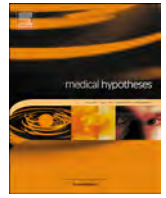




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The effects of allium sativum on immunity within the scope of COVID-19 infection



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ABSTRACT

The severity of coronavirus disease 2019 (COVID-19) infection is quite variable and the manifestations varies from asymptomatic disease to severe acute respiratory infection. Fever, dry cough, dyspnea, myalgia, fatigue, loss of appetite, olfactory and gustatory dysfunctions are the most prevalent general symptoms. Decreased immune system cells such as suppressed regulatory T cells, cytotoxic and helper T cells, natural killer cells, monocytes/macrophages and increased proinflammatory cytokines are the characteristic features. Compounds derived from *Allium sativum* (garlic) have the potential to decrease the expression of proinflammatory cytokines and to reverse the immunological abnormalities to more acceptable levels. *Allium sativum* is suggested as a beneficial preventive measure before being infected with SARS-CoV-2 virus.

Allium sativum is a functional food well-known for its immunomodulatory, antimicrobial, antiinflammatory, antimutagenic, antitumor properties. Its antiviral efficiency was also demonstrated. Some constituents of this plant were found to be active against protozoan parasites. Within this context, it appears to reverse most immune system dysfunctions observed in patients with COVID-19 infection. The relations among immune system parameters, leptin, leptin receptor, adenosin mono phosphate-activated protein kinase, peroxisome proliferator activated receptor-gamma have also been interpreted. Leptin's role in boosting proinflammatory cytokines and in appetite decreasing suggest the possible beneficial effect of decreasing the concentration of this proinflammatory adipose tissue hormone in relieving some symptoms detected during COVID-19 infection.

In conclusion, *Allium sativum* may be an acceptable preventive measure against COVID-19 infection to boost immune system cells and to repress the production and secretion of proinflammatory cytokines as well as an adipose tissue derived hormone leptin having the proinflammatory nature.

Introduction

Coronavirus disease 2019 (COVID-19), is related to a RNA virus of the coronavirus family, severe acute respiratory syndrome coronavirus (SARS-CoV), which is renamed as SARS-CoV-2 [1,2]. The severity of COVID-19 infection is quite variable and the manifestations varies from asymptomatic disease to severe acute respiratory infection. Persons with obesity are also at high risk due to chronic diseases associated with obesity. Quarantine in obese subjects should likely be longer than normal weight individuals [3]. On the other hand, obese people already avoid social contact and experience high rates of depression. Health care providers are expected to fight obesity more than ever. The COVID-19 pandemic and the obesity epidemic threat the world in unprecedented ways [4]. This pandemic disease has also destroyed the lives of children and their families [5]. World Health Organization

declares that although all age groups are at risk of COVID-19, older people face significant risk of developing severe illness, due to physiological changes, especially in immune system, associated with ageing and presence of chronic diseases [6].

At the first instance, fever, dry cough, respiratory distress, myalgia, fatigue and loss of appetite are the most prevalent general symptoms. Most of patients have reported olfactory and gustatory dysfunctions. The anosmia or ageusia are also recognized as important symptoms of the COVID-19 infection. In critical patients, at least one of the following three criteria may be observed: respiratory failure, septic shock, multiple organ failure [7–11]. The relationships among symptoms and consequences are shown in Fig. 1.

The profiles of some immune cells in COVID-19 have also been clarified. Decreased regulatory T (Treg) cells, cytotoxic and helper T cells, natural killer (NK) cells, monocytes/macrophages have been

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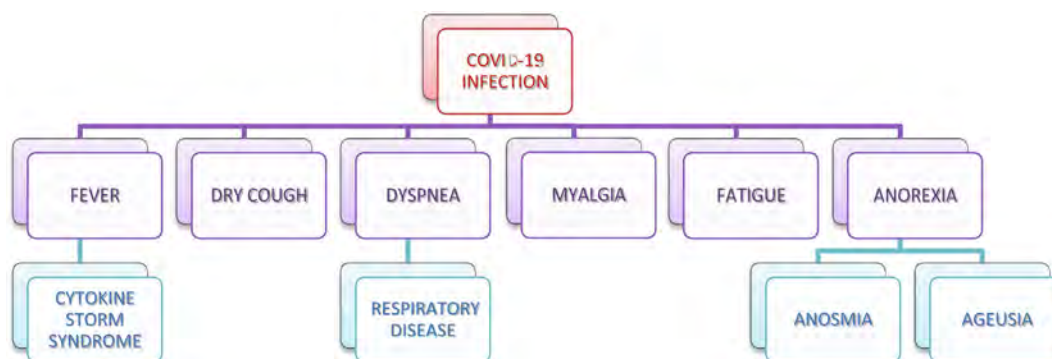


Fig. 1. Signs and general symptoms of COVID-19 infection.

pointed out. On the other hand, proinflammatory cytokines such tumor necrosis factor-alpha (TNF- α), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interferon gamma (IFN- γ), as well as leptin are among those exhibiting an increasing tendency [12–14].

The beneficial effects of *Allium sativum* (garlic) on health have been emphasized for centuries. Garlic contains numerous compounds that have the potential to influence immunity [15,16]. In recent reports, garlic and its complex constituents have been investigated as promising candidates for improving immune system. Garlic extracts and compounds isolated were examined in terms of their immunoregulatory functions in detail. It is a well-known fact that immune dysfunction plays an important role in the development and progress of several diseases and this functional food may contribute to the prevention and treatment of pathologies such as obesity, metabolic syndrome, cardiovascular disorders, gastric ulcer, and even cancer [17,18]. Aged garlic extract (AGE) might be used as herbal medicine with few side effects as compared to chemotherapy in treating cancers caused by substances like aflatoxin B1 [19].

Garlic participates in cytokine secretion modulation, which may provide a mechanism of action for many of its therapeutic effects. Alliin is the main organosulfur compound in garlic and has been shown to induce a decrease in the expression of proinflammatory cytokines [17,18].

COVID-19 infection is of a fast-spreading and easy-transmitting disease properties. The world is in the need of pharmacological treatment protocols including wide variety of medicines to cure patients. The investigations on the development of vaccine are underway. There are some suggestions towards increasing hemoglobin production and increasing hemoglobin availability for oxygen binding [20]. It is also hypothesized that patients with severe COVID-19 infection may have a severely compromised immune response and could be treated by monalizumab, interferon α , chloroquine, and other antiviral agents [21]. In the current emergency state, there are some suggestions related to the use of all available therapeutic tools and the potential anti-retroviral activity of hesperidin as well as routine as co-treatment or as preventive treatment in patients with COVID-19 infection [22]. Today, it is well understood that prevention of this infection will be much more plausible than treatment to get rid of the outbreak of this epidemic disease. *Allium sativum* seems to counteract most of the negativities caused by COVID-19 infection. Within the scope of preventive measures, this functional food may prevent this viral agent from spreading over the body. We suggest that administration of this plant will contribute to the immune system elements during the fight against this pathogen.

The hypothesis

T cell immunity plays important roles in chronic inflammatory diseases such as obesity and asthma. Regulatory T cells exhibit anti-inflammatory nature. Decreased numbers of Treg cells observed in

COVID-19 infection might represent a challenge of these cells. The helper T cells (CD4 + T cells) and the cytotoxic T cells (CD8 + T cells) are two major types of T cells. Helper T cells 'help' other cells of the immune system, while cytotoxic T cells kill virally infected cells and tumours. Both are important soldiers of the immune system. Natural killer cells are a group of immune cells possessing cytolytic activity against virus-infected and tumor cells [23–26].

In patients with COVID-19 infection, suppression of these cells were noted. In severe cases, they are even much lower. As one of the results of this profile, a reduction in IFN- γ was also observed [12–14].

Garlic supplementation causes significant increases in CD4 + and CD8 + cells. This plant also stimulates NK cells [18,27]. Decreased leptin, leptin receptor, peroxisome proliferator activated receptor-gamma (PPAR- γ) and IL-6 concentrations were also detected as the other beneficial immunological and hormonal effects of the garlic [17,28,29].

Leptin leads to appetite decrease [30]. Decreased leptin concentrations caused by garlic may be helpful to alleviate appetite loss observed in patients with COVID-19 infection.

When all these findings including antimicrobial and antiviral features of garlic were evaluated together, our hypothesis is that it is plausible to consider this plant as a preventive measure to alleviate harmful effects of the disease.

Evaluation of the hypothesis

The recently emerged coronavirus mainly act on lymphocytes. The SARS-CoV-2 infection affects primarily T lymphocytes particularly CD4 + T and CD8 + T cells, resulting in decrease in IFN- γ production. Absolute number of T lymphocytes, CD4 + T and CD8 + T cells decreased in nearly all the patients, and were markedly lower in severe cases than moderate cases. Patients with COVID-19 exhibit lower level of Treg cells, and more obviously damaged in severe cases. Natural killer cells decreased in COVID-19 patients, and severe cases had a lower level than mild cases. These immunological markers may be of importance due to their correlation with disease severity in COVID-19 [12–14]. Fig. 2 shows alterations observed during COVID-19 infection.

On the other hand, significant increases in CD4 + and CD8 + T cells were observed following a short-term garlic extract supplementation. This finding showed that using garlic extract supplementation promoted cellular immune system of karate athletes [27].

Alliin is the major biologically active component of garlic and shows anti-microbial activity. In recently performed studies, it was also reported that this functional food also combats against malaria parasite, tumor growth and cytomegalovirus [15,31–34]. Allicin is also active against protozoan parasites including Plasmodium, which is thought to be mediated by inhibiting cysteine proteases. It was demonstrated that allicin reduced parasitaemia in malaria. The absolute numbers of CD4 + T cells, dendritic cells (DC) and macrophages were significantly higher in allicin-treated mice with malaria infection [31]. In a recent



Fig. 2. Some elements in immunological profiles of patients with COVID-19 infection.

report, the use of antimalarial drugs have been recommended for prophylaxis and treatment of high-risk individuals with SARS-CoV-2 infection [32]. Allitridin (Allyl trisulfide) can promote cytomegalovirus (CMV)-induced Treg expansion and Treg-mediated anti-CMV immunosuppression. Therefore, allitridin may be useful as a therapeutic agent fighting against CMV [34].

Garlic has antiinflammatory, antimutagenic and antitumor properties. Intra-tumor injection of a protein fraction purified from fresh garlic bulbs significantly increased CD8 + T lymphocytes in the peripheral blood, augmented CD8 + T cell infiltration into the tumor site, decreased tumor size and inhibited tumor growth in experimental studies [15,33].

Garlic seems to increase the immune system functions. It stimulates macrophages, lymphocytes, NK cells, DC and eosinophils, by mechanisms including modulation of cytokine secretion, immunoglobulin synthesis, phagocytosis and macrophage activation [18]. After 45 days of AGE consumption, $\gamma\delta$ -T and NK cells proliferated better and were more activated than cells of the placebo group [16].

CD4 + T cell and total white blood cell count were significantly increased in garlic treated rats. This showed the immune system boosting capability of garlic [35].

In a study, salad lowered participants' desire to eat and caused satiety compared to the garlic bread. Dieters were less hungry after the salad compared to the garlic bread [36]. This data showed the appetite increasing effect of garlic.

It has been reported decreases in gene expression and serum protein levels of the adipocytokines leptin and resistin, as well as decreased serum IL-6 concentrations. Treatment with alliin reduces metaflammation markers in diet induced obesity mice and improves some metabolic parameters without affecting others [17]. The effects of *Allium sativum* on various systems are shown in Fig. 3.

Aged garlic extract suppresses the production of proinflammatory cytokines such as TNF- α and CRP in the liver [37]. In the hypothalamus, aged black garlic (ABG) treatment induced a decrease in leptin receptor (LepR) mRNA levels. In subcutaneous adipose tissue, ABG treatment decreased adipose weight and downregulated the gene expression of PPAR- γ and LepR. In brown adipose tissue, PPAR- γ mRNA levels were significantly decreased in ABG-treated rats [28]. Leptin activates adenosin mono phosphate-activated protein kinase (AMPK) and activated AMPK inhibits PPAR- γ transcriptional activity in hepatoma cells [29]. Peroxisome proliferator activated receptor- γ is also suggested to be involved in the treatment protocols of obesity and depression [38].

Leptin secreted from adipose tissue stimulates AMPK in the liver. AMPK phosphorylates acetyl CoA carboxylase. The phosphorylation



Fig. 3. The effects of garlic (*Allium sativum*) upon biological and immunological components of the systems.

inactivates this enzyme, which converts acetyl CoA to malonyl CoA. Inhibition of this reaction prevents the synthesis of fatty acids. The increase in AMPK activity stimulates fatty acid oxidation enzymes and leads to reduced triacylglycerols. Depending upon these effects, fatty acid synthesis will be inhibited and fatty acid oxidation (FAO) will take action. This leads to reductions in triacylglycerol (TAG) levels [39]. The physiological, biochemical and immunological effects of leptin are shown in Fig. 4.

On the other hand, leptin stimulates Th1 cells and at the same time, inhibits CD4⁺ CD25⁺ FoxP3⁺, Treg cells, which inhibit the activity of Th1 cells. As known Th1-type cytokines produce proinflammatory responses.

Consequences of the hypothesis and discussion

Garlic is a functional food, whose antimicrobial effect was known for a long time [15]. In recent years, the studies emphasizing its immunomodulatory effect are being introduced. During historical periods, in various cultures, it was used to increase the taste of the meals. The appetite increasing effect of garlic is being reported [36]. There are investigations on which it increases the numbers of Treg cells; CD4⁺ CD25⁺ FoxP3 [19].

Coronavirus disease 2019 is a novel disease caused by a new and a fully aggressive version of coronavirus. Since December 2019, numerous publications giving information related to patients with COVID-19 are being introduced. In these publications, symptoms such as anosmia and ageusia as well as complete loss of these senses are being reported. Besides, severe reductions in the number of Treg cells as well as cytotoxic and helper T cells were also observed in these patients. Upon evaluation of some proinflammatory cytokine levels, in addition to IL-1 and IL-6, an increase was recorded in the levels of leptin, the best known and the most investigated adipokine, with proinflammatory properties [1,7–14].

Leptin is an anorexigenic hormone, which commands hypothalamic neurons not to eat and to metabolize more by way of alpha-melanocyte stimulating hormone. Leptin, by increasing proopiomelanocortin (POMC) and cocaine amphetamine regulated transcript expression, and at the same time, decreasing agouti related peptide (AgRP) and

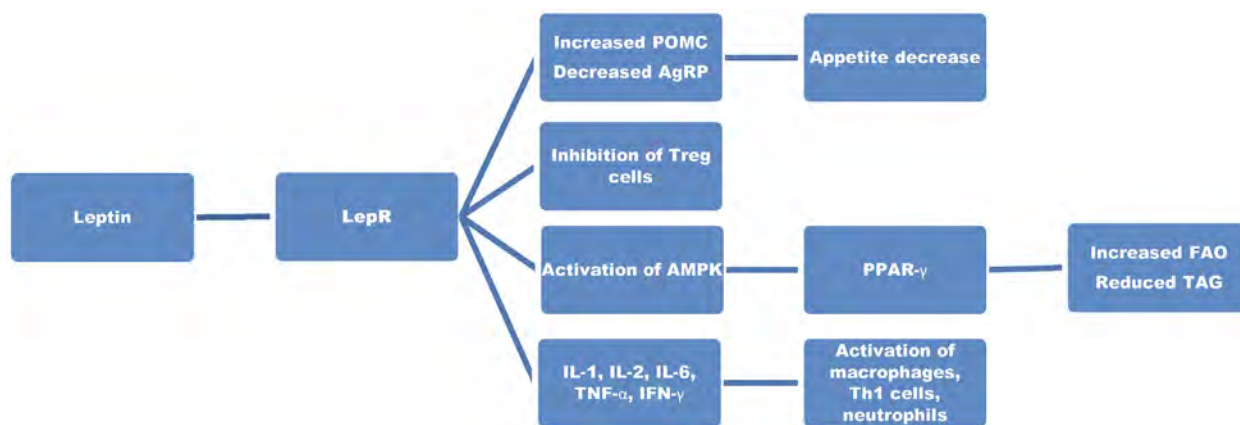


Fig. 4. Leptin, hypothalamic leptin receptor and associated processes.

neuropeptide Y expression, causes appetite decrease. Increase of POMC expression by way of α -melanocyte stimulating hormone and decrease of AgRP expression lead to activation of melanocortin (MC4) receptors and decrease of MC4 receptor inhibition, respectively, which in turn, both, cause appetite decrease. Garlic decreases leptin by decreasing LepR. It is also reported that garlic increases appetite [17,36,39,40].

The investigations related to the developmental trials of the vaccine, which may require a long period of time are still in progress. Isolation and social distancing are primary interventions [3]. However, under the light of these findings, garlic may also be proposed during the prevention of COVID-19.

Garlic was known as a powerful antibacterial agent for years, and also has been shown to be beneficial in cancer and hypertension therapies [15,31,34]. It is known that both cancer and hypertension are also the main morbidity and mortality risk factors for COVID-19 infection. In this viral infection, most of the immune-compromised patients have losted their lives during the medical treatments in intensive care units because of bacterial sepsis exhibiting high C-reactive protein levels, septic shock and multiorgan failures. This information concerning garlic has a special importance on this issue.

This food may be helpful 1. to reverse some signs and symptoms observed in these patients, 2. to reincrease or regain the decreased or lost gustatory sense, 3. to increase the number of Treg cells, 4. to increase cytotoxic and helper T cells, 5. to decrease the levels of leptin, leptin receptor and PPAR- γ 6. to increase appetite, 7. to prevent the inhibition of CD4⁺ CD25⁺ FoxP3⁺, Treg cells, 8. to decrease IL-6 concentrations, 9. to stimulate NK cells, 10. to suppress TNF- α and C-reactive protein.

Immuno-compromised clinical status is life-threatening for patients with COVID-19 infection, therefore, these beneficial effects of *Allium sativum* on immune system are quite important.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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