influence the onset of concomitant disorders, arterial hypertension, and metabolic disorders [9]. Secretion of Pro-inflammatory cytokines by macrophages and lymphocytes in response to infection-induced cell damage in lung tissue may increase the severity of the infection and even lethal rate in COVID-19 infected persons.

The present study aimed to evaluate the anti-inflammatory and antiviral effects of an especially formulated apple vinegar called D.SAP on mouse models with COVID-19 infection. The expression level of the three most expressed genes in the SARS-COV-2 virus was assessed for analysis of antiviral effects and the expression level of IL-1, IL-6, and TNF-α genes in lung tissue models were assessed for analysis of anti-inflammatory effects.

Material, methods, and methodology

Animal modeling and grouping

Inbred BALB/c mice choose because of their small size, clear genetic background, and ease of genetic manipulation to design the COVID-19 animal model. Mouse ACE2 (mACE2) has a low affinity for the S protein of SARS-CoV-2that reduces the infection rate of wild-type viruses [10]. Due to the incompatibility of mACE2 with SARS-CoV-2 S protein, the use of mouse-adapted SARS-CoV-2 strains, and the expression of human ACE2 (hACE2) in mice used for the production of COVID-19 animal model [11]. Mouse-adapted SARS-CoV-2 strain MASCp6 (mouse-adapted strain at passage 6), with mutation of the strain, at the RBD site of viral S protein which increase the binding affinity of the protein to mACE2 and was confirmed to be infectious to mice with inflammatory responses and moderate pneumonia in them used in this study. Inbred BALB/c mice were housed under specific pathogen-free conditions under controlled temperature and humidity in a Biosafety Level-2 laboratory and the institutional committee for animal care and use approved all procedures following the standards established by the US Animal Welfare Act [12].

For the production of SARS-CoV-2 infected mice, MASCp6 mice were intranasally challenged using inocula of 103 plaque-forming units (PFU) for three days. Intranasal-induced COVID-19 lung infection assessed in the upper respiratory tract based on detection of viral RNA in nasal secretions using quantitative Real-time PCR. Animal modeling was conducted based on protocols proposed in previous studies [13, 14]. Mice were grouped into nine groups, each group including six male inbred BALB/c mice. Details of the groups named from A to I (based on English alphabetic order) and also their viability rate during the study are presented in table 1.

Table 1: Group's names and viability rate during the infection and treatment process

Group name	Group description	Total number of mice	Dead number of mice	Date of death
A	Controls with no infection and no treatment	6	0	-
B	Covid19 infected with no treatment	6	2	4th day
C	Covid19 infected with treatment by 12.5% D.SAP	6	0	-
D	Covid19 infected with treatment by 25% D.SAP	6	0	-
E	Covid19 infected with treatment by 50% D.SAP	6	1	7th day
F	Covid19 infected with treatment by distilled water	6	1	6th day
G	Pretreated with 12.5% D.SAP and then Covid19 infected with treatment by 12.5% D.SAP	6	0	-
H	Pretreated with 25% D.SAP and then Covid19 infected with treatment by 25% D.SAP	6	0	-
I	Pretreated with 50% D.SAP and then Covid19 infected with treatment by 50% D.SAP	6	0	-

Protocols of treatment with D.SAP

D.SAP is used for treatment in three different concentrations, 50 percent, 25 percent, and 12.5 percent of concentration by distilled water as solvent. Intranasal inoculation of 106 PFU per mouse with three different dosages at seven days intervals for treatment groups, seven days before infection process, and seven days after infection for pretreatment groups.

Scarification and Lung tissue sampling

All animals were sacrificed for endpoint analysis on day seven post-infection. Lung tissues of sacrificed mice were collected, homogenized, and subjected to viral load determination. All viral challenge experiments and treatments had been operated in a Biosafety Level-3 animal facility.

Expression analysis of viral RNA and inflammation genes

RNA was extracted from homogenized samples immediately after samplings according to standard protocols using by RNA Purification kit (GeneJET™ RNA Purification Kit#K0732, Thermo scientific - Fermentas, Latvia). Genomic DNA contamination of extracted RNA was removed using DNase Treatment & Removal Reagents (DNase I, RNase-free (#EN0521) Fermentas, Latvia), according to the kit protocol. The quality and quantity of RNA were assessed by one percent Agarose gel electrophoresis and UV- spectroscopy respectively. Synthesis of cDNA was conducted by a Transcription First Strand cDNA Synthesis Kit (RevertAid Premium First Strand cDNA Synthesis Kit #K1652, Thermo scientific -Fermentas, Latvia) based on the manufacturer's protocol and previous studies [15].

Specific primers and probes were designed by "oligo7" software and were blasted on the NCBI website. Standard curves for each gene had been prepared by serial dilutions (1: 4) of pooled cDNA from total RNA extracted from samples of mice from the control group. In each experiment, the R2 value of the standard curve was more than 0.99 and no-template control assays showed no detectable signal. In addition, the efficacy of PCR reaction calculates using Lin-Reg PCR free software (Amsterdam, Netherland). Real-time PCR was conducted by The TaqMan® PCR Starter Kit, Thermo scientific - Fermentas, Latvia). CFX96 Touch Real-Time PCR Detection System (BIO-RAD, California, US) used for triplicate method Quantitative Real Time-PCR. Gene expression examinations are conducted based on previous studies [16]. Livak formula was used to calculate the ratio. Primers' sequences are presented in table 2.

Table 2: Sequence of Premiers Used For Real-Time PCR

Gene	Forward primer	Reverse primer
N	5'CCTCTTCTCGTTCCTCATCA3'	5'CCTGGTCCCCAAAATTTCCT3'
E	5'GAAGAGACAGGTACGTTAA3'	5'AAGGTTTTACAAGACTCACG3'
RDRP	5'CATCTCACTTGCTGGTTCCT3'	5'CCTTAATAGTCCTCACTTCTCTC3'
IL-6	5'CAGTTGCCTTCTTGGGACTGA3'	5'GAAGTCTCCTCTCCGGACTTG3'
IL-1b	5'AATCTCGCAGCAGCACATCA3'	5'ACGGGAAAGACACAGGTAGC3'
TNF-α	5'CCAACGGCATGGATCTCAAAG3'	5'TCCCTTGAAGAGAACCTGGGA3'
GAPDH	5'GTCAAGGCCGAGAATGGGAA3'	5'GGCCTCACCCCATTTGATGT3'

Statistical examinations

Normal distribution for continuous variables was evaluated with the Kolmogorov-Smirnov test. Descriptive data are expressed as mean ± SD (range) and the level of statistical significance was set at $P < 0.05$. Statistical differences between groups were calculated by one-way ANOVA. Multiple comparison corrections were performed by the Bonferroni correction test. Statistical analysis was performed using SPSS software version 24.

Results

Analysis of D.SAP antiviral effects

Results showed significant antiviral effects of D.SAP on covid-19 infection. While group B shows a decrease in cycle threshold in all three genes and an increase in viral load after seven days, all treated groups with different concentrations became SRARS-COV-2 negative. Group F which included Covid-19 infected mice that were treated with distilled water for seven days and used as the sham group showed no significant difference in viral load compared with the B group in any gene. The p-values of B group versus F group in each gene were N gene (p-value: 0.3), E gene (p-value: 0.2), and RDRP gene (p-value: 0.3). It should be noticed that the increase of viral load in F group was slightly but not significantly slower in comparison to B group. All pretreatment groups showed no positive mouse after the seven days of pretreatment before infection time and seven days after the infection. While both treatment and pretreatment groups in all concentrations became negative after treatments, it should be noticed that pretreatment groups had non-significant but interesting lower viral load after infection compared with other groups. Results of SARS-COV-2 viral RNA presence and level in each group after infection and after the treatment with D.SAP were presented in Tables 3 and 4 respectively.

Table 3: Viral load rate in groups based on the expression level of three SARS-CoV-2 main genes (mean cycle threshold value of N, E, and RDRP intragroup) before treatment with D.SAP

Group	N gene ct value	E gene ct value	RDRP gene ct value	Final result
A	not detected	not detected	not detected	not infected
B	31.6	33.3	32.3	infected
C	32.5	33.1	32.5	infected
D	31.2	32.4	33.6	infected
E	32.5	33.2	32.1	infected
F	31.1	33.6	33.2	infected
G	37.3	not detected	38.7	weakly infected
H	37.5	not detected	39.2	weakly infected
I	38.1	not detected	38.3	weakly infected

Ct: cycle threshold, N: Nucleocapsid, E: Envelope, RDRP: RNA-dependent RNA polymerase

Table 4: Viral load rate in groups based on the expression level of three SARS-CoV-2 main genes (mean cycle threshold value of N, E, and RDRP intragroup) after seven days of treatment with D.SAP

Group	N ct value	E ct value	RDRP ct value	Final result
A	not detected	not detected	not detected	not infected
B	27.3	26.1	26.4	infected
C	not detected	not detected	not detected	not infected
D	not detected	not detected	not detected	not infected
E	not detected	not detected	not detected	not infected
F	28.1	28.1	27.7	infected
G	not detected	not detected	not detected	not infected
H	not detected	not detected	not detected	not infected
I	not detected	not detected	not detected	not infected

Ct: cycle threshold, N: Nucleocapsid, E: Envelope, RDRP: RNA-dependent RNA polymerase

Analysis of D.SAP anti-inflammatory effects

Overexpression of IL-1β ،IL-6 and TNF-α genes in lung tissue was detected in all infected groups compared to group A. highest overall upregulation of three genes was revealed in groups B and F and all pretreatment groups, especially group G showed the lowest dysregulation and over expression of inflammatory genes compared to controls. All treatment and pretreatment groups showed significant down-regulation of inflammatory genes in comparison with the B group indicating the anti-inflammatory effects of D.SAP nasal treatment. Findings of the expression level of inflammatory genes in lung tissue of each group after scarification are presented in Tables 5 and 6.

Table 5: Gene expression level of IL-1β ،IL-6 ، TNF-α genes in lung tissue of COVID19 infected mice compared with control mice (calculated by comparative method and Livak formula)

Comparisons	*IL-6 Ratio*	*IL-1β Ratio*	*TNF-α Ratio*
B Vs. A	4.42	3.88	4.18
C Vs. A	2.34	2.22	2.03
D Vs. A	1.93	1.62	2.02
E Vs. A	2.05	1.55	1.92
F Vs. A	3.94	3.61	3.86
G Vs. A	1.25	1.3	1.2
H Vs. A	1.22	1.33	1.77
I Vs. A	1.68	1.46	1.74

Table 6: Statistical analysis of IL-1β ،IL-6 ، TNF-α gene expression level in lung tissue of infected mice models in each group versus control mice

Comparisons	*IL-6 p-value*	*IL-1β p-value*	*TNF-α p-value*
B Vs. A	0.004*	0.006*	0.008*
B Vs. C	0.03*	0.01*	0.01*
B Vs. D	0.01*	0.004*	0.01*
B Vs. E	0.01*	0.004*	0.01*
B Vs. F	0.12	0.14	0.18
B Vs. G	0.01*	0.01*	0.01*
B Vs. H	0.005*	0.009*	0.01*
B Vs. I	0.01*	0.01*	0.01*

* used for significant corrected p-value

Discussion

The findings of the present study revealed that different doses of D.SAP is firstly biocompatible and harmless and do not lead to animal mortality as well as able to prevent the continuation of the disease in all doses after one week of smoking and the virus in mice after treatment. Pretreatment of patients also leads to low pathogenicity in mice and makes treatment easier. SARS-COV-2 is a new respiratory virus that has caused a major pandemic and high mortality in the world due to the way its symptoms appear and the possibility of its clinical symptoms remaining hidden for seven to fourteen days, as well as the ability to transmit quickly from person to person. Rapid transmission of this virus and its high mutagenicity may cause a constant pandemic with a high mortality rate in this disease. Severe inflammation in the lung tissue, which leads to the destruction of air sacs, was the main cause of death in patients with Covid-19.

Findings showed firstly, inhalation of D.SAP does not lead to any inflammation or adverse effects leading to death or disease as well as reduction of significant inflammation rate in D.SAP recipients. It has been reported that inflammation has a major role in the severity of COVID-19 infection and inflammation overwhelming could cause death [17]. An effective therapeutic strategy to suppress or modulate inflammation in COVID-19 which target the immune system as well as strength it in combat with the virus is a great discovery.

Small pro-inflammatory proteins called cytokines are released by immune cells and regulate the immune response to pathogens. Chemokines, interleukins, monokines, tumor necrosis factors (TNF), and interferons (IFN) act on the cells that secret them and neighboring cells or cells that are distant from the site of secretion [18]. Massive and dysregulated cytokine release may cause a cytokine storm [19]. Cytokine storms are common in viral infections and had been reported in MERS-CoV, SARS-CoV, influenza, and recently in SARS-Cov-2 [20]. The prevalence of cytokine storms in COVID-19 is high and it may relate to delayed viral clearance, the late response of interferon type I, up-regulation of neutrophil extracellular traps, and pyroptosis [21]. It has been determined that a negative coronary test in mice after one week was associated with a reduction in inflammation and thus a reduction in air sac damage. Also, the best results were observed in the pretreatment group G, which showed that smoking 12.5% concentration of D.SAP for seven days before the onset of pneumonia lead to a significant reduction in pneumonia viral load. Antiviral effects of D.SAP may lead to faster viral clearance that could be one of the mechanisms of cytokine storm inhibition which in turn reduce the severity of symptoms during and after relief from the disease, as well as an increase in resistance to infection.

Other potential protective effects of D.SAP that may explain its potential immune-regulator effects could be associated with probiotics. It has been indicated that probiotics have an immune-boosting quality that effectively fights influenza-like respiratory infections and the common cold [22]. Recent studies provide evidence supporting the antiviral and general immune-strengthening health effects of probiotics. prophylactic and immune-boosting probiotics have potential effects on the prevention of respiratory viral infections such as COVID-19 [23]. Several lines of evidence should nutraceuticals such as Zn, vitamin D, vitamin C, curcumin, and cinnamaldehyde, have an-

tioxidant and anti-inflammatory effects. Grouping these nutrients in the right combination may suppress the lethal inflammation providing both prophylactic and therapeutic support against COVID-19 [24]. It may explain the potential immune-boosting and immune regulatory role of D.SAP as apple based formulation.

Conclusion

In conclusion, although there is a great lack of information about SARS-Cov-2 and its treatments, new strategies such as taking or inhaling D.SAP, especially daily and before infection may reduce the side effects of infection and help the reduction of the lethal rate of the pandemic. Extensive studies on D.SAP and its role in improving respiratory infections can help us better understand the mechanisms of function and how they might be used for possible treatment.

Acknowledgment

We would like to thank the Islamic Azad university laboratories sections.

Conflict of Interests

Authors declare that there is no conflict of interest.

References

1. Satuna, R. K., Negi, A., & Satuna, R. (2020). Intuitive vision and indigenous immunity boosting approaches for COVID19: From the literature of Pandit Shriram Sharma Acharya. Dev Sanskriti Interdisciplinary International Journal, 16, 01-15.
2. Vianello, A., Guarnieri, G., Braccioni, F., Lococo, S., Molena, B., et al. (2022). The pathogenesis, epidemiology and biomarkers of susceptibility of pulmonary fibrosis in COVID-19 survivors. Clinical Chemistry and Laboratory Medicine (CCLM), 60(3), 307-316.
3. Di Micco, P., Di Micco, G., Russo, V., Poggiano, M. R., Salzano, C., et al. (2020). Blood targets of adjuvant drugs against COVID19. Journal of Blood Medicine, 11, 237.
4. Kolberg, E. S. (2020). ACE2, COVID19 and serum ACE as a possible biomarker to predict severity of disease. Journal of Clinical Virology, 126, 104350.
5. Sarowska, J., Wojnicz, D., Jama-Kmiecik, A., Frej-Mądrzak, M., Choroszy-Król, I. (2021). Antiviral potential of plants against noroviruses. Molecules, 26(15), 4669.
6. Lim, J. S., & Ha, J. W. (2021). Effect of acid adaptation on the resistance of Escherichia coli O157: H7 and Salmonella enterica serovar Typhimurium to X-ray irradiation in apple juice. Food Control, 120, 107489.
7. Kadyan, S., & Pradhan, D. (2020). Antifungal Lactic Acid Bacteria (LAB): Potential use in food systems. In Novel Strategies to Improve Shelf-Life and Quality of Foods (pp. 73-94). Apple Academic Press.
8. Topol, E. J., & Iwasaki, A. (2022). Operation Nasal Vaccine—Lightning speed to counter COVID-19. Science Immunology, eadd9947.
9. Popko, K., Gorska, E., Stelmaszczyk-Emmel, A., Plywaczewski, R., Stoklosa, A., et al. (2010). Proinflammatory cytokines Il-6 and TNF-α and the development of inflammation in obese subjects. European journal of medical research, 15(2), 1-3.
10. Wan, Y., Shang, J., Graham, R., Baric, R. S., & Li, F. (2020). Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. Journal of virology, 94(7), e00127-20.
11. Zheng, J., Wong, L. Y. R., Li, K., Verma, A. K., Ortiz, M. E., Wohlford-Lenane, C., ... & Perlman, S. (2021). COVID-19 treatments and pathogenesis including anosmia in K18-hACE2 mice. Nature, 589(7843), 603-607.
12. Gu, H., Chen, Q., Yang, G., He, L., Fan, H., et al. (2020). Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. Science, 369(6511), 1603-1607.
13. Zheng, J., Wong, L. Y. R., Li, K., Verma, A. K., Ortiz, M. E., Wohlford-Lenane, C., ... & Perlman, S. (2021). COVID-19 treatments and pathogenesis including anosmia in K18-hACE2 mice. Nature, 589(7843), 603-607.
14. Le Bras, A. (2022). A new lethal mouse-adapted SARS-CoV-2 for COVID research.
15. Abbasy, S., Shahraki, F., Haghighatfard, A., Qazvini, M. G., Rafiei, S. T., et al. (2018). Neuregulin1 types mRNA level changes in autism spectrum disorder, and is associated with deficit in executive functions. EBioMedicine, 37, 483-488.
16. Haghighatfard, A., Andalib, S., Amini Faskhodi, M., Sadeghi, S., Ghaderi, A. H., et al. (2018). Gene expression study of mitochondrial complex I in schizophrenia and paranoid personality disorder. The World Journal of Biological Psychiatry, 19(sup3), S133-S146.
17. Wong, R. S. (2021). Inflammation in COVID-19: From pathogenesis to treatment. International journal of clinical and experimental pathology, 14(7), 831.
18. Zhang JM, An J. (2007). Cytokines, inflammation and pain. International anesthesiology clinics, 45(2):27.
19. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. (2012). Into the eye of the cytokine storm. Microbiology and Molecular Biology Reviews, 76(1), 16-32.
20. Wong, J. P., Viswanathan, S., Wang, M., Sun, L. Q., Clark, G. C., & D'elia, R. V. (2017). Current and future developments in the treatment of virus-induced hypercytokinemia. Future medicinal chemistry, 9(2), 169-178.
21. Soy, M., Keser, G., Atagündüz, P., Tabak, F., Atagündüz, I., & Kayhan, S. (2020). Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clinical rheumatology, 39(7), 2085-2094.
22. Ashaolu, T. J. (2020). Immune boosting functional foods and their mechanisms: A critical evaluation of probiotics and prebiotics. Biomedicine & Pharmacotherapy, 130, 110625.
23. Singh, K., & Rao, A. (2021). Probiotics: A potential immunomodulator in COVID-19 infection management. Nutrition Research, 87, 1-12.
24. Mrityunjaya, M., Pavithra, V., Neelam, R., Janhavi, P., Halami, P. M., & Ravindra, P. V. (2020). Immune-boosting, antioxidant and anti-inflammatory food supplements targeting pathogenesis of COVID-19. Frontiers in Immunology, 2337.