

Immunostimulatory Properties of Common *Chlorella* (*Chlorella vulgaris*) Solutions

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Abstract This study reports the isolation and characterization of various strains of *Chlorella vulgaris* obtained from plant-based sources. Based on the outcomes of preliminary analyses, a comprehensive experimental framework was developed to evaluate the therapeutic potential of natural biologically active solutions derived from *Chlorella vulgaris*. The investigation focused on elucidating the mechanisms underlying the quantitative biological activity of these phyto-genic solutions. Special emphasis was placed on their immunomodulatory properties and their effectiveness in mitigating immunodeficiency conditions in animals. The experimental results suggest that bioactive compounds present in *Chlorella vulgaris* exhibit promising immunotherapeutic effects, thereby offering a natural and sustainable alternative for enhancing immune responses in veterinary contexts. The findings contribute to the growing body of research on algae-based medicinal agents and their role in the development of plant-derived immunostimulants.

Keywords Biostimulant, *Chlorella*, Beneficial properties, Composition, Caloric content, Antigen, Immunostimulant, Immunocorrector, Serum, Algae, Blood cells, Monocytes, Lymphocytes

1. Relevance

Chlorella is a freshwater microalga with an emerald-green color. It was first studied after World War II as an alternative protein source for the population. *Chlorella* shares many similarities in composition and properties with *Spirulina*, but contains higher amounts of vitamins and minerals. Its caloric content ranges from 250 to 400 kcal per 100 g, depending on the form in which it is consumed (e.g., powder, tablets, etc.). The plant contains up to 70% high-quality protein (by dry weight), comprising all nine essential amino acids. Moreover, *Chlorella* is a rich source of vitamin C, vitamin B12, and iron—covering 6% to 40% of the recommended daily intake of this mineral. Like other algae, it also provides omega-3 fatty acids and antioxidants that support disease prevention.

The rich composition of *Chlorella* has been shown to reduce the toxicity of heavy metals in the brain, liver, and kidneys. Its components neutralize many highly toxic substances found in food, including dioxins, which are known to disrupt hormonal function. In one study, *Chlorella fusca* was shown to neutralize up to 90% of bisphenol A (BPA), a chemical commonly leached from plastic food containers. *Chlorella* contains a wide range of antioxidants, including vitamin C, β -carotene, chlorophyll, and lycopene, which reduce the

formation of advanced glycation end products (AGEs)—compounds that contribute to inflammation, chronic diseases, and diabetic complications.

Algal supplements have been found to enhance antioxidant defense in chronic smokers and individuals at high risk for cancer. Supplementation also improves liver function in patients with various hepatic disorders, leading to a reduction in enzyme levels that are harmful to the liver. However, the benefits for healthy individuals remain unclear. In healthy subjects, *Chlorella* intake increases the production of antibodies that help combat foreign agents, enhancing immune defense. Nevertheless, one study indicated that the supplement stimulated immune responses in men and women aged 50–55, but showed no effect in individuals older than 55.

Regardless of the form of intake, *Chlorella* has been shown to reduce levels of low-density lipoprotein (LDL) cholesterol and triglycerides in individuals with hypertension. Scientists suggest that this improvement is due to a combination of beneficial compounds, including dietary fiber, antioxidants, and vitamin B3. *Chlorella* supplements support cardiovascular and renal health, both of which are essential for maintaining normal blood pressure. They also contribute to reduced arterial stiffness. Researchers hypothesize that nutrients such as omega-3 fatty acids, potassium, calcium, and the amino acid arginine may play a role in preventing arterial hardening. Furthermore, *Chlorella* intake lowers blood glucose concentrations in individuals at high risk of diabetes and enhances insulin sensitivity, particularly in those with liver conditions [16,17,41,44].

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Received: Aug. 1, 2025; Accepted: Aug. 20, 2025; Published: Aug. 22, 2025
Published online at <http://journal.sapub.org/ijvmb>

Studies conducted in Kyoto, Japan, demonstrated that *Chlorella* contributes to weight reduction by regulating gene expression and facilitating the conversion of genetic information from DNA nucleotide sequences into functional products such as RNA or proteins. This process leads to a reduction in fat accumulation. Researchers also identified that the genes influenced by *Chlorella* are involved in signaling pathways, lipid transport, glucose and insulin uptake. Among them are genes associated with metabolism, signaling, receptors, transporters, and cytokines, particularly those involved in fat and insulin metabolic pathways. Given the distinct gene expression profiles related to glucose uptake, it is likely that *Chlorella* activates insulin signaling pathways, which may explain its blood sugar-lowering effects [22,23,24,42].

Scientists currently advise caution in placing excessive

hope in dietary supplements (DS) and do not recommend replacing conventional treatments with them. However, they acknowledge the potential effectiveness of such supplements when used in combination with pharmaceutical therapies. Certain components of this superfood, particularly antioxidants, have been shown to reduce inflammation in respiratory diseases, including asthma. These compounds improve the antioxidant status of patients with chronic pulmonary conditions but have not been shown to significantly restore respiratory function. Antioxidants such as lutein and zeaxanthin protect the eyes from strain and fatigue, and they reduce the risk of age-related macular degeneration. Lutein is a potent carotenoid synthesized in dark leafy green plants. It protects the eyes from harmful blue light emitted by digital devices such as smartphones and computers [37,38,39].



Figure 1

Table 1. Chlorella Composition, Properties, and Health Effects

Parameter / Aspect	Details
Type and Appearance	Freshwater microalga, emerald-green color
Historical Background	Studied after WWII as alternative protein source
Comparison with Spirulina	Similar composition, but higher in vitamins and minerals
Caloric Content	250–400 kcal per 100 g (varies by form: powder, tablets, etc.)
Protein Content	Up to 70% high-quality protein (dry weight), contains all 9 essential amino acids
Key Vitamins and Minerals	Rich in vitamin C, vitamin B12, iron (6–40% RDI)
Other Nutrients	Omega-3 fatty acids, antioxidants
Heavy Metal Detoxification	Reduces toxicity in brain, liver, kidneys; neutralizes dioxins
Chemical Detoxification	Neutralizes up to 90% of bisphenol A (BPA)
Antioxidant Content	Vitamin C, β -carotene, chlorophyll, lycopene; reduces AGEs formation
Effects on Smokers and Cancer Risk	Enhances antioxidant defense, reduces oxidative stress
Liver Health	Improves function, lowers harmful enzyme levels
Immune System Effects	Increases antibodies in healthy individuals; limited effect over age 55
Cardiovascular Effects	Lowers LDL cholesterol and triglycerides, supports heart and kidney health, reduces arterial stiffness
Blood Glucose Control	Lowers blood sugar, enhances insulin sensitivity
Weight Management	Regulates gene expression, reduces fat accumulation, affects lipid/glucose metabolism genes

According to unofficial data, *Chlorella* may alleviate symptoms of premenstrual syndrome (PMS). Researchers studying the composition of the algae suggest that B vitamins and calcium are primarily responsible for the observed positive effects on women's health. Additionally, the iron, folic acid, and vitamin B12 contained in the plant may help lower high blood pressure and provide other benefits during pregnancy. These conclusions are supported by studies involving 32 and 70 pregnant women, respectively. Participants reported fewer signs of hypertension and a lower incidence of anemia [40,43,46].

The implementation of the Presidential Decree of the Republic of Uzbekistan dated March 28, 2019, No. PF-5696 "On Measures for the Fundamental Improvement of the Public Administration System in the Field of Veterinary Medicine and Animal Husbandry" and the Resolution of the Cabinet of Ministers of the Republic of Uzbekistan dated November 11, 2017, No. 905 "On the Approval of the General Technical Regulation on the Safety of Veterinary Medicinal Products and Feed Additives" has established that veterinary medicinal products include ready-to-use chemical-pharmaceutical and biological preparations, as well as feed additives.

In light of the above, the development of a technology for obtaining biologically active ingredients from *Chlorella vulgaris*, a naturally derived preparation, can be considered an environmentally friendly solution. Organizing its industrial-scale cultivation offers opportunities for the real economy to produce new technologies and competitive, eco-friendly biostimulants. The use of *Chlorella*-based feed has been shown to increase milk yield by 15%, meat production by 25%, and poultry egg output by 20%. It also promotes increased body biomass in young cattle, thereby enhancing the efficiency of agricultural development in the Republic of Uzbekistan.

Research Goals and Objectives. The main objective of this research is to investigate the mechanisms of action of bioactive compounds extracted from *Chlorella vulgaris* on the immune system and to evaluate their effects on hematopoiesis. Additionally, the study aims to induce secondary immunodeficiency disorders in laboratory rats and to explore treatment methods using specific compounds derived from *Chlorella vulgaris*. The research also includes studying, via localized hemolysis methods, the formation of antibody-producing cells (APCs) in experimental animals and monitoring quantitative changes in blood cell components as a result of serum solution exposure.

Scientific Novelty. This study focuses on the development of a technological approach for extracting natural medicinal bioactive substances derived from *Chlorella vulgaris*. A model of secondary immunodeficiency was induced in rats through disease, followed by treatment with various doses of the extracted bioactive compounds to assess their effects. The study involved monitoring changes in erythro- and leukopoiesis (red and white blood cell formation) in response to the natural medicinal compositions extracted from different

strains of *Chlorella vulgaris*. Based on the obtained results, it is proposed that these natural medicinal solutions may be applied to develop quantitative mechanisms of action and therapeutic strategies for treating various immunodeficiency conditions in animals.

2. Research Methods

The study employed the identification of antibody-forming cells in the spleen. These cells were detected using the local hemolysis in agarose method, as described by Nordin and Jerne (1963). A model of secondary immunodeficiency was established using toxic hepatitis. Rats were administered a 20% solution of carbon tetrachloride (CCl₄) at a dose of 0.2 mL for three consecutive days. On the final day, immunization with sheep red blood cells (SRBCs) was performed, followed by a repeated intraperitoneal injection of CCl₄ to verify model consistency. The model effectively induced secondary immunodeficiency.

Sheep erythrocytes (SRBCs) were used as antigens for immunization. The study hypothesized that the number of antibody-forming cells (AFCs) in the spleen and the mean count of hematopoietic cells could be statistically analyzed to assess the effects of treatment. The reliability of the results was set at a 95% confidence level. Red and white blood cells were counted using standard hematological methods and evaluated microscopically with a Goryaev chamber.

3. Results and Discussion

The activity of natural medicinal bioactive compounds derived from *Chlorella vulgaris* was found to play a significant role in restoring immune function. Therefore, the effects of *Chlorella vulgaris*-based preparations on the immune system were investigated experimentally. Additionally, the study included *Timalin*, a thymus-derived peptide medication, as a comparison.

The experimental groups were organized as follows:

- **Group I** – Intact (healthy control);
- **Group II** – CCl₄ control (immunodeficient model);
- **Group III** – CCl₄ + Timalin;
- **Group IV** – CCl₄ + *Chlorella vulgaris* (0,1%);
- **Group V** – CCl₄ + *Chlorella vulgaris* (0,5%);
- **Group VI** – CCl₄ + *Chlorella vulgaris* (1,0%).

All animals were housed under identical conditions with temperatures maintained at 20–28°C and humidity at 50%, in separate cages under constant monitoring. No significant external changes were observed, although rats in the CCl₄-treated groups showed reduced appetite and skin changes. The animals treated with *Chlorella vulgaris* received therapeutic doses for five consecutive days. Following treatment, the number of AFCs in the spleen was quantified, and the immunostimulatory effects of the preparations were evaluated.

Experimental Results:

- In the intact control group, the average number of AFCs in the spleen was (**7378 ± 503**).
- In the immunodeficient (CCl₄-only) control group, the number dropped significantly to (**1032 ± 203**), a **6.1-fold decrease** ($p < 0.05$), confirming the severity of the induced immunodeficiency.
- In the group treated with **Timalin**, the AFC count was (**5241 ± 637**), reflecting a **5.0-fold increase**.
- In the group receiving 0.1% *Chlorella* extract, the AFC count was (1303 ± 385), a 1.1-fold increase.
- The **0.5% *Chlorella* group** showed an AFC count of (**6150 ± 687**), representing a **4.5-fold increase** ($p < 0.05$).

- The **1.0% *Chlorella* group** demonstrated the highest immune response, with an AFC count of (**8052 ± 673**), a **6.1-fold increase** (see Table 2).

In addition to assessing the immunomodulatory properties of *Chlorella*, the study also examined its effects on erythropoiesis and leukopoiesis. In intact (control) animals, the leukocyte count averaged **5.8 × 10³ cells/μL**, while in immunodeficient animals administered CCl₄, this value decreased to **3.4 × 10³ cells/μL**. In the group treated with **Timalin**, leukocyte levels recovered to **4.1 × 10³ cells/μL**. The group that received *Chlorella*-based compounds demonstrated a restoration of leukocyte count to **4.7 × 10³ cells/μL**. No significant changes were observed in erythrocyte counts.

Table 2. Effects of *Chlorella*-Based Preparations on Immune and Hematopoietic Parameters

No.	Experimental Group	NCSC (million, M ± m)	Index (i/s)	AFC (M ± m)	Index (i/s)	Splenocyte Count (×10 ⁶ cells, M ± m)	Index (i/s)
1	Intact (<i>control</i>)	143 ± 16	-	7378 ± 503	-	36.0 ± 4.0	-
2	Control (CCl ₄)	93 ± 0.8	-1.6	1032 ± 203*	-6.1	6.4 ± 1.5*	-5.6
3	Immunodeficient (CCl ₄) + <i>Timalin</i>	120 ± 13	+1.3	5241 ± 637*	+5.0	24.0 ± 2.1*	+3.7
4	Immunodeficient (CCl ₄) + <i>Chlorella vulgaris</i> (0.1%)	127 ± 10	+1.4	1303 ± 385	+1.1	6.5 ± 1.9	+1.0
5	Immunodeficient (CCl ₄) + <i>Chlorella vulgaris</i> (0.5%)	105 ± 11	+1.1	6150 ± 687*	+4.5	26.0 ± 2.4*	+4.1
6	Immunodeficient (CCl ₄) + <i>Chlorella vulgaris</i> (1.0%)	121 ± 12	+1.3	8052 ± 673**	+6.1	35.2 ± 3.7**	+5.5

Notes: * Significant difference compared to Group 1 ($p < 0.05$).
 ** Significant difference compared to Group 2 ($p < 0.05$).
 i/s – index of ratio (relative change vs. Group 1).
 “-” or “+” indicates decrease or increase, respectively
 NCSC: Nucleated spleen cells.
 AFC: Antibody-forming cells.

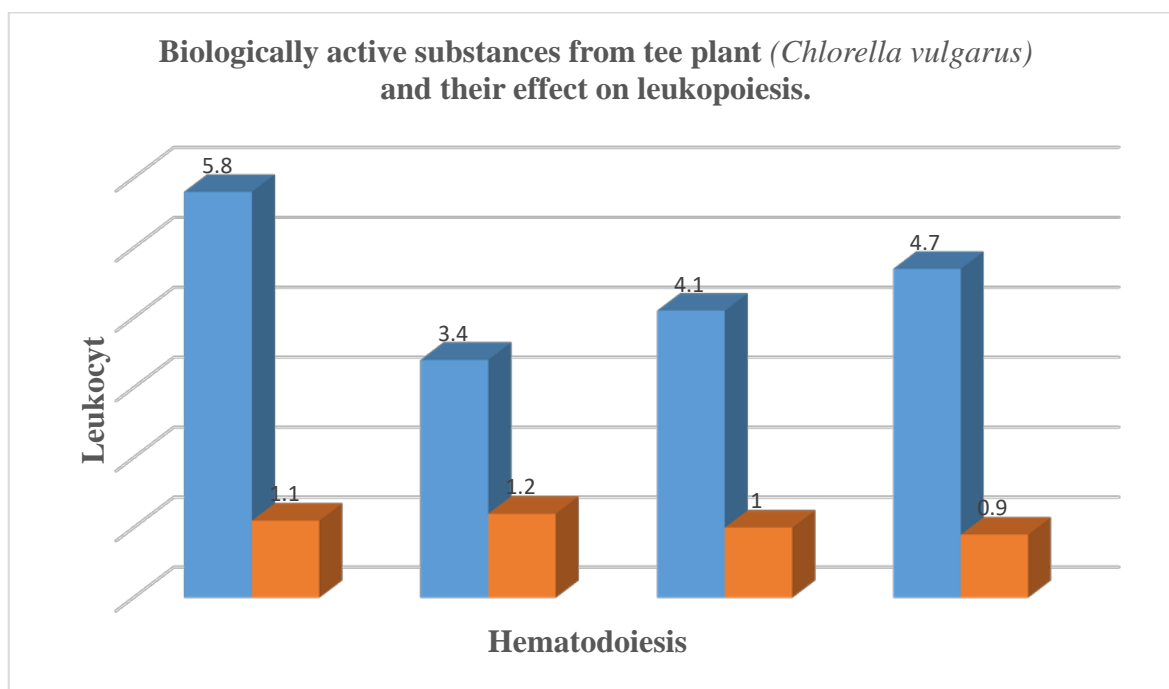


Figure 2. Further Investigation of the Effects of *Chlorella*-Based Preparations on Erythropoiesis and Leukopoiesis



Figure 3

The study evaluated the immunological and hematopoietic impact of a natural medicinal bioactive substance derived from *Chlorella vulgaris*. Secondary immunodeficiency was induced in laboratory rats through intraperitoneal injections of a 20% carbon tetrachloride (CCl₄) solution (0.2 mL) over three consecutive days. The number of antibody-forming cells (AFCs) in the spleen was measured to assess immune function.

Natural immune-regulating agents, including **Timalin** (a thymus-derived peptide), and plant-based bioactive substances from *Chlorella vulgaris* were compared in terms of their therapeutic efficacy. Experiments were conducted under controlled humidity and temperature conditions, with animals housed in individual cages.

Throughout the study, no marked external changes were observed in the animals' appearance. However, immunodeficient animals displayed decreased appetite and skin alterations. Animals receiving the *Chlorella*-based bioactive compound were treated for five days with various dosages. The resulting number of AFCs in the spleen and the intensity of immune response were then evaluated.

According to the experimental data (see Table 2):

- In the intact control group, the mean number of AFCs

was (**7378 ± 503**).

- In the immunodeficient control group (CCl₄-only), this value decreased drastically to (**1032 ± 203**) — a **6.1-fold decrease** ($p < 0.05$), indicating severe immunodeficiency.
- In the group treated with **Timalin**, AFC levels increased to (**5241 ± 637**)—a **5.0-fold increase**.
- The **0.5% *Chlorella vulgaris*** extract group showed an increase to (**6150 ± 687**) AFCs—**4.5 times higher** than the immunodeficient group ($p < 0.05$).
- The **1.0% *Chlorella vulgaris*** extract group demonstrated the highest immune response, reaching (**8052 ± 673**) AFCs—a **6.1-fold increase**, nearly equivalent to intact controls ($p < 0.05$).
- In the course of investigating the effect of *Chlorella vulgaris* on leukopoiesis and erythropoiesis, a notable restoration in leukocyte levels was observed:
 - Intact animals: **5.8 × 10³ cells/μL**
 - Immunodeficient animals (CCl₄): **3.4 × 10³ cells/μL**
 - Timalin group: **4.1 × 10³ cells/μL**
 - *Chlorella* group: **4.7 × 10³ cells/μL**

The treatments had minimal effect on erythrocyte counts.

4. Conclusions

The results of this study demonstrate that natural medicinal bioactive substances derived from *Chlorella vulgaris* exhibit immunostimulatory properties and can contribute to the restoration of leukocyte counts in immunodeficient states. The evidence suggests that these compounds support immune system recovery and hematopoiesis, making them a promising candidate for therapeutic applications in veterinary medicine and possibly beyond.

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