



INTERGOVERNMENTAL INSTITUTION FOR THE USE OF MICRO-ALGAE SPIRULINA AGAINST MALNUTRITION

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IN SUPPORT OF THE UNITED NATIONS MILLENNIUM DEVELOPMENT GOALS & THE POST 2015 DEVELOPMENT AGENDA

IIMSAM'S STRATEGIC PARTNER HASHBIOTECH'S SCIENTIFIC TEAM IN INDIA REVIEW PAPER TO SYNTHESIZED THE INTERNATIONAL COMMUNITY ON THE BENEFITS OF INCLUDING SPIRULINA AND PHYCOCYANIN IN THE FIGHT AGAINST EBOLA VIRUS IN WEST AFRICA. (LIBERIA, GUINEA, SIERRA LEONE , NIGERIA)



(Picture from left to right) Dr. Satyam Agrawal, Dr. Madhunika Agrawal and the Hashbiotech Scientific R&D Team at the IIMSAM/Hashbiotech Spirulina Cultivation Farms and Scientific Research Facilities, Punjab-India.

<<The paper was prepared after reviewing almost two hundred International Journals, some of them are in the reference section, explicitly written on Impact of Spirulina & Phycocyanin on the treatment, prevention of Ebola or Similar Viruses, their impact, death causing factors, spreading factors, spreading mechanism, treatments, cure, prevention, clinical trials, in-vivo & in-vitro studies etc. The paper is written in a simplest possible way to make it understandable for everyone>>

FIGHT AGAINST EBOLA VIRUS WITH SPIRULINA AND PHYCOCYANIN:

Review Paper Released on 25 September 2014

Ebola virus disease (EVD), also known as Ebola haemorrhagic fever is caused by the Ebola Virus (Ebov). Ebola Virus is an enveloped virus consisting of a single stranded RNA (ssRNA) belonging to the Filoviridae family. Ebola Virus infection is highly lethal and leads to severe immune suppression. It is a rare and deadly virus that causes hemorrhagic fever and bleeding both inside and outside the body which proves to be fatal to the infected individuals. As the virus spreads through the body, it damages organs and makes the body prone to other invading infections. Ultimately, it renders the body extremely weak and the levels of blood-clotting factors severely drop thereby causing uncontrollable bleeding. Mortality due to EVD, as per WHO, has been calculated to be somewhere between seventy to ninety percent! Because of its high mortality rate, EBOV has been listed as a select agent in the World Health Organization Risk Group 4 Pathogen (requiring Biosafety Level 4-equivalent containment); a U.S. National Institutes of Health/National Institute of Allergy and Infectious Diseases Category; a Priority Pathogen; U.S. CDC Centers for Disease Control and Prevention Category; a Bioterrorism Agent, and listed as a Biological Agent for Export Control by the Australia Group.

ROLE OF PHYCOCYANIN ENRICHED SPIRULINA IN EBOLA VIRUS: Ebola virus causes an acute, serious illness which is often fatal if untreated. As the virus spreads through the body, it damages the immune system and vital organs. Early symptoms include fever, sore-throat, muscle-pain and headaches followed by vomiting, diarrhea and rash. It causes levels of blood-clotting to drop. This leads to severe, uncontrollable bleeding. There is no specific treatment available for the disease yet.

Patients are treated by supportive care-rehydration with oral or intravenous fluids- and treatment of specific symptoms is done which improves survival. However, mother nature offers a potential anti-EboV compound Cyanovirin-N (CVN) found in the blue green algae SPIRULINA. Cyanovirin-N, an 11 kDa peptide present in Spirulina, is a novel cyanobacterial carbohydrate-binding protein shows a significant antiviral activity against enveloped viruses such as Ebola. Spirulina not only provides the anti-viral compound but also helps in rebuilding the body's strength providing the required complete nutrition and further Phycocyanin, a vital protein, helps counter all other EVD adjoining auto-immune degenerations in the body by its super anti-inflammatory, anti-oxidant and other various medicinal virtues. The minimum recommended dose, as gathered from various credible journals, to keep away as well as counter the deadly Ebola Virus is:

PHYCOCYANIN: 6 TABLETS A DAY OF 200mg EACH I.E 1.2 GRAMS SPIRULINA: 6 TABLETS A DAY of 500 mg EACH I.E.3 GRAMS

As inferred from various research papers and articles, this dose if taken regularly may be beneficial in case of Ebola Virus infection by remediating and preventing the downstream consequences of the infection that otherwise often prove to be fatal. It may also be inferred that the above regular dose may also be able to provide protection against Ebola infection by strengthening general body and arm the immune system with CVN- the potential anti-Ebola virus soldier.

SPIRULINA FOR COMBATING EBOLA

In the world where there is no specific cure for Ebola virus so far, Spirulina and Phycocyanin by exhibiting their outstanding functions may prove to be a great savior for the Ebola infected patients. CVN (Cyanovirin-N), present in Spirulina has been studied by various researchers for its anti-viral action on most encapsulated viruses like HIV, Ebola etc. The action involves CV-N binding to N- linked high-mannose oligosaccharides on the viral glycoprotein rendering it ineffective.

CVN (Cyanovirin-N), a protein with a highly complicated structure, binds to sugars attached to the cover of the Virus, envelopes its proteins and prevents it from binding to the mucosal cell surfaces. This interaction inhibits binding of virus to cell and reduces their ability to further infect healthy cells. This compound has been studied by scientists to be active against almost all encapsulated viruses like HIV, Influenza and even Ebola. This antiviral protein can help the Ebola- infected individuals to over the infection and thus creating a hope against fatalities.

There is currently no specific or licensed medicine for treatment of Ebola virus disease (EVD). "CV-N found in Spirulina platensis is the first molecule known to inhibit Ebola infection by interfering with the virus' ability to enter cells" says the scientist who studied the effects of Spirulina on Ebola in-vivo on animal models. It can therefore be concluded that Spirulina and its vital proteins would be extremely beneficial for prevention of the infection and may aid in treatment of Ebola – preventing the downstream consequences of the infection that often proves to be fatal. While the CV-N protein is not currently available in pure form, Spirulina itself along with its derived vital proteins may offer the same antiviral protection in addition to numerous other health benefits. Spirulina platensis has been successfully studied for its anti viral properties especially in case of encapsulated viruses. This antiviral activity, in a large part, is attributable to the richness of S. platensis in vital proteins like Phycocyanin and Cyanovirin-N. These proteins contained in Spirulina have the ability to slow down the progression of viruses, including HIV and Ebola, by reducing their ability to infect healthy cells. Primary mechanism of action of Cyanovirin-N seems to occur through specific binding with N-Linked mannose oligosaccharides on glycoproteins of viral surface. Ebola Virus Disease causes the patient to exhibit vomiting and diarrhea, it impairs kidney and liver function, and in severe cases internal and external bleeding may occur (e.g. oozing from the gums, blood in the stools etc.) During this period of time, the body gets weakened and has already lost its nutrition. Spirulina provides nutrition to the body so that body cells can easily revive. Spirulina contains unusually high amounts of protein, containing all essential amino acids. It provides all the essential vitamins, minerals which the human body uses as fuel or as catalyst. Spirulina is immuno- stimulating. It acts directly on myeloid lineages and either directly or indirectly on Lymphocytes, Macrophages, Hematopoietic stem cells and NK cells. It also stimulates the production of Interferon gamma (IFN- γ) and other.

Its bioactive proteins stimulate the intestinal immune system and enhance the responsiveness to vaccines. Antioxidants like Vitamin E, SOD, Phycocyanin and β -carotene have potent antioxidant activity to scavenge free radicals, including alkoxyl, hydroxyl and peroxy radicals. Polysaccharides of Spirulina improve the immune system to combat against severe infections. It is a rich source of GLA, which has a specific effect on the endocrine system, helping restore hormone, health and aiding in normalizing insulin activity. Spirulina is having natural iodine and Selenium, which nourishes the thyroid, protects all glandular tissues and ultimately supports both immune and metabolic function. Along with all the above benefits it is highly digestible!

MODE OF ACTION OF CYANOVIRIN-N ON EBOLA

Cyanovirin-N (CVN), a cyanobacterial lectin, is a potent 'viral entry inhibitor' currently under development as a microbicide against a broad spectrum of enveloped viruses. CV-N was originally identified as a highly active anti-HIV agent and later, as a virucidal agent against other unrelated enveloped viruses such as Ebola, and possibly other viruses. CVN's antiviral activity appears to involve unique recognition of N-linked high-mannose oligosaccharides, Man-8 and Man-9, on the viral surface glycoproteins. Due to its distinct mode of action and opportunities for harnessing the associated interaction for therapeutic intervention, a substantial amount of research on CV-N has accumulated since its discovery in 1997. Initial results of several researches have revealed that CV-N had both in vitro and in vivo antiviral activity against the Ebola virus. Addition of CV-N to the cell culture medium at the time of infection showed inhibited development of viral cytopathic effects (CPEs) i.e. it showed no damage done to cell which is usually done when the Virus replicates inside the cell and the breaks open the cell to release the replicated viruses; this indicates no further spread of the Ebola virus.

In simple words, CV-N inhibits HIV and Ebola infection by binding to the outside of the virus and physically blocking it from entering healthy cells. The protein attaches to a particular sugar molecule on the virus surface making it ineffective. In yet another research report, Cyanovirin-N (CV-N) has been shown to inhibit Ebola Zaire virus (EboZV) infection, both in vitro and in vivo, through its ability to bind to oligomannoses-8/9 on the EboZV surface glycoprotein (GP). Evidence is provided showing that CV-N can effectively inhibit DC-SIGN-mediated EboZV infection. These reports suggest use of CV-N as a potential molecule for blocking viruses that show carbohydrate-dependent interactions at viral entry. In addition, study reinforces that CV-N acts uniquely to prevent essential interactions between the envelope glycoprotein and target cell receptors and further supports the potential broad utility of CV-N as a microbicide to prevent the transmission of HIV and Ebola.

PHYCOCYANIN FOR EBOLA

After transmission to a new host, the ebola virus enters a cell. Once inside the host cell's cytoplasm, the ebola virus uncoats itself and releases transcriptase, which is contained in the virion. Transcriptase transcribes the viral -ssRNA into the complimentary +ssRNA. This positive, single stranded RNA will then be used as the template for the new viral genomes. Soon after the infection, the cell develops cytoplasmic inclusion bodies that contain the highly structured viral nucleocapsid (the nucleocapsid contains the genome and can sometimes have other proteins in it as well). After the nucleocapsid has been formed, the new virus will self-assemble and bud from the cell membrane stealing some of the membrane for its envelope. Ebola attacks cells important to the function of lymphatic tissues. It can be found in fibroblastic reticular cells (FRC) among the loose connective tissue under the skin and in the FRC conduit (FRCC) in lymph nodes. This allows the virus to rapidly enter the blood and leads to disruption of lymphocyte homing at high endothelial venules (HEV). Ebola virus seems to be most active in infecting fibroblasts of any type (especially fibroblastic reticular cells).

The next most frequent cell types are mononuclear phagocytes with dendritic cells more affected than monocytes or macrophages. Endothelial cells become infected after the connective tissue surrounding them is fully involved. Then, epithelial cells of any type are infected. In general, epithelial cells become infected only if they contact other cells that amplify the virus such as fibroblastic reticular cells (FRC) and mononuclear cells. This would be true for skin appendages like hair follicles and sweat glands because they are heavily vascularized and have a lot of FRC networks associated with them. Liver cells and adrenal gland epithelial cells have fibroblastic reticulum as their main connective tissue and both have resident mononuclear cell phagocytes hanging on FRC cells near the blood/epithelial cell interface.

Phycocyanin inhibits the transmission of affected cell to another healthy cell. Studies have shown that Phycocyanin inhibits Ebola Zaire virus (EboZV) infection, both in vitro and in vivo, through its ability to bind to oligomannoses-8/9 on the Ebola Virus surface glycoprotein (GP). Here, we report the in vitro potency of Phycocyanin to inhibit Ebola Virus GP from mediating gene transduction. Therefore, if it is not able to bind the cell then it may not transfer to other healthy cells.

The highly destructive Ebola virus weakens the whole immune system and causes many kinds of additional infections and auto-responses in the body. Sepsis, a potentially fatal whole-body inflammation caused by severe infection, has been known to be one such infection associated with Ebola. Further, though sepsis is triggered by gram negative bacteria and Ebola by an RNA virus, these both have similar pathogenic mechanisms involving heavy release of pro-inflammatory cytokines from macrophages, circulatory shock, vascular hyper-permeability with edema, and disseminated intravascular coagulation. Phycocyanin aids the immune system in the patients suffering from ebola virus as:

- ☒ **Phycocyanin assists the stem cells found in bone marrow, which produce white blood cells that make up the cellular immune system and red blood cells that oxygenate the body.**
- ☒ **Phycocyanin emulates the affect of the hormone erythropoetin, (EPO), which is produced by healthy kidneys and regulates bone marrow stem cell production of red blood cells (Kozlenko and Henson, 1998).**
- ☒ **Phycocyanin enhances secretory IgA antibody response and suppresses allergic IgE antibody response in mice immunized with antigen-entrapped biodegradable microparticles (Nemoto-Kawamura et. al., 2004).**
- ☒ **The particular types of immune cells involved in cancer control, cytotoxic T lymphocytes and NK cells, function more effectively with increased intakes of Phycocyanin (Arias et. al., 2011).**

The Ebola virus is normally transmitted by direct contact with infected body fluids or skin/mucus membrane contact. Once inside the body, the virus attacks macrophages and monocytes, relying upon host antibodies and complement component 1 for efficient infection. The white blood cells respond by releasing large amounts of pro-inflammatory cytokines that increase permeability of the vascular endothelium, which facilitates easier entry into the virus's secondary targets, endothelial cells. These cytokines also recruit more macrophages to the area, maximizing the number of cells that Ebola can use to spread throughout the body. In such a situation Phycocyanin may act as an anti inflammatory molecule which further inhibits transmission of infected cells by different ways as it may Scavenge free radicals, including alkoxy, hydroxyl and peroxy radicals, Suppresses inducible nitric oxide synthase (iNOS) expression, Inhibits liver microsomal lipid peroxidation, Inhibit proinflammatory cytokine formation, such as TNF α , Suppresses cyclooxygenase-2 (COX-2) expression, Decreases prostaglandin E(2) production, Reduce myeloperoxidase production and Decreases nitrite production thereby relieving inflammation and assisting the body to over the infection without damaging the body's cells and organs.

On being infected by Ebola Virus, hepatocytes are being destroyed by the virus, ensuring that these cell signals cannot be cleared from the bloodstream. Thus to respond to a weak immune response and to counter the above mentioned additional infections as well as severe body responses to infections, the vitalizing protein 'Phycocyanin' is highly recommended. Phycocyanin acts as a powerful anti-oxidant inhibits the reactive oxygen species as well as scavenges free radicals without altering liver functions.

Phycocyanin reduces lipid peroxidation and helps in maintaining structural organisation of liver. In addition, Phycocyanin is a selective COX-2 inhibitor and prevents the prostaglandins synthesis, which is the major precursor of inflammation in body. Hence, Phycocyanin may acts as a **potent hepato-protective molecule**.

After infection by Ebola Virus, signature of Ebola virus infection i.e. the presence of the virus's double-stranded RNA, when detected by immune system proteins, triggers a full immune response. Phycocyanin helps to induce this immune response in the patients suffering from ebola virus, since the virus can hide its molecular signatures using its VP35 protein and can suppress immune responses and thus replicate unchecked. Meanwhile, the immune system is going haywire. Interferons are not being made, white blood cells are trapped inside the circulatory system because sGP limits their movement, macrophages and monocytes are releasing a cocktail of proinflammatory cytokines that destroy the vascular endothelium, but also activate the coagulation cascade. This puts the human body in a paradoxical state in which the patient can die of hypovolemic shock from massive hemorrhage, or from catastrophic thrombosis, the formation of blood clots around the body. Antioxidants present in Phycocyanin interact with and neutralize free radicals, thus preventing them from causing damage to cellular structure. It is also beneficial in preventing lipid-peroxidation caused by them and hence prevents oxidative stress associated injuries to the organs. Phycocyanin is also a powerful anti-oxidant which removes all the intoxications from the body. In addition, antioxidant therapy is vital in scavenging free radicals and ROS preventing degeneration in post-oxidative stress scenario.

Therefore, there may be concluded that Phycocyanin itself may become an effective treatment for ebola infection. Scientists are optimistic that this knowledge eventually may lead to useful therapies. Meanwhile consumption of both Spirulina and Phycocyanin could not only help prevent Ebola virus attack but may also assist in fighting back the infection with a healthy body. This therapy may support the infected individual to over the infection without causing too much damage or without pushing it towards fatality. This information has been concluded from various research papers that have successfully reported the natural protective ability of super-food Spirulina and the vital protein Phycocyanin without causing any side effects. The natural origin and no known side effects indicate that this therapy may prove highly beneficial as indicated by numerous researched beyond doubts.

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