# Nutrition Bio-Shield Superfood: Healthy and Live Herbal Supplement for Immune System Enhancement

Azam Bayat, Aref Khalkhali, Ali Reza Mahjoub

Abstract—Healthy and viable herbal supplement were prepared from wheat by a green route. This organic biomaterial was named Nutrition Bio-shield Superfood (NBS). The NBS supplement had various vitamins, macro and micro molecules, and ingredients. In this study, 20 small Balb/C labile specimens were used in a weighing 30  $\pm$  5 range. The samples were randomly divided into different groups, then the groups were divided into 5 groups. According to the results of this study, the mean number of white blood cells and neutrophil percentage in the experimental group receiving healthy and live dietary supplement showed a significant increase at the 5% probability level in all three groups received 50, 100 and 150 mg/ kg body weight of the mouse compared to the control group. In general, the dietary supplement increases the level of immunity.

Keywords-Healthy and live herbal supplement, biomaterial, immune system, enhancement.

#### I. INTRODUCTION

THE entry or spread of a pathogenic infectious agent into L the human or animal body is called infection. The word infection also implies that the body responds in part to an invasion of the pathogen, and this can be either an immune response (evidence may not be easily obtained) or a disease. Microbial agents are one of the most common pathogens in humans today. In order to inhibit and eliminate them, antibiotics must be used that each acts with different mechanisms [1]. On the other hand, pathogenic bacteria have been resistant to specific antibiotics after a while, so research and discovery should be continued in order to discover new antibiotics [2].

Pathogenic microbial theory states that some diseases are caused by microorganisms. The growth, development and reproduction of these microorganisms can lead to disease. The microorganisms that cause the disease are called the pathogen and their category of diseases is called infectious diseases. Even when a pathogen causes illness, factors such as the environment, genetics, and the severity of the disease and whether it affects a person often affect the condition. This theory was expressed following theories such as Miasma Theory (air pollution) to explain the causes of infectious diseases in the 19th century. Experiments on discovering the relationship between germs and infectious diseases were carried out by Louis Pasteur and another French scientist

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between 1860 and 1864 [3]. With the discovery and pathology of microorganisms that cause Puerperal and Vibrio Pyogenic fever in the blood, he concluded that boric acid could be used to kill these microorganisms. Robert Berkey and another scientist German played an important role in shaping the theory of pathogenic microbes. According to Koch's theory [18], to prove that microorganism is the cause of the disease:

- The pathogenic microorganisms should be found to be 1 high in all living organisms affected by the disease, but should not be found in healthy species.
- 2. The microorganisms causing the disease must be isolated from a patient organism and can be grown in pure culture.
- 3. When a similar living creature becomes infected to the microorganisms of the culprit, it must become ill.
- 4. Disease-causing microorganisms isolated from the patient's organism should be the same with the primary cultured disease-causing microorganisms.

Although this theory contains some fundamental shortcomings, it has played a significant role in the development of the pathogens microbes' theory. The first extensive explanation for the new use of disinfectants in surgical operations was published by Joseph Lister in 1867. It was adapted from the theory of pathogenic microbes expressed by Louis Pasteur and published in an essay called the principles of disinfection in surgery [4]. In this article it was stated that the use of substances such as phenol can kill many microbes and disinfect the surgical site.

Nowadays, natural compounds play a very important role in medical and pharmaceutical sciences, and the role of natural compounds of plant origin is very important in recent years. It seems that the presence of phenolic and flavonoid compounds in their constituents makes them very different from other natural compounds [5]. The use of plant compounds for the treatment of diseases is an old way in many parts of the world, especially developed countries. In the current century, due to the increased complications of chemical drugs and more bacterial resistance to these substances, there is a lot of attention to medicinal plants with antimicrobial properties that can eliminate common problems with antibiotic use.

Considering the mentioned issues and the increasing prevalence of antibiotic resistance, this study was conducted to evaluate the effect of a type of healthy and live dietary supplement on the increase of immunity level in rat mice. For this purpose, healthy and viable food supplement was synthesized by a green route. This organic biomaterial was named NBS. The NBS food supplement has various vitamins, macro and micro molecules, and ingredients such as B1, B2, B3, B5, B6, B9, C, K, A, E, D, phosphorus, potassium, sulfur,

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magnesium, calcium, boron, iron, manganese, zinc, copper, omega-3, omega-6, omega-9 etc.

### II. METHODOLOGY

In this study, 20 small Balb/C laboratory specimens were used at a weight of  $30 \pm 5$  from Knowledge-Based Company of Green Drug Researchers. The samples were randomly divided into different groups. The samples were placed in lab conditions for 1 week to adapt to the laboratory environment. During the maintenance period of light and darkness, the rats were exposed to 12 hours of humidity and sufficient light at a temperature of 30-25 °C. Feeding the mice was done with standard food without any restrictions on access to water and food. Then, the groups were divided into 4 groups in the following sections.

- Control group: These groups were used without any injections of extract and were only used to receive sheep red blood cells (SRBC), and compare with the treatment groups. In total, they only SRBC received 10 milliliters for 10 days.
- Treatment group 1: The mice in this group were the same as the control group receiving SRBC, but with the addition of SRBC, treated rats received the NBS fresh and healthy dietary supplement in the form of water-soluble 50 mg/kg body weight in the form of gavage.
- Treatment group 2: In this group, treated rats received the SRBC and the NBS food supplement in the form of water-soluble 100 mg/kg body weight in the form of gavage.
- Treatment group 3: In this group, treated rats received the SRBC and the fresh and healthy dietary supplement in the form of water-soluble 150 mg/kg body weight in the form of gavage.

After the end of the injection period, all blood samples were taken, the blood was poured into microtubes containing an anticoagulant 5% (EDTA) and placed in a rotator for 20 minutes, and then counting operations of White blood cell was done.

In order to analyze the data in this study, we first make sure that the distribution of data is normal, using Kolmogorov-Smirnov test. Also, in this study, in order to evaluate the significance of the data, it is recommended to use ANOVA test. ANOVA test was used to investigate the differences between and within groups. P < 0.05 indicates statistical significance. In order to clarify this issue, by Scheffe post hoc test, this significance was tested one by one between groups.

## III. RESULTS AND DISCUSSION

The effect of SRBC on the amount of white blood cells in small experimental mice and the effect of the NBS healthy and live dietary supplement with concentrations of 50, 100 and 150 mg/kg on the amount of white blood cells in the SRBC recipients were investigated (Fig. 1).

Study and count of white blood cells and their comparison in experimental and control groups showed that the mean number of white blood cells in all three experimental groups receiving healthy and live dietary supplement with concentrations of 50, 100 and 150 mg/kg body weight of the rat showed a significant increase to the control group at a 5% probability level (Fig. 2). Also, the results of the effect of SRBC on neutrophil count of small experimental mice and the effect of the NBS healthy and live dietary supplement with concentrations of 50, 100 and 150 mg/kg on the amount of neutrophil counts of SRBC-receptor mice were investigated (Fig. 3).

Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied	Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied
1	4/3	gr 29	1	6/4	28 gr
2	4/7	34 gr	2	6/3	29 gr
3	4/4	30 gr	3	6/4	32 gr
4	4/6	28 gr	4	6/6	27 gr
5	4/5	30 gr	5	6/4	30 gr
a	4/5	Average	b	6/42	Average
Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied	Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied
1	7/1	31 gr	1	8/4	33 gr
2	7/4	30 gr	2	8/6	29 gr
2 3	6/9	30 gr 28 gr	2	8/6 8/5	29 gr 32 gr
-		100			
3	6/9	28 gr	3	8/5	32 gr

Fig. 1 (a) The results of the SRBC effect on the amount of white blood cells in small experimental mice and the effect of the NBS healthy and live dietary supplement with concentrations of (b) 50, (c) 100 and (d) 150 mg/kg on the amount of white blood cells in the SRBC recipients

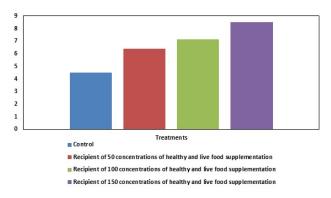


Fig. 2 Comparison of white blood cell count in experimental groups

The results showed that neutrophil percentage in all three experimental groups receiving the healthy dietary supplement was significantly increased at 5% probability level (Fig. 4), and also, lymphocyte counts decreased significantly (Fig. 5). However, the level of lymphocytes in the recipients groups of the healthy and live food powder is higher than that of the control group. Comparison of lymphocytes in experimental groups was shown as Fig. 6. Therefore, it can be stated in general that the dietary supplement studied in this research

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increases the level of immunity of the body.

Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied	Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied
1	29	29 gr	1	41	28 gr
2	28	34 gr	2	39	29 gr
3	30	30 gr	3	44	32 gr
4	32	28 gr	4	42	27 gr
5	30	30 gr	5	40	30 gr
а	29/8	Average	b	41/2	Average
	0				
Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied	Number of mice	Count ×10³/µL	Mouse weight studied
of mice	×10 <sup>3</sup> /µL	studied	of mice	×10 <sup>3</sup> /µL	studied
of mice	×10 <sup>3</sup> /µL	studied 31 gr	of mice	×10 <sup>3</sup> /µL 56	studied 33 gr
of mice	×10 <sup>3</sup> /µL 51 54	studied 31 gr 30 gr	of mice	×10 <sup>3</sup> /µL 56 59	studied 33 gr 29 gr
of mice	×10 <sup>3</sup> /µL 51 54 56	31 gr 30 gr 28 gr	of mice 1 2 3	×10 <sup>3</sup> /µL 56 59 61	studied 33 gr 29 gr 32 gr

Fig. 3 (a) The results of the SRBC effect on neutrophil count of small experimental mice and the effect of the NBS healthy and live dietary supplement with concentrations of (b) 50, (c) 100 and (d) 150 mg/kg on the amount of neutrophil counts of SRBC-receptor mice

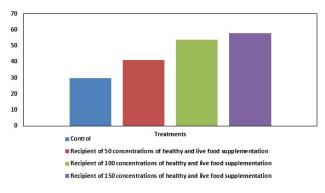


Fig. 4 Comparison of neutrophil levels in experimental groups

## IV. DISCUSSION

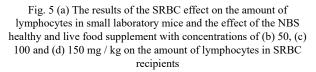
The immune system protects individuals through different cellular and molecular mechanisms. The system is designed to detect its own cells and substrates, but also can to be able to detect and destroy external organisms and their products [4], [6]. The main plug of the immune system is subdivided into a non-specific (inherent) and specific (acquired) immune system [6]. Intrinsic and natural immunity include the following components: physical barriers and molecular membrane, complement systems, antimicrobial substrates such as lysosomes, and other inflammatory mediators such as phagocytes (neutrophils and macrophages) and other leukocytes (such as natural killer cells) that are capable of virus lysis and tumor cells. In other words, the acquired immune system acts on the basis of previous communication and memory, which is usually divided into two categories:

1. Homural immunity, which synthesizes B lymphocytes and releases specific antibodies, such as 5 types of different

immunoglobulins.

2. Cell-mediated immunity that is dependent on the lymphocyte subtypes processed by the thymus (lymphocyte T and their products).

Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied	Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied
1	74	29 gr	1	62	28 gr
2	76	34 gr	2	64	29 gr
3	72	30 gr	3	59	32 gr
4	79	28 gr	4	63	27 gr
5	73	30 gr	5	60	30 gr
a	74/8	Average	b	67/6	Average
Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied	Number of mice	Count ×10 <sup>3</sup> /µL	Mouse weight studied
1	54	31 gr	1	43	33 gr
2	52	30 gr	2	46	29 gr
	51	28 gr	3	40	32 gr
3				10	32 gr
3 4	54	32 gr	4	42	52 gr
	54 50	32 gr 31 gr	4	42	32 gr 27 gr



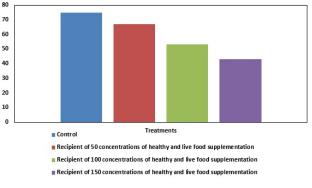


Fig. 6 Comparison of lymphocytes in experimental groups

Lymphokines are effective against a large number of antigens. The subtype of T lymphocytes is typically referred to T-lymphocytes TH of the worker (HELPER) or TC-CD suppressor T lymphocytes to the CD8 + and CD4 + dependent clusters of differentiation. In this context, T-lymphocytes, especially antigen-releasing cells, are destroyed by viruses or other intracellular microorganisms, while hemorrhagic immunity creates a great defense mechanism against extracellular microorganisms [7]. Two levels of the immunocomoetence system are not separated, so that they create a coherent model for the development of an immune system against harmful infections, cellular degeneration, or pathogenic mechanisms [7]. Examples of these interactions are the stimulation of T-cell lymphocytes by the antigens for

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the production of lymphokines, which promote the proliferation and differentiation of macrophages, as well as the interaction between Th and the CD19 (B +) lymphocytes, which induces the production of immunoglobulins by B cells. Antigenic cells (such as macrophages) facilitate detection of antigens over B and T cells, while for new and former antigens, responding to delayed skin sensitivity is another lymphatic-mediated response. The practical result of this complete defense of living organisms is the collaboration of immunoglobulins various in protecting individuals. Interactions between nutrition, immune function and pathological conditions are multifactorial [8], [9]. Therefore, nutrient intakes and nutritional status affect host immunocompetence and response to the disease or infection; immunodeficiencies have harmful effects on the nutritional value of the body, while also affect the outbreak of the disease or infection, as well as a number of pathogenic microorganisms or illness that causes malnutrition and immunedeficiency. Therefore, biochemical and anthropometric indices are related to dietary intakes, clinical examinations and also immunological scales [10], [11]. In this paper, epidemiological and clinical evidence has shown that immune copmutativeness is dependent on nutritional status, as malnutrition is associated with damage to immune responses and increased prevalence of infection in the lysis [12]. Excessive intake of certain nutrients may increase immune competence, but it may also induce a decrease in immune deficiencies [8], [13]. In addition, the quality and quantity of nutrient composition of the diet are also effective in regulating the immune response [14], [15]. Lymphoid tissues are one of the rapidly recurrent tissues that are highly susceptible to nutrient imbalances affecting the pathways and metabolic functions involved in immune defense. Additionally, infections are associated with reduced intake of food, catabolic bowel stress, increased nutrient loss from feces, urine and sweat, and defects in protein synthesis (immunoglobulin) and cell proliferation [16]. Assessing the possible nutritional status was performed through a large number of immunological measurements, such as counting lymphocyte subsets, stimulating the propagation of lymphoblast by various antigens, leukocyte migration and phagocyte function, delayed skin sensitivity reaction, plasma concentration of different immunoglobulins (antibodies), cytokines and other intermediates such as interferon ( $\alpha$ ,  $\beta$ ,  $\gamma$ ), internucleicans, differentiation factors of CELL-B, and monocinases [7]. In each case, skin tests such as counting lymphocytes and the performance determine are the most commonly performed tests in nutritional assessment [17].

## V.CONCLUSION

Based on the results of this study, it can be said that this compound significantly increases the white blood cell and neutrophil count and also reduces the amount of lymphocytes, which better protects the body against the pathogenic agent.

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