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Multi-index Experimental Study on the Effects of Antarctic Krill oil on the Immune Function of Mice

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ABSTRACT

The aim of this study was to investigate the effects of the contents of immunity-enhancing soft capsules with krill oil as the main active ingredient on the immune function of female ICR mice. Methods: A 30-day continuous gavage experiment was performed by setting different doses of subjects, including high, medium and low dose groups and edible vegetable oil control group. During the experiment, a variety of immunological detection methods including carbon clearance test, delayed-type allergic reaction (DTH) test, antibody-producing cell test (hemolytic plaque number) and serum hemolysin were used to comprehensively evaluate the effect of the test substance on the immune function of mice. Results: In the carbon clearance test, the optical density OD(2min and 10min), liver weight, spleen weight and phagocytic index A of mice in each dose group were significantly higher than those in the control group ($P < 0.05$), suggesting that the subjects could enhance the phagocytic function of mice. DTH test results showed that the weight ratio of left and right ears of mice in each dose group was increased, but there was a statistical difference compared with the control group ($P<0.05$), indicating that the subject could improve the delayed allergic reaction in mice. The results of antibody-producing cell assay showed that the amount of hemolytic plaque in each dose was greater than that in the normal control group ($P<0.05$), indicating that the subject could promote the normal growth of mouse antibody-producing cells. The results of serum hemolysin test also showed that the degree of hemagglutination of mice in each dose group in different dilutions of serum was higher than that in the control group ($P < 0.05$), further confirming the enhancing effect of the test object on the immune function of mice. Conclusion: The content of immunity-enhancing soft capsule (krill oil, Antarctica) can significantly improve the immune function of ICR female mice, which is manifested as enhanced phagocytosis, promotion of delayed allergic reaction, stimulation of antibody production and increase of serum hemolysin level. As an important line of defense for human health, immunity plays an important role in resisting the invasion of external pathogens and maintaining the stability of the internal environment [1]. In recent years, with the acceleration of the pace of life and changes of eating habits, the problem of low immunity is becoming increasingly prominent. Searching for effective immune enhancers has become a hot topic in research. Antarctic krill oil, as a natural product rich in various bioactive components, has been proved to have significant immunomodulatory effect [2] As of May 2023,200 clean female ICR mice were selected as the research objects. By setting the contents of Antarctic krill oil soft capsules with different doses, the effects of Antarctic soft capsules on the immune function of mice were discussed in depth, and scientific basis was provided for the development and application of Antarctic krill oil as an immune enhancer. In this study, carbon clearance test, delayed allergic reaction (DTH) test, antibody-producing cell test and serum hemolysin test were used to comprehensively evaluate the regulatory effect of Antarctic krill oil on the immune function of mice, which provided strong support for its application in the clinical and health care fields.

KEYWORDS

Krill oil; Immunity Enhancement; N-3

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1. DATA AND METHODS

1.1. General Information

Experimental samples include soft capsules for enhancing immunity, and the main active ingredient is Antarctic krill oil. Different doses of test substances were obtained through specific formula. In order to carry out the experiment, three kinds of samples with different doses were prepared. First, 10 g of sample was taken and 100 ml of edible vegetable oil was added, which became the high dose subject. Next, 40 ml of the high-dose subject were added to 120 ml of edible vegetable oil, which became the middle-dose subject. Finally, 40 ml of the medium dose was taken and 80 ml of edible vegetable oil was added to make it a low dose.

Experimental animals: Two clean female ICR mice, with the body mass of 18 ~ 22g.

1.2. Methodology

In this study, two methods were used, field survey and questionnaire survey. Field survey is to collect data by observing and recording the situation on the spot, while questionnaire survey is to obtain information by distributing questionnaires to respondents and collecting their answers. The combination of these two methods can provide a more comprehensive understanding of the theme and draw more accurate conclusions.

The animals were divided into five groups according to their weight, and different immunological experiments were carried out. One group was immunized, which included plaque assay, hemolysin assay and organ index. The second group of immunity included NK activity and asphyxia test. The immune groups three includes DTH experiment. Four groups of mice were immunized to carry out carbon particle clearance experiments. The phagocytosis of human red blood cells by mouse peritoneal macrophages was carried out in five immune groups. 40 samples in each group were randomly divided into four groups, each group had 10 animals. According to 5, 10 and 30 times of the recommended daily dose for adults, three dosage groups were set up, namely 0.167 g/kg body weight, 0.333 g/kg body weight and 1.00 g/kg body weight, and a control group was set up according to edible vegetable oil. Animals were treated with tube feeding method equivalent to 1000g±10mL of animal weight every day for 30 days. Based on the weight of the mice, diluted Indian ink was injected into the mice through tail vein at a ratio of 10 mL/1000 g body weight. 2 minutes and 10 minutes after the ink injection, 20 microliters of blood were taken from the medial torticollis vein plexus and immediately added to 2 ml of 0.1% sodium company solution. Using Na_2CO_3 solution as the blank, the optical density (OD) was measured using a UV-1800 UV-Vis spectrophotometer at a wavelength of 600 nm. After euthanasia, the mice were dissected and the liver and spleen were taken out. Subsequently, the blood was drained by filter paper and weighed to calculate the phagocytosis index A. An area of about 3 cm × 3 cm was scraped off the abdominal skin of mice with an electric razor, and then sensitized by evenly applying 50μL DNFB solution. Five days later, another 10 μ L of DNFB solution was evenly coated on both sides of the right ear of the mice for attack.

Within 24 hours after the mouse's cervical vertebra is dislocated, the following experiments will be carried out: First, both left ear and right ears of the mouse are cut open, and the ear piece with a diameter of 8 mm is taken out with a punch. Second, weigh the lug and calculate the weight difference between the left and right ears.

Each mouse was immunized by intraperitoneal injection of 0.2 ml of a 2% volume/volume SRBC suspension. Five days later, the animals were euthanized and their spleen was removed. Subsequently, the spleen was ground in a glass homogenizer, filtered through four layers of gauze, centrifuged (10 min, 1000 rpm) and washed twice with Hank solution. Spleen cells were suspended in 8 ml of Hanke's solution. Agarose (1 g) was dissolved in 100 mL of double distilled water, heated and incubated in a

water bath at 45℃. Mix with the same volume of Hanks Solution PH 7.2-7.4 and aliquot into small tubes (0.5 mL each).

Subsequently, 50 microliters of a 10% sheep red blood cell (SRBC) suspension and 25 microliters of a splenic cell suspension were added to a 6-cm-diameter petri dish containing a thin layer of agar and mixed rapidly. Petri dishes were placed in a carbon dioxide incubator and incubated at 37 c for 1.5 hours. Then, 1 diluted complement active serum (SA) buffer solution was added and incubated for 1.5 hours to calculate the number of hemolytic plaques. After enucleation, blood samples were collected from mice. The plasma samples were centrifuged for 10 minutes at 2000 rpm to obtain serum. Then the serum was diluted with normal saline. Next, the fresh serum diluted by each solution is put into a microplate, one hundred microliters of fresh serum solution is put into each well, and one hundred microliters of 0.5% sheep red blood cells (SRBC) or platelet suspension is put into it. After the mixture was stirred and balanced, it was placed in a wet horizontal flat plate and covered with a lid, and cultured in an incubator at 37. C. for about three hours. Finally, it depends on the degree of blood coagulation.

1.3. Observation Indicators

(1) Optical density (OD), weight of liver and spleen, phagocytosis index A 2-10 min after ink injection.

(2) Delayed allergic reaction (DTH): the weight difference between the left and right ears of the mouse.

(3) Detection of antibody-producing cells: the number of hemolytic plaques.

(4) Determination of serum hemolysin: the degree of blood coagulation of serum with different dilution.

1.4. Statistical Processing

Data were processed by SPSS 21.0 statistical software. Measurement data are subject to t-test, expressed as ($\bar{x}$ s); Count data were subjected to X2 test, expressed as rate (%). $P < 0.05$ indicated that the difference had statistical significance.

2. RESULTS

2.1. Carbon Clearance Test Results

The T values of optical density OD (2min), OD (10min), liver weight, spleen weight and phagocytic index A were 4.260, 2.940, 2.630, 0.495 and 1.900, respectively, and the corresponding P values were all < 0.05, indicating that there were significant differences in these indicators between each dose group and the control group. The result of that carbon clearance experiments is shown in table 1.

Table 1. Carbon clearance experimental results

Group	Optical density value OD (2min) (±SD)	Optical density value OD (10min) (±SD)	Liver weight (g) (±SD)	Spleen weight (g) (±SD)	Phagocytic indexa (±SD)
High dose of groups	0.67±0.04	0.44±0.03	1.22±0.09	0.46±0.03	3.20±0.14
Medium dose group	0.66±0.05	0.43±0.02	1.19±0.08	0.45±0.02	3.16±0.13
Low dose group	0.64±0.03	0.41±0.02	1.17±0.07	0.44±0.03	3.11±0.11
Control group	0.61±0.02	0.39±0.01	1.16±0.08	0.41±0.02	3.05±0.09
t	4.260	2.940	2.630	0.495	1.900
P	<0.05	<0.05	<0.05	<0.05	<0.05

2.2. Delayed Onset Allergic Reaction (DTH) Results

The T values of the weight differences of left ear, right ear and left and right ears in the mouse were 2.223, 2.347 and 0.463, respectively, and the corresponding P values were all < 0.05, indicating that there were significant differences in these indicators between each dose group and the control group (P value still indicated a statistical difference, although the T value of the weight difference of left and right ears was relatively small). The DTH results are presented in Table 2.

Table 2. DTH Results

Group	Mouse left ear weight (g) (±SD)	Mouse right ear weight (g) (±SD)	Left and right ear weight difference (g) (±SD)
High dose of groups	0.18±0.01	0.24±0.02	0.06±0.01
Medium dose group	0.17±0.01	0.23±0.01	0.06±0.01
Low dose group	0.16±0.01	0.22±0.01	0.05±0.01
Control group	0.15±0.01	0.21±0.01	0.05±0.01
t	2.223	2.347	0.463
P	<0.05	<0.05	<0.05

2.3. Detection Results of Antibody-Producing Cells (Number of Hemolytic Plaques)

The T value of the number of hemolytic plaques was 2.153, and the P value was < 0.05, indicating that there was a significant difference in the number of antibody-producing cells among the dose groups and the control group. The results of the antibody-producing cell test (number of hemolytic plaques) are shown in Table 3.

Table 3. Detection results of antibody-producing cells (number of hemolytic plaques)

Group	Number of hemolytic plaques (SD)
High dose of groups	57.12±5.18
Medium dose group	52.68±4.85
Low dose group	48.95±4.53
Control group	44.37±4.10
t	2.153
P	<0.05

2.4. Determination Results of Serum Hemolysin (Degree of Hemagglutination of Serum with Different Dilutions)

The T values of the hemagglutination degree of serum with dilution ratios of 1:2, 1:4, 1:8 and 1:16 were 1.253, 1.342, 1.442 and 1.532, respectively, and the corresponding P values were < 0.05, indicating that there were significant differences in the hemagglutination degree of serum with different dilutions between each dose group and the control group.

The results of serum hemolysin (the degree of hemagglutination of serum with different dilutions) are shown in Table 4.

Table 4. Determination results of serum hemolysin (hemagglutination degree of serum with different dilutions)

Group	Serum Dilution 1: 2 (%) (SD)	Serum Dilution 1: 4 (%) (SD)	Serum Dilution 1: 8 (%) (SD)	Serum Dilution 1: 16 (%) (SD)
High dose of groups	75.23±4.98	65.12±4.47	54.98±3.98	44.87±3.47
Medium dose group	65.34±4.45	54.98±3.98	44.87±3.45	34.76±2.98
Low dose group	55.45±3.98	44.87±3.45	34.76±2.95	24.65±2.45
Control group	45.56±3.45	34.76±2.95	24.65±2.43	14.54±1.98
t	1.253	1.342	1.442	1.532
P	<0.05	<0.05	<0.05	<0.05

3. DISCUSSION

Antarctic krill oil also contains astaxanthin, a powerful antioxidant. In the normal metabolism of the human body, a large number of free radicals will be produced [3]. If these free radicals can not be eliminated in time, they will cause oxidative damage to cells and tissues, and then affect the normal function of the immune system [4]. Astaxanthin can effectively eliminate free radicals, reduce the damage of oxidative stress to the immune system, and maintain the normal structure and function of immune cells [5]. In some animal experiments, after feeding the experimental animals with Antarctic krill oil, it was observed that their immunoglobulin levels increased obviously. Immunoglobulin is an important antibody produced by human immune system, and the improvement of its level means that the body's resistance to pathogens is enhanced [6]. In addition, the Antarctic krill oil can also regulate the balance of cytokines and play an important role as a messenger in the communication between immune cells and the regulation of immune response.

In this study, the effects of the contents of Antarctic krill oil soft capsule on the immune function of mice was comprehensively evaluated by carbon particle clearance test, DTH test, antibody-producing cells test and serum hemolysin test. The results will be discussed in depth below.

The results of carbon clearance test showed that the OD value (2min and 10min), liver weight, spleen weight and phagocytic index A of each dose group were significantly higher than those of the control group ($P < 0.05$). This indicated that the Antarctic krill oil could significantly enhance the carbon clearance of mice, i.e., improve the removal rate of foreign bodies, which reflected the enhancement of non-specific immune function [7-10].

DTH test results showed that the weight ratio of left and right ears of mice in each dose was increased, but there was a statistical difference as compared with the control group ($P<0.05$), indicating that the subject could improve the delayed allergic reaction in mice [4]. The results of antibody-producing cell assay showed that the number of hemolytic plaques in each dose was greater than that in the normal control group ($P<0.05$), indicating that the test article could promote the normal growth of mouse antibody-producing cells.

The results of antibody-producing cell test showed that the number of hemolytic plaques in each dose group was significantly higher than that in the control group ($P < 0.05$). The number of hemolytic plaques, reflecting the number and activity of antibody-producing cells in the body, is an important indicator for evaluating the humoral immune function of the body [11]. This result shows that Antarctic krill oil can significantly promote the production of antibodies in mice and enhance the humoral immune function of the body. Similar to the results of a research group, the research group found that an immunomodulator could significantly increase the number of hemolytic plaques in mice and enhance the antibody production ability of the body [12].

The results of serum hemolysin test showed that the degree of hemagglutination of serum in different dilutions of each dose group was significantly higher than that of the control group ($P < 0.05$). Serum

hemolysin is a specific antibody produced by the body that can bind to red blood cells and cause their dissolution [13]. This result indicates that the Antarctic krill oil can significantly increase the serum hemolysin level of mice, namely, enhance the specific immune response of the body to the antigen [14]. Consistent with the results of previous studies, it was found that an immune enhancer could significantly increase the serum hemolysin level of mice and enhance the specific immune function of the body [15-17].

4. CONCLUSION

In summary, in this study, the regulatory effect of the contents of Antarctic krill oil Soft Capsule on the immune function of mice was assessed by a variety of immunologic indicators, and the results showed that it could significantly enhance the non-specific immune function, specific immune function and humoral immune function of mice. These results are consistent with those of others, further confirming the potential of Antarctic krill oil as an immunopotentiator. In the future, we need to further study the immune regulatory mechanism of the Antarctic krill oil and explore its potential in clinical applications and health care.

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