

Spirulina Extract Enhances T-Cell Responses Targeting Spike Protein of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): A Potential Drug Candidate for Treatment of COVID 19

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Abstract: Globally, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) killed more than 323k people and trimmed the economic growth significantly. Vaccine development is not soon and people may require living with the SARS-CoV-2 virus. T helper cells target the spike protein of SARS-CoV-2. And a strong T cell response is directly proportional to the rate of SARS-CoV-2 suppression. Spirulina augmented interferon-gamma (IFN γ) & Natural Killer (NK) cell cytotoxicity in humans. Spirulina was effective in inhibiting several viral replications like HIV-1 and potentially induced IFN γ in healthy volunteers. Immunity is the ultimate protective shield combating with various infectious diseases including COVID-19. A nutrient supplement Spirulina being an effective immunomodulator showed promising results in proliferating cell-mediated immunity and activation of T-cells. Spirulina extract degrades histone deacetylases responsible for the inflammatory gene expression. Spirulina is associated with a wide range of adverse events and in few people it may be fatal. So, people with allergies, autoimmune disorders and other underlying medical conditions should consult healthcare professional before consuming. Pure Spirulina extract is safe (not regulated by the US FDA) and a potential drug candidate in combating with the SARS CoV-2. This review recommends well-controlled, randomized clinical trials to confirm the effectiveness of Spirulina in inhibition and proliferation of the novel virus in the human body.

Key words: Cell-Mediated Immune Response • Inhibition of Viral Replication • Cytokine Storm • Cytotoxic Potential • Stimulation of NK Cells

INTRODUCTION

The damage caused by the COVID 19 has been considered as the world war 3. It killed more than 323k people and the health crisis related economic impacts lead the risk and volatility in financial markets. Approximately, 13-32% fall in the global trade could be expected depending on the duration of this health crisis related economic downturn [1].

Many pharma companies and research & development centers are putting ceaseless efforts in developing treatment drugs or a preventable vaccine. There were many theories, observations and assumptions that evolved as the number of COVID 19 cases is increasing. Many countries announced lockdown and several countries shut down their normal life since March 2020. Despite several efforts, there was no change in the number of COVID 19 infections, the reproduction number

is coming down. Except for a few countries like South Korea, New Zealand, Wuhan-China etc., the lockdown was not successful in many countries. As the economy is severely affected, industries closed, people have lost jobs, & hunger deaths in few countries rose, most of the countries in the world decided to relax on restrictions in phases. Though the number of cases is increasing & WHO is a warning, countries have no options to handle this deepened economic crisis.

There are approved drugs (HCQ and remdesivir) to use in the emergency conditions and vaccine trials are ongoing but chances are dim getting approved therapy for COVID 19 by the end of 2020. Then what is the alternate? Shall we live in the lockdown & wait until the vaccine comes in place?.

Many industrialists and scholars are saying that COVID 19 will not go naturally but people should live with it until vaccine development takes place. Interestingly,

Princeton university revealed that there is no effect of climate over the spread of the COVID 19 transmission, but it may be a mitigating factor when the population becomes resistant to the virus. As more immunity in the population develops, the more we expect the increased sensitivity of the virus to climate [2]. People with underlying medical conditions and poor immunity are more sensitive to any infection including Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) [3]. So, immunity in the human body is playing a crucial factor in protecting from various infections.

The natural development of immunity to a pathogen is a multi-step process that usually occurs in 1-2 weeks after infection. A viral infection triggers a non-specific response within the human body producing macrophages (δ), neutrophils and dendritic cells which delays the progression of the virus progression and further stopping disease symptoms. This non-specific response is an adaptive response, where antibodies (immunoglobulins) are produced and directly bind to the surface of the virus [4]. People are ready to wear masks, maintain social distance and all guidance to avoid SARS CoV-2 infection, however, most of the population trying differently to develop or enhance immunity internally so that accidental contact with the virus will not succumb them to the SARS CoV-2 infection.

Spirulina can be consumed as a healthy food because of its rich nutritional qualities. Spirulina is not regulated by the United States Food and Drug Administration (US FDA) however, after reviewing a petition to consider Spirulina extract as a coloring agent in chewing gums and candies, concluded as safe [5]. Spirulina as a dietary supplement can be recommended at doses of 3 and 4.5 grams/day [6]. Several human & animal studies showed that Spirulina is an effective immunomodulator and can influence the activation of immune cells. Colossal bibliography is available on nutritional & therapeutically applications of Spirulina.

Through research, we are discovering just how Spirulina strengthening the immune system and how it is beneficial if added to our diet [6]. This present review focusses on the immunomodulating activities of Spirulina providing innate protection against SARS-CoV-2 infection.

MATERIALS AND METHODS

Literature related to immunomodulating effects of Spirulina in animal & human models are reviewed. The review centered on the immunoproliferation effects of Spirulina extract on different viral infections including

SARS-CoV-2 while suggesting as a potential drug candidate for COVID-19 care.

RESULTS AND DISCUSSION

Currently, vaccine development is focusing on immune response antibodies, the proteins produced by B-cells which ideally bound SARS-CoV-2 and prevent them from entering cells. In contrast, T-cells prevent infections in 2 ways; by activating B-cells into action (T-helper cells) and destroying the infected cells (T-killer cells). The severity of infection is inversely proportional to the strength of T cell responses.

Immunologists, Shane Crotty and Alessandro Sette at the La Jolla Institute for Immunology portrayed that specific viral protein particles would provoke strong T cell responses. In a study, 15 out of 18 COVID patients reported T helper cells targeting the spike protein of SARS-CoV-2 [6]. From the results, it is obvious that a strong T cell response will suppress SARS-CoV-2 infection further arresting its proliferation. Before these results come into the limelight, it was unclear whether T cells eliminate SARS-CoV-2 or event whether they provoke the immune system. According to Crotty, the results were encouraging as T helper cell responses against SARS-CoV-2 were well established [5].

In a study conducted at UC Davis [7], immunologists collected blood samples from 12 healthy volunteers and separated peripheral blood mononuclear cells (PBMC) including macrophages, monocytes and lymphocytes (B & T cells). Spirulina potentially induced interferon-gamma ($IFN\gamma$) and moderately induced interleukin-4 (IL-4) and interleukin beta (IL-1 β) after 72 hours of incubation.

Replication of Human Immunodeficiency Virus- 1 (HIV-1) replication in human-derived T cells and in PBMC was inhibited with Spirulina at a concentration of 5-10 mg/ml. The study concluded that even a small concentration of Spirulina is enough to reduce viral replication, however larger amounts with >100 therapeutic index, would completely arrest its replication [8].

A purified extract of Spirulina (calcium-spirulan) inhibited replication of HIV-1, herpes, human cytomegalovirus (HCMV), influenza A virus, mumps and measles virus. The extracts proved to be safe for human & monkey cells in cell culture 8.

In another study, authors demonstrated augmentation of $IFN\gamma$ & Natural Killer (NK) cell cytotoxicity in humans when given with hot-water extract of Spirulina platensis [9]. They established similar results in mice, however, $IFN\gamma$ is poorly induced

in mice compared to humans [9]. These results endorse the efficiency of Spirulina in inducing the immune system in humans.

In a double-blind controlled study, it was elicited that Spirulina extract may protect athletes from deterioration of immunity caused due to strenuous exercises and prevents [10]. The extract of Spirulina platensis triggered a significant effect on the stimulation of NK cells and their cytotoxic potential [11, 12]. A pilot randomized; comparative clinical trial conducted in Hepatitis C (HCV) patients showed a significant decrease in the viral load in Spirulina treatment group [13].

In Baby Hamster Kidney cell cultures and in baby mice, non-toxic doses of *S. platensis* (50 ug/ml) showed reductions in the type O, A and SAT2 titers of foot and mouth disease virus (FMDV) by 35.7%, 28.5% and 31% respectively [14].

Between 1979 and 1998, several animal studies majorly including mice have demonstrated enhanced immune response after administration of Spirulina extract [15-25].

From the above results, it is apparent that T cells have a strong action against SARS-CoV-2, not only suppressing the infected cell but also arresting its proliferation. The results were certain that T cells are active against various viruses like HIV-1.

In consolidation, a nutrient supplement Spirulina, an effective immunomodulator showed promising results in proliferating cell-mediated immunity and activation of T-cells; both helper & killer cells. Spirulina extract in high concentrations can inhibit & arrest viral replications completely.

In both human and animal models, Spirulina showed positive immunological results in acting against various viruses like HIV-1, HCV, Influenza, FMDV, Measles, Mumps, etc., So, there is no doubt that Spirulina can strengthen our immune system and provides good protection against various viral infections including SARS-CoV-2. Viruses may show different mechanism of actions, but the immune responses exerted by the human body is the same against all viruses. The strength of the immune response will decide the chances of getting infected and showing symptoms of the disease.

For ages, Spirulina has been a lifesaver and considered a miraculous single-cell protein with many therapeutic applications. Though, limited research was conducted on Spirulina and its immunological effects against SARS-CoV-2, the outcomes from the studies

conducted by Crotte and Thiel provides substantial evidence to the discussion.

The above investigations demand additional studies to determine these effects in the patients consuming Spirulina. The data is limited and no appropriate method was used to interpret the results. Amazingly, even secondary metabolites produced by Spirulina have exhibited therapeutic applications [26].

Immunity plays a crucial role in determining the health of a human being. A strong immune system can keep human beings away from all infectious diseases and viruses. In general, the preferential increase in IFN γ production over IL-4 would shift the immune system to mounting a cell-mediated immune response rather than a humoral response. A cell-mediated response involves activating T-cells and antibodies which work with macrophages. The moderate increase in IL-1 β secretion, a cytokine that acts on almost every cell in the body to promote inflammation, works to support the overall immune response.

COVID-19 & Cytokine Storm: When a pathogen enters into the human body, hyperactive immune system sometimes may activate destructive overacted reaction termed a cytokine storm [27]. Exaggerated immune cells spread across and start attacking healthy cells; red blood cells and white blood cells finally damaging the liver. Blood vessels may become leaky accumulating fluid in the lungs & blood clots may chock the flow of blood causing shock, organ damage and finally death. From a study of 29 severe Covid 19 patients, Wuhan physicians reported cytokine storm reporting high levels of the cytokines (pro-inflammatory) IL-2R and IL-6. High levels of IL-6 may be an early indicator for cytokine storm [27]. Another team in Wuhan analyzed 150 patients and reported that IL-6, C-Reactive Protein and ferritin were in high levels in patients died than survived [27].

Will Spirulina Neutralize the Cytokine Storm? From above results, Spirulina potentially induced IFN γ and moderately induced IL-4 and IL-1 β . Spirulina extract proved to be having anti-inflammatory effect by inhibiting histone deacetylases (HDACs) which are responsible for expression of inflammatory gene in macrophages. Spirulina extract enhances global histone H3 acetylation and degrades HDAC protein suppressing pro-inflammatory cytokines [28]. Results demonstrated that Spirulina extract decreased p65 binding and H3K9/K14 acetylation at the promoters of IL-1 β and tumor

necrosis factor α (Tnf α) thus exerting anti-inflammatory effect [28].

Adverse Events: Consumption of Spirulina may be associated with a few adverse events (AEs) related to its nutrient ingredients and conditions of its cultivation. In general, minor AEs associated including nausea, insomnia, headaches etc., [29]. It may be associated with contaminants like heavy metals, bacteria and microcystins [27]. Spirulina may help in thinning the blood and cause increased blood coagulation time [29]. Some studies do not agree that spirulina affects blood coagulation time [30, 31]. In people with food & different allergies, spirulina consumption may be fatal [32].

After consuming 2000 mg daily for 15 days with fish oil capsules, I have experienced bloating, feeling hot, stomach upset and insomnia. However, lifestyle, food and other nutrient supplements may have caused these events. Spirulina may have some detrimental effects in a few people. So, consult healthcare professional before consuming it.

Avoid [29-34]: The following people should strictly avoid spirulina consumption & suggested to consult with healthcare professionals.

- Pregnant women and children
- People with autoimmune disorders
- People with food & other allergies
- People with liver disorders
- People with phenylketonuria
- People with vitamin K deficiency

To highlight, most of the vaccines under development are focusing on different immune responses compared to Spirulina. As Spirulina extract without impurities was considered as safe to use color additive mixtures in coloring foods by the US FDA (21CFR73.530) [35]. However, Spirulina is not approved for the treatment of any disease by the US FDA and like other dietary supplements not regulated by the US FDA. So, there is no guarantee that the product is accurate in dose and free of contaminants. This review does not endorse Spirulina as an alternate therapeutic application to any vaccine or gold standard therapy for COVID 19 but strongly recommends multiple randomized & controlled studies establishing its efficacy against SARS-CoV-2 when administered as a standalone or as a combination therapy. As Spirulina enhances immune response, people with autoimmune

disorders and other underlying medical conditions should consult healthcare professional before consuming as dietary supplement.

Limitations: The review focused on research outcomes including Spirulina in proliferating immune response against various viruses. No appropriate study design & the methodology used to impact the interpretation of the findings. Limited data on the safety and efficacy of spirulina in children and pregnant women. Advised to consult healthcare professionals before consuming for any specific indication.

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