COVID-19: Potential of microalgae derived natural astaxanthin as adjunctive supplement in alleviating cytokine storm

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Abstract

The pandemic outbreak of Coronavirus disease (COVID-2019) is a potentially fatal and highly contagious disease. Given that in absence of definitive COVID-19 treatment, and the presence of asymptomatic carriers, the conventional intervention measures to curb the rate of infection and deaths will be highly challenging. Accumulating evidences suggest that excessive reactive inflammation, oxidation, and an exaggerated immune response very likely to contribute to its pathology, leads by a violent immune response cytokine storm and subsequent progression to life threatening acute respiratory distress syndrome (ARDS)/acute lung injury (ALI). Microalgae derived natural astaxanthin (nASX), a well-known potent anti-oxidant and broad-spectrum anti-inflammatory compound with impressive safety profile, is protective against cytokine storm, ALI/ARDS. This article summarizes the most likely benefits of nASX may provide as an adjunctive in attenuation of COVID-19 induced health adversaries based on its putative pathogenesis. There is rationale, pre-clinical evidences of effectiveness and evidence of safety from long-time use for other indications to justify possible inclusion of nASX as adjunctive in combination with primary anti-viral drugs therapy will hugely benefit COVID-19 patients by improving their health and reducing recovery time.

Key Words:

SARS-CoV-2, COVID-19, Astaxanthin, *Haematococcus pluvialis*, Cytokine Storm, ARDS, Sepsis, ALI, Anti-oxidant, Anti-inflammatory

1. Introduction

In December 2019, emergence of a distinctive coronavirus (CoV), later termed as Severe Acute Respiratory Syndrome (SARS-CoV-2) was determined as responsible for the outbreak of highly contagious and potentially fatal atypical pneumonia, called coronavirus disease (COVID-19) (1-2). Since its first report in Wuhan, China, the virus has rapidly spread over 200 countries all over the world by April 2020 causing over 1.75 million infected and more than 1,00,000 fatalities so far. Considering the unprecedented emerging global situation, the World Health Organization (WHO) on January 30, 2020 declared the COVID-19 outbreak as a Public Health Emergency of International Concern urging for an urgent necessity to contain the spread of SARS-CoV-2

infection to curtail on the global death toll (2). Without any definitive treatment for COVID-19 at present, repurposing antiviral therapy, corticosteroid therapy and mechanical respiratory support have been considered as currently applied measures to treat COVID-19 patients (2). Respiratory involvement, presenting as mild flu like illness to potentially lethal acute respiratory distress syndrome or fulminant pneumonia resembling that of SARS-CoV is the dominant clinical manifestation of COVID-19 (2). Like other respiratory tract infection, pre-existing comorbidities are reported to enhance vulnerability to COVID-19 patients (2). Although the pathophysiology of SARS-CoV-2 is not well studied yet, existing evidences suggest likely resembles to other SARS-CoVs infection; the acute lung injury resulting from aggressive inflammation initiated by viral replication (2, 4).

Microalgae derived natural astaxanthin (nASX) is a *keto*-carotenoid with strong antioxidant and anti-inflammatory activities known for its wide array of health-promoting and clinical benefits as nutritional and functional food supplement. Clinically, natural astaxanthin has shown diverse benefits with excellent safety and reported to block oxidative DNA damage, lowered C-reactive protein (CRP) and other inflammation biomarkers (5-6). Previous studies reported that nASX exert positive effects in alleviating cytokine storm, acute lung injury, acute respiratory syndrome, etc. (7). Herein, we aimed to review available literature and summarize underlying relevant evidences indicating that natural astaxanthin will have beneficial or supportive role as adjunctive utility in alleviating SARS-CoV-2 induced disease symptoms.

2. Clinical presentation and pathogenesis of COVID-19

SARS-CoV-2 infected symptomatic patients are reported to present mostly with upper respiratory infection symptoms included, fever, fatigue, nonproductive cough, dyspnea, myalgia, diarrhea, normal or decreased leukocyte counts, elevated C-reactive protein and lactate dehydrogenase (LDH) (2-4, 8-11). In severe progression, the disease results in radiographic evidence of pneumonia, with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), respiratory failure, sudden cardiac arrest, sepsis, etc. (2, 8-11). However, it was reported that not all people exposed to SARS-CoV-2 develop similar COVID-19 disease severity, although the exposure history was similar (2, 8-18). Patients with chronic comorbidities, compromised immunity and respiratory status were reported to be the primary driver of the COVID-19 disease severity with worse outcome of higher chances of fatality (2, 8-11, 16-18). Wang et. al (9) reported that the non-survivors when compared to the survivors began with reduced lymphocyte count 3 days after onset of the disease, followed by increasing leukocytes, neutrophil and D-dimer after 5 days before an abrupt rise in creatinine and blood urea after 9 days of disease onset (9, 11, 17). Plasma concentration of IL2, IL7, IL10, MCPI, MIP1A, and TNFa were reported higher among ICU admitted COVID-19 patients, suggest the involvement of a cytokine storm (2, 8-11). Higher lung injury was associated with lymphopenia and higher levels of C-reactive protein, neutrophil and LDH (9, 11-14). COVID-19 ARDS patients showed bilateral diffuse alveolar damage with cellular fibromyxoid exudates and presence of cytopathic-like changes in the intra-alveolar spaces (10, 17). A summary of clinical presentation of COVID-19 patients is given in Table 1.

Although the complex pathological mechanism involved in COVID-19 that produces pneumonia is not yet clearly understood, accumulating evidences from early reports on clinical and laboratory features from COVID-19 infected critically ill patients revealed the presence of a Cytokine Storm Syndrome (CSS), resulting in Acute Respiratory Distress Syndrome (ARDS), Sepsis

and multi-organ failures (2). ARDS is the common immunopathological event for SARS-CoV-2, SARS-CoV and MERS-CoV infection (13). Currently available reports suggest ARDS is the principal cause of fatality in COVID-19 (2, 8-18). One of the main mechanisms for ARDS is the cytokine storm, the deadly uncontrolled systemic inflammatory response resulting from the release of large amounts of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-8, IL-9, IL-10, IL-12, IL-18, IL-33, TNF-α, TGFβ, etc.) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, etc.) by immune effector cells in SARS-CoV infection (2, 8-23). Huang *et al*. (2) reported increased cytokine levels in 41 COVID-19 inpatients (including 13 ICU patients and 13 non ICU patients), IL-1B, IL-1RA, IL-7, IL-8, IL-9, IL-10, IFNγ, TNF-α, vascular endothelial growth factor (VEGF), granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), fibroblast growth factor (FGF), interferon-γ-inducible protein (IP10), monocyte chemoattractant protein (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), platelet derived growth factor (PDGF). Individuals with severe COVID-19 infection show elevated levels of IL-2, IL-7, IL-10, G-CSF, IP10, MCP1, MIP1A, TNFα IL-6, IFN-α, and CCL5, CXCL8, CXCL-10 in serum compared to those with mild or moderate infection, potential indicators of a cytokine storm (2, 8-14,16-18, 23). The level of IL-6 was reported to be significantly higher in non-survivors than survivors, which was confirmed in critically ill patients (18). The cytokine storm triggers a violent attack by the immune system to the body, causing acute lung injury (ALI), RNAaemia, acute cardiac injury, sepsis and multiple organ failure, and finally lead to death in severe cases of SARS-CoV-2 infection (2, 8-19).

Table 1. Demographics and Clinical Presentation of COVID-19 (17)

Demographics	Range of best estimates
Age	30-69 years
Incubation period	3 – 5 days
Transmission route	Contact, droplet, fomite & suspected aerosol
Common symptoms	Fever, fatigue, dry cough, upper airway congestion, shortness of breath, myalgia/arthralgia
Diagnosis (Biochemistry)	Lymphopenia, elevated C-reactive protein, elevated lactose dehydrogenase
Radiology	Ground glass opacity or bilateral patchy shadows
Median time from first symptoms to: (days)	
- Hospitalization	7 (4 – 8)
- ARDS	9 (8 – 14.0)
- ICU admission	10.5 (8 – 17)
Severity/critical rate	7-10%
Major complications	ARDS, arrhythmia and septic shock
Risk factor associated with poor prognosis	

-	Demographics	Older, underlying comorbidities (hypertension, diabetics, coronary heart disease, chronic obstructive pulmonary disease)
-	Clinical presentation	Presented with dyspnea, anorexia, rash, greasy fur on tongue
-	Chronological order for non-survivors	**Since onset of disease** Day 3: reduced leukocyte, lymphocyte, neutrophil count, increased D-dimer Day 5: increased leukocyte, neutrophil count Day 9 onwards: abrupt rise in creatinine and blood urea
-	Biochemistry	Increased plasma concentration of IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, TNFa, C-reactive protein, neutrophil percentage, lactate dehydrogenase, lymphopenia

Autopsy Histology

Bilateral diffuse alveolar damage with cellular fibro-myxoid exudates; pneumocyte desquamation and formation of hyaline membrane; interstitial lymphocyte-dominated mononuclear inflammatory infiltrates; multi-nucleated syncytial cells with atypically enlarged pneumocytes in intra-alveolar spaces

The cytokine release syndrome (CRS) results because of an accentuated immune response triggered by SARS-CoV-2 infection in severe COVID-19 patients, diagnosed with lymphocytopenia, a differential diagnostic criterion for COVID-19 (13, 18). The CRS caused by SARS-CoV-2 was reported to mediated by leukocytes other than T cells, which may be overcome by blocking IL-1, IL-6 and TNFα (13). Hence, destroying the immune evasion of SARS-CoV-2 is imperative in its treatment and specific drug development (8). Based on understanding of COVID-19 triggered cytokine storm, it is very important to treat the infection (with appropriate anti-viral therapy), and it may be even more critical to treat the host with appropriate therapeutic modalities to dampen the overly exuberant immune responses responsible for CSS-induced multi-organ dysfunction syndrome (MODS) and death (2, 8-11, 13-14, 16-19). This suggests a strategy of intervention of COVID-19 with Antiviral Therapy along with CSS-Targeted Therapy (13, 17).

Current understandings based on accumulated evidences suggest that SARS-CoV-2 induces a potential amplified inflammatory response to sequential consequences of ALI, ARDS to a life-threatening dire consequence of potential septic shock with elevated expression of inflammatory related genes along with inevitable secondary infections, rather than increased viral load. And if a similar, although, the most likely pathological mechanism exists in SARS-CoV-2 induce COVID-19, the attenuation of the cytokine storm by targeting key steps in the process may deliver improved outcomes (8, 13, 17-18, 21-28).

3. Treatment of COVID-19

There is no known definitive treatment for SARS-CoV-2 induced COVID-19. Supportive management with specific respiratory and ventilator supports are current mainstays of treatment. Sequential progression of respiratory compromise was the predominance of respiratory malfunction in overall clinical demise (2, 8-19, 28).

Undoubtedly, antiviral and supportive treatments are essential for patients with COVID-19 (24). As cytokine storm is relatively a very common manifestation in severe patients and often lead to exacerbation, intervention with anti-inflammation therapy may help in preventing further injury (8, 13, 17). It is therefore becoming apparent that a combination therapy approach involving an antiviral along with an effective immunotherapy may produce a more favorable outcome, and this is of intense investigation. Current treatment approaches are based on treatments for SARS-CoV infection and the ensuing chronic inflammation include anti-viral medications as well as steroidal and non-steroidal anti-inflammatories (NSAIDS), glucocorticoids', hydroxychloroquine or chloroquine, TNF-inhibitors and antibiotics. (8, 13, 15, 17-18, 24-30). Shi *et al.* (13) suggested a two-phase approach for potential treatments of COVID-19 patients: the first immune defense-based protective phase for non-severe COVID-19 cases and the second, inflammation-driven damaging phase for severe COVID-19 patients. Zhang *et al*. (30) presented a critical review on perspectives of anti-inflammatory drug treatment based on clinical and immunological characteristic of severe COVID-19 patients, stating that blocking the CS at right time followed by initiation of anti-inflammation therapy is very critical for reducing the rate of fatalities. There are multiple reports, stated that COVID-19 infectivity is likely to be higher in adult persons with chronic comorbidities due to their weaker immune function (2-4, 8-11, 13-19, 28-31). Zhou et al (18) reported that the results from treatment of COVID-19 with anti-viral such Lopinavir and Ritonavir was not obvious, longer in duration for viral shedding. Alternative treatment with nebulized inhalation of interferon along with antiviral Chinese Traditional Medicine (CTM) were reported to relived faster and effects better (17, 25-28, 30). Glycyrrhizin, the bioactive constituent of licorice root (*Glycyrrhiza glabra*), a potent anti-inflammatory, anti-oxidant and known antiviral (anti-SARS) reported to be have encouraging results (17, 28). Zhang *et al.* (31) speculated Melatonin, a proven anti-inflammatory and anti-oxidant as adjuvant for COVID-19. Overall, the prognosis and recovery from critical hyperinflammation stage of illness is very poor, and prompt recognition and application of preventive therapy may yield better results (17). A range of antioxidants as supplements will offer a window of quick recovery of patients by reducing post treatment side-effects.

While the anti-viral approaches and vaccines being considered as immediate countermeasures are unavailable and undergoing multiple clinical trials, there may be needed to consider treatment regimens from analogous disease patterns (17, 24, 29-30). Matching clinical dispositions may be considered in efforts to develop therapeutic interventions. Additionally, dire outcomes of illness may be overcome with adjunctive measures that do not necessarily cure the underlying disease (17, 28-31). Repurposing an old malaria drug chloroquine or hydroxychloroquine was reported to show potent activity against COVID-19 and used as preventive medicine to treat COVID-19 patients and medical professionals (24, 26-27, 31).

More than a supportive care, an adjunctive measure may assist critically ill COVID-19 patients health (30). Based on supportive evidences there are multiple natural compound which may have advantages to use as adjunctive countermeasures for rapid recovery of COVID-19

patients (17, 26-31). Herein, we outline the strong evidences of natural astaxanthin as a potent anti-inflammatory and anti-oxidant compound supporting its potential beneficial application as adjunctive in patients with COVID-19.

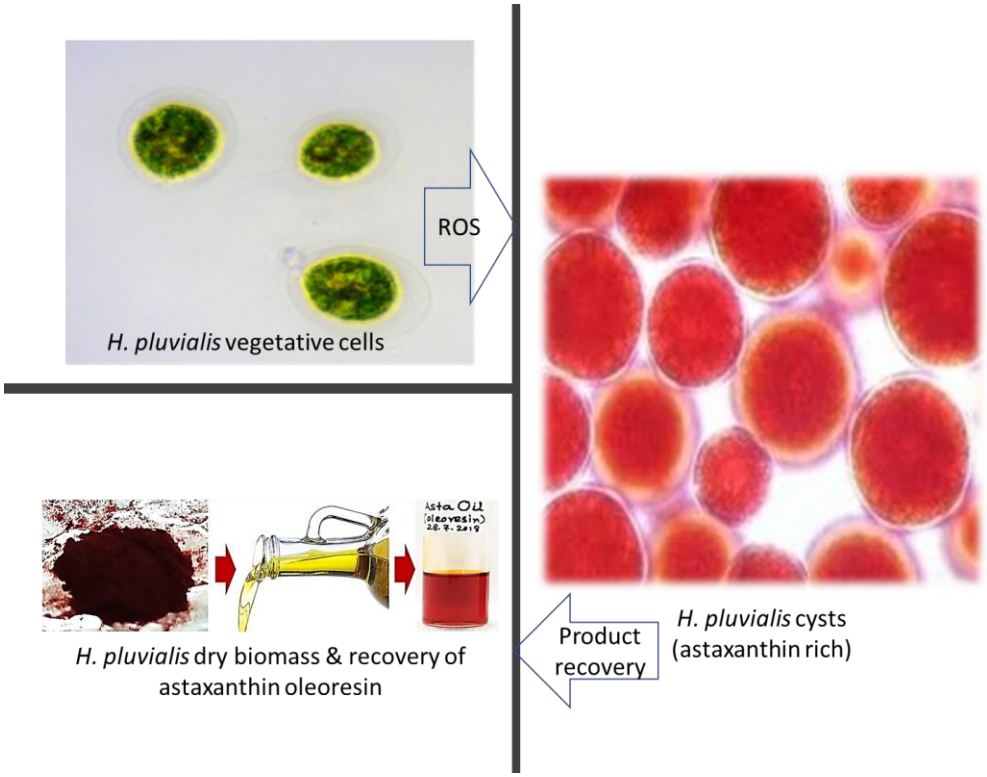

Figure 1. An outline of astaxanthin production in microalgae *H.pluvialis*. Green vegetative cells undergo morphogenetic changes in response to stress induced unfavorable condition, mainly mediated by ROS. Massive accumulation of astaxanthin is the result of its protective mechanism under unfavorable conditions, which can be easily recovered with vegetable oil. Commercially a two stage process is applied for production of astaxanthin oleoresin from microalgae *H.pluvialis.*

4. Rationale for use of astaxanthin as potential countermeasure in COVID-19 treatment

Microalgae *Haematococcus pluvialis* derived natural astaxanthin (nASX), a xanthophyll carotenoid (3,3'-two hydroxyl-4,4'-two ketone-beta, beta'-carotene) is a nutrient with unique cell membrane actions and diverse clinical benefits (5-7, 32-37, 39-56, 61-69). Previous studies reported that nASX, which the microalga *H. pluvialis* accumulated in response to ROS driven adverse conditions, can play major roles in regulating immunity and disease etiology, suggesting its wide array of potential therapeutic and nutritional supports in human health benefits including, anti-oxidant, immune-booster, anti-inflammatory, anti-aging, neuroprotective, immunomodulatory, anti-proliferative, anti-bacterial, anti-apoptotic, etc., (5-7, 32, 36, 39-49). The antioxidant activity of nASX is reported to far exceeding the existing antioxidants with ROS-scavenging capacity of 6000 times that of vitamin C, 800 times that of coenzyme Q10, 550 times that of vitamin E, 200 times that of polyphenols, 150 times that of anthocyanins, and 75 times that of α-Lipoic acid (45). Most importantly, no apparent side effects or negative results have been reported for astaxanthin (32, 39). Pharmacokinetic studies demonstrated that after ingestion of nASX (esterified), the ester bond was broken down by the hydrolytic activity of the

digestive enzyme and only the unesterified astaxanthin appears in the blood (38). Absorption into the intestinal lining cells (enterocytes) occur by passive diffusion, facilitate in presence of fat or other lipids, then incorporate in chlymomicrons, which transport it into the liver (38). The recent human studies elaborated on the safety perspectives of nASX, and so far, no documented negative effects were found over its 20 years of consumption as a dietary supplement (5-7, 32, 36-39-42). Addition of nASX as a potential adjunctive supplement along with repurposing antivirals may likely to improve effectivity and physical health of the patients.

4.1. Astaxanthin as anti-oxidant and anti-inflammatory compound

Reactive oxygen species (ROS) play a crucial role in the host inflammatory response and cytokine outbreak during virus infections, cardiovascular disease, cancer, neurodegenerative disease and diabetes, suggesting anti-oxidant as an important medicine to ROS-related diseases (33-37, 39-45). nASX with its unique molecular structure stretches through the bilayer membrane, providing resilient protection against oxidative stress (5). It can scavenge and quench ROS and free radicals (superoxide anion, hydrogen peroxide, singlet oxygen, etc.) in both the inner and outer layers of the cellular membrane unlike most antioxidants, which work either in the inner (e.g., vitamin E and β-carotene) or the outer side of the membrane (e.g., vitamin C) (5, 37, 40-46, 48). The anti-oxidative effect of nASX cooperates with its anti-inflammatory actions by up-regulating anti-oxidative enzymes (e.g. superoxide dismutase) and down-regulating pro-oxidative enzymes (e.g. nitric oxide synthetase) (7, 37, 46-52). Astaxanthin is also known to protect pancreatic beta cells by reducing oxidative stress and sugar toxicity, improve the levels adiponectin and HDL, and enhances blood flow and circulations (5).

In addition to a strong natural antioxidant, nASX also exerts broad-spectrum anti-inflammatory activities through multiple pathways (5-7, 34-36, 40, 46-55). nASX significantly attenuate pathological elevation, critical inflammatory cell signaling nuclear factor kappa-B (NF-kB) pathway both *in vitro* and *in vivo* and reduce TNF-α in humans, resulting decrease in multiple pro-inflammatory cytokine level, which may potential in maintaining lung health and minimizing the impacts of SARS-CoV-2 infection (7, 30, 46-52, 54, 56). nASX also known to significantly decreased other important mediators of inflammation in animal models, including IL-6, IL-1b, COX-2, CRP, PGE-2, iNOS, and nitric oxide (NO) (5-7, 33-35, 44-51, 55). Miyachi *et al*. (47) reported that localization of nuclear factor κB/p65 and the level of inflammatory cytokines (IL6, TNF-α) tended to decrease following treatment with astaxanthin, and significant improvement of cell proliferation *in vitro*. nASX also reported to inhibit apoptosis in alveolar epithelial cells (7, 46, 56). Additionally, to the inhibition of NF-kB pathway activation, reduction in the M1/M2 macrophage phenotype ratio is important in decreasing levels of inflammatory cytokines (48, 52).

Zhang *et al*. (46), reported that nASX exert anti-inflammatory effect through increasing Sirtuin 1 (SIRT1) and inhibiting the Toll-like receptor 4 (TLR 4) signaling pathway resulting reduction of pro-inflammatory response and secondary brain injury. TLRs are a group of pattern recognition receptors that play a critical role in the innate immune system (46). TLR4 express mainly in microglia and plays a pivotal role in triggering inflammatory response in the CNS (46). In sepsis-induced ALI, application of nASX can play a vital role in proper regulation of SIRT1 to attenuate lung injury and inflammation. Nuclear factor kappa-B (NF-κB) is closely associated with pro-inflammatory and pro-oxidative responses while being an inflammatory mediator in ALI (7,

52, 56). Previous studies reported that the anti-inflammatory effect of nASX involves the suppression of NFkB activation in ARDS (7, 47-49).

Acute inflammatory disease such as sepsis and acute lung injury (ALI) is a systemic inflammatory syndrome induced by infection and involves damage to multiple organs and tissues (7). ALI is one of the major causes of SARS-CoV-2 induced COVID-19 fatalities (2, 8-11, 57-60). ARDS is the acute condition of a COVID-19 infected patients leading to sudden breathlessness, rapid heart rate, severe hypoxia, and requiring immediate intensive care facilities. The severity is diagnosed by bilateral ling opacities on chest imaging (2, 8-11, 17, 57-60). COVID-19 patients suffering from ARDS end up with damaged alveolar walls called diffuse alveolar damage leading to serious scarcity of oxygen supply to the other organs through the blood resulting potential multiple organ failures (2, 9-10, 13, 18, 58-60).

Research efforts currently have been largely focused on innate immune system and conceptually viewed sepsis and ALI as syndrome of hyperinflammation (8, 10-19, 30-31, 57-60). Increasing evidences indicate that dysregulation of cytokines in acute inflammation is the most important step in mediating, amplifying and perpetuating the process of sepsis or ALI (7, 8, 11-14, 18, 52). Evidences from lipopolysaccharide (LPS)-induced acute lung injury and sepsis, suggest the activation of pro-inflammatory signaling pathways, including transcription factor nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB) and mitogen activated protein kinases (MAPKs), triggering the production of a variety of inflammatory cytokines (7, 47, 51-52). The excessive release of various pro-inflammatory cytokines mainly including TNF-α, IL-6, IL-1β, IL-12 and IL-8 rapidly initiate a systemic inflammatory response leading to simulation of adaptive immune response and cytokine storm resulting the acute cellular injury to form sepsis or ALI (7-8; 12-13, 17, 23, 30). These observations along with currently accumulating evidences related to COVID-19, strongly suggest that the inflammatory process involve in association with ARDS related ALI and sepsis (7, 13, 17-18, 30, 52, 57-60). This warrants that effective anti-oxidant and anti-inflammatory treatments must be given strategically to treat COVID-19 patients (13, 17, 28-30, 52, 57-60). Implications of anti-inflammatory / anti-oxidants candidates to intervene the excessive production of cytokines, including IL6 and TNF-α can be a promising strategy for the prevention and treatment of COVID-19 induced ARDS related ALI and sepsis (7-8, 13, 17, 24-30, 52, 57-60). Recently, natural ant-inflammatory compounds are being considered highly promising candidates in this strategy of intervention (17, 28, 30, 31). nASX with its proven anti-inflammatory and anti-oxidant activity backed by multiple preclinical and human trials and with its extraordinary safety profile can be one of the most promising candidates to be tried against COVID-19. Potential implications of nASX in the treatment of avian influenza virus infection was reported and stated that nASX can exhibit significant benefit in a combinatorial drug along with antiviral compound to be used for the treatment (7, 52). Although, detail clinical result is lacking at this moment, pre-clinical trials in animal model was demonstrated encouraging results. Cai *et. al*. (7) demonstrated that nASX can prevent LPS-induced ALI/ARDS and sepsis by inhibiting the activation of pro-inflammatory signaling pathway of MPAK/NF-κB. Experimental results demonstrated that nASX significantly inhibits the production IL6 and TNF-α. The results showed that treatment with nASX not only significantly reduced the death rate due to sepsis but also exerted protective effects on lung tissues. nASX treatment also decreased alveolar wall swelling and lessened the decline in the number of pulmonary alveoli in lung tissue (7).

With the absence of definitive treatment in acute COVID-19 infected patients, ARDS/ALI are leading with high mortality rate (2, 11, 17, 30, 60-63). Sepsis syndrome is the most frequent causes of ARDS, leading to increased lung permeability, enhanced polymorphonuclear neutrophil (PMN) sequestration and respiratory failure causing sudden rise in death toll, as indicated by current pandemic worldwide (11, 30, 58-60). During acute COVID-19 treatment in intensive care, high dose of vitamin C was suggested as a "rescue therapy" whenever necessary, along with high pressure flow nasal oxygen (58). Administration of anti-inflammatory substance to potentially avert the existing ARDS condition is not known (30). Taken together, we speculate that implications of nASX as adjunctive countermeasure in the treatment of COVID-19 may exert dual purpose of both as anti-oxidant and anti-inflammatory compound with beneficial outcome of reduce fatality and rapid recovery (**Figure 2**.).

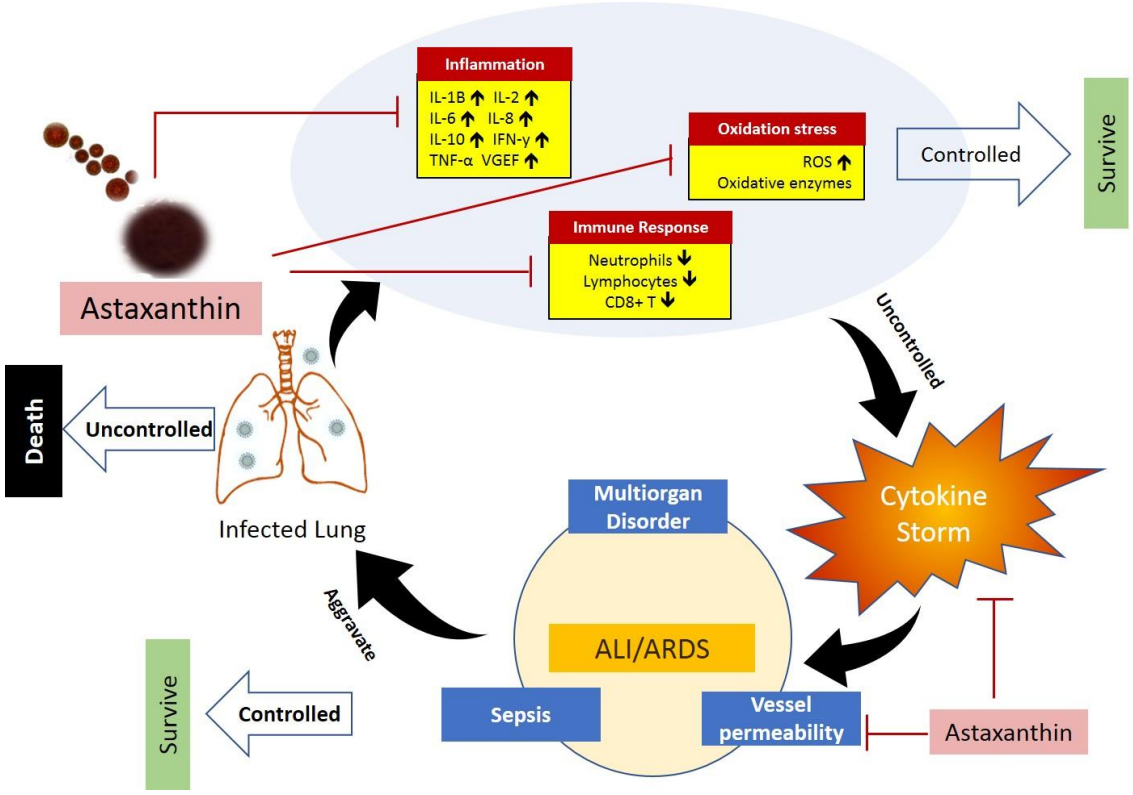

Figure 2. Pathogenesis of COVID-19 and potential adjunctive use of natural astaxanthin. We presumed that lung infected by SARS-CoV-2 elevated oxidation stress, elevated ROS mediated inflammation and a suppressed immune response proceed unabated resulting violent cytokine storm. ARDS/ALI may ensue, accompanied by series of complications, which vary according to the disease severity. Astaxanthin may play a major role in regulation of the immune response, downregulation of pro-inflammatory components and maintaining oxidation stress, resulting in alleviation of cytokine storm. Astaxanthin may also provide support for patients with ALI/ARDS and related complications.

4.2. Astaxanthin as immunomodulatory and immune booster impacts on ARDS

With the virus infection of respiratory epithelial cells, dendritic cells phagocytose the virus and present antigens to T cells. Effector T cells function by killing the infected epithelial cells, and cytotoxic CD8+ T cells produce and release pro-inflammatory cytokines which induce cell

apoptosis (12, 23). Both the pathogen and cell apoptosis trigger and amplify the host innate immune response. The exacerbation of cytokine production, excessive recruitment of immune cells and the uncontrollable epithelial cells damage generates a vicious circle for infection related to ARDS or ALI. Clinical characteristics of COVID-19 suggest a reduced level of neutrophils, lymphocytes, CD4+ T and CD8+T cells in peripheral blood indicates disease severity (8, 11-14,18, 23, 30).

nASX a potent antioxidant and anti-inflammatory carotenoid, plays a pivotal role in modulating the immune response. The immune boosting activities of nASX have been well documented and supported by multiple pre-clinical and clinical trials including human as well as animal models (6-7, 37, 46-51, 53-56). Park *et al*. (37) reported that dietary supplement of nASX was reported to stimulate mitogen-induced lymphocyte proliferation, increase natural killer cell cytotoxicity and the delayed-type hypersensitivity response, and increase the number of total T and B cells in the peripheral blood. It was reported that astaxanthin is absorbed after oral administration subsequently utilized by blood leukocyte subcellular organelles, mostly by the mitochondria (37, 61). Lin *et al.* (53) reported that nASX modulates the production of T helper 1 cytokines, such as IL-2 and IFN-γ, without causing significant cytotoxic effects in primary cultured lymphocytes. nASX exerts regulatory actions on the immune system and directly enhances the immune response by improving proliferation and maturation of natural killing cells, T and B lymphocytes, granulocytes and monocytes (23, 37, 61).

NOD-like receptor 3 (NLRP3) inflammasome is part of the innate immune response during lung infection. The pathogen, including a virus (CoVs have not yet been tested), triggers NLRP3 activation to amplify the inflammation. There is probably a balance of the protective and damaging actions of NLRP3 in the lung. Gao *et al*. (54) demonstrated that nASX can attenuate iohexol-induced human proximal renal tubular epithelial cells injury *via* the mechanism related to the inhibition of ROS production and down-regulation of NLRP3 inflammasome and its downstream apoptosis and inflammatory response. The experimental results confirmed that pretreatment with nASX can inhibit the overexpression of ROS in HK2 cells, thereby inhibiting the NLRP3 inflammasome and its downstream apoptosis and inflammatory response (54).

Immunomodulation by natural bioactive compound can provide additional therapeutic support to conventional chemotherapy for a range of diseases including COVID-19, especially when selective immunosuppression is needed for autoimmune disorders. There are several diseases where immunostimulatory drugs are needed to overcome the immunosuppression induced by drugs or environmental factors, and immunosuppressants are required when there is undesired immunopotentiation. Moreover, drugs that can improve the immune system are needed to quell the immunosuppressive effects produced by stress and chronic diseases, and in situations where immune responsiveness is impaired. Multiple experimental results demonstrated the role of nASX in enhancing immune response (6, 37, 61-64). Using a human model, Park *et al.* (37) reported that dietary nASX regulate immune response, protect oxidative damage and inflammation simultaneously. nASX was reported to enhanced both cell-mediated and humoral immune responses. Significant increase of immune markers including T cell and B cell mitogen-induced lymphocyte proliferation, IFN-γ and IL-6 production, and LFA-1 expression were reported (37). Notable, dramatic decreased of DNA damage biomarker (plasma 8-OHdG), along with significant reduction of plasma C-reactive protein concentrations were reported (37).

4.3. Astaxanthin effects in cytokine levels and ALI

As of now there is no report on the use of nASX in COVID-19 patients, however, available data suggest potential implications of nASX in relation to the treatment of human infection by pathogenic Avian Influenza Virus (52). Available publications on potential use of nASX in the treatment with the subject related to other disease-causing systemic inflammation, ROS burst, etc., reported to exert promising results in attenuation of circulating cytokines levels (7, 40, 44, 46, 48, 51-54, 56). Cai *et al.* (7) demonstrated the potential use of nASX in the treatment of clinical sepsis and reported that treatment with nASX exhibited significant protection against ALI. nASX exhibited its protection against ALI mostly through:

i. inhibition of LPS- induced secretion of pro-inflammatory cytokine,
ii. inhibition of TNF-α and IL-6 secretion,
iii. protective effects against lung injury, repressed alveolar wall swelling and attenuated the decline in the number of pulmonary alveolar,
iv. inhibition of MAPK/NF-kB signaling pathway,
v. significantly suppressed the degradation of IκB-α and phosphorylation of ERK1/2, P38 and JNK in sepsis and ALI, attenuation of ALI,
vi. significant inhibition of bronchial alveolar lavage fluid (BALF) in ALI, suppressed lung edema,
vii. significant decrease in Myeloperoxidase, (MPO) activity,
viii. reduction of macrophage infiltration evidenced by reduced CD38 expression
ix. anti-inflammatory and anti-ALI activities.

Sepsis and ALI, which lack an effective clinical therapy is characterized as the most common cause of death in acute COVID-19 patients worldwide (2, 10-11, 30, 60). Steroidal (e.g. Corticosteroids) and nonsteroidal anti-inflammatory drugs (NSAIDs) were reported to be either therapeutically ineffective or exacerbate symptoms in the treatment of sepsis and ALI in acute COVID-19 patients (30, 57-59). Application of nASX can be a potential approach for the treatment and prevention of septic shock or ALI and its associated diseases can be the intervention of the NF-kB mediated inflammatory response (7, 30, 52, 57-60).

4.4. Astaxanthin and other supportive effects

nASX is considered as a broad-spectrum bioactive compound with its myriad of health beneficial activities exerts prudently through multiple pathways. Besides its potent anti-oxidant, reactive oxygen species (ROS), immunomodulatory and ant-inflammatory activities, nASX is also reported as potential neuroprotective. In this regard, and mainly due to its capability to cross blood-brain barrier, nASX has gained growing interest as a multi-target pharmacological agent against neurological disorders including Parkinson's disease, Alzheimer's disease, brain and spinal cord injuries, neuropathic pain, aging, depression, and autism (61-64). Potential antiviral activities of nASX against influenza virus and hepatitis C infection were also reported (52, 65-66).

Wang *et al*. (67) demonstrated the anti-lung fibrosis functionalities of astaxanthin *in vivo* and *in vitro*. Xu *et al* (68) reported that astaxanthin protect lung injury from ochratoxin induced inflammation and oxidative damage via regulating Nrf2 and NF-kB pathway. Lung fibrosis is associated with inflammation characterized by the recruitment of macrophages, neutrophils and lymphocytes in the airways. Available data suggest that ROS, such as superoxide, hydrogen peroxide (H_2O_2), peroxy-nitrite and hydroxyl radical are major mediators of lung inflammatory

processes triggering apoptosis in the alveolar epithelial cells type-II (AEC-II). *In vivo* and *in vitro* studies revealed that nASX alleviates renal fibrosis (69) and potential therapeutic agent in cardiovascular disease (40, 70).

Integrity of vascular endothelial barrier is crucial in the immunoregulation within alveoli. Severe inflammation and immune responses induce epithelial and endothelial cell apoptosis, as well as increasing the production of VEGF, which aggravates edema and the extravasation of immune cells from blood vessels. Experimental evidence suggests that astaxanthin potentially mediates the suppression of VEGF in vascular endothelial cells (71). nASX also reported to has therapeutic and prophylactic potential in the airway inflammatory response associated with chronic obstructive pulmonary disease (COPD). Published reports suggest that nASX may support with preventive measures against COVID-19 induced renal injury, septic cardiomyopathy, and liver injury.

4.5. Astaxanthin safety aspects

nASX is proven to be safe for human consumption, orally bioavailable, and a natural bio-active compound notified generally recognized as safe (GRAS) and approved by the United States Food and Drug Administration (USFDA) for human consumptions in dosages up to 12 mg per day and up to 24 mg per day (32, 36, 39).

5. Conclusion

In view of the current global scenario, an efficient intervention strategy with decisive countermeasures are pivotal to curb COVID-19 deaths worldwide. Clinical manifestations of COVID-19 include fever, cough, fatigue, muscle pain, diarrhea, and pneumonia. Respiratory supportive along with anti-viral therapies are the mainstream of treatments for severe cases. As disease progression to acute stage induced by CS in severe cases, leads to hyperinflammatory ARDS/ALI, Septic shock and multiple organ failures, to potential fatalities. This suggest a definitive anti-viral treatment along with a timely supportive anti-inflammatory therapy at the right window time is pivotal importance to contain the disease status advancing to acute condition and potential fatality. However, at present a definitive COVI-19 intervention strategy is lacking altogether with a specific anti-SARS-CoV-2 drug.

The myriad of potential therapeutic and health beneficial effects of natural astaxanthin as anti-inflammatory, anti-oxidative, immune booster and immunomodulator, were repeatedly demonstrated against respiratory disorder models induced by infections and associated complications. These evidences may suggest a likely beneficial implication of microalgae sourced natural astaxanthin in SARS-CoV-2 infected individual. Its potential beneficial role in COVID-19 patients is yet to be explored, may considered for further studies as adjunctive supplement with specific beneficial activity against CS among others. There is rationale, pre-clinical evidences of effectiveness and evidences of safety related to its long-term use as dietary and therapeutic supplement for multiple health indications to justify its possible benefits and may necessary to include for clinical research in patients with COVID-19.

Declaration competing interest

None

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