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Investigating the immunological responses of AB7 natural solution

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Abstract

Introduction: The immune system is the strongest and most efficient mechanism of the body to defend itself against all kinds of invading and non-invasive foreign agents. However, a malfunctioning immune system can play a devastating role by causing many diseases and disorders in the body. Diseases and disorders of the immune system are classified depending on the activity of the immune system. An example of an important disorder of the immune system is the cytokine storm, for example, in COVID-19, which leads to severe respiratory distress. An overactive immune system has the potential to compromise health just as much as an underactive immune system. The use of natural compounds will be effective in modulating immune system disorders. One of the terms that is mentioned in Persian medicine, including Hakim Aghili Khorasani Shirazi (author of the book " **Kholase** Al-Hekmat ") in the book " **Makhzan al-adviyeh** " as a fortifying factor for body, is "**Padzahr**". In this study, boiled water of **Padzahr** was used to investigate the mentioned effects.

Methods: The studied population were included 14 male Balb/c mice that were randomly divided into two equal groups and immunized with SRBC antigen. Mice in the treatment group received padzahr water (200 µl) daily for two weeks from the beginning of the study. Humoral and cellular immunity, respiratory burst ability, nitric oxide production rate and proliferation index of spleen cell were measured.

Results: The obtained results indicate a significant increase in the anti-SRBC antibody titer in the serum of mice in the treatment group at the same time as a decrease in the intensity of the cellular reaction compared to the control group. The proliferation rate of spleen cells was significantly increased compared to the control group. However, the respiratory burst ability and nitric oxide production in the population of phagocytic cells in the spleen of mice in the treatment group were significantly reduced compared to the control mice.

Conclusion: Since the intensity of respiratory explosion and the ability to produce nitric oxide in phagocytic cells are significantly reduced, it seems that padzahr water can be considered as a natural compound that modulates the immune system.

Keywords: Padzahr, Humoral Immunity, Cellular Immunity.

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Introduction

Natural compounds with medicinal properties have been of special interest to people since the distant past due to their use in curing diseases. These compounds have unique and valuable properties and have played an important role in the general health of the society until today[1]. The historical discoveries made in India, China, Egypt and Iran show that natural compounds with medicinal properties have been used in various forms by people, and with the rise of Islamic civilization, they have developed more rapidly[2]. Padzahr is a stone-like mass found in the digestive tract (usually the stomach) of animals, in traditional medicine, a set of healing properties were attributed to this padzahr, the most famous of which is the belief that this material is an antidote[3]. In Persian and Arabic texts, antidote refers to a substance It may be able to prevent the effects of poison in the body, but physicians call anything that reverses the harmful effects of other substances an antidote. In the

reference books of traditional medicine, doctors and pharmacists usually called single antidote drugs that were found naturally, antidote, and compound antidote drugs called antidotes[4]. The padzahr is black[5]. One of the characteristics of this is that the weight of padzahr never decreases[6]. It agrees with all moods (persons) and repels most of the hot poisons of animal, plant and vegetable temperament. It strengthens the body and the five senses, the heart, the brain, and the liver, and it strengthens the sexual powers, and it is uplifting, happy, and removes sadness. It can be used for the sting or bite of poisonous animals and for the wound caused by it, and it causes the poison to be removed through secretion from the wound. And it is also effective in wound healing. You can put it in a milk container and use the milk after some time.

The immune system is the body's defense mechanism against various diseases and infections. Naturally, the deficiency of the immune system causes a long list of immune system disorders[7]. The immune system is the strongest and most efficient mechanism of the body to defend itself against all kinds of invading and non-invasive foreign agents. However, a malfunctioning immune system can play a devastating role by causing many diseases and disorders in the body[8]. Diseases and disorders of the immune system are classified depending on the activity of the immune system. An overactive immune system has the potential to endanger health as much as an underactive immune system[9].

According to the mentioned materials and the literature review, no study has been conducted on the effects of antidote (padzahr) on immune system disorders, and the purpose of this study is to investigate the effect of padzahr on the amount and direction of immune system responses.

Method

The present study was conducted after obtaining the code of ethics from the ethics committee of Baqiyatallah University of Medical Science (IR.BMSU.REC.1399.065) in the laboratory animal breeding center of the university.

Preparation of the extract

The prepared extract was prepared from Attari and was used per microliter unit in the design of the present study.

Immunization and treatment of animals

The population studied in this experimental study, which was conducted as a case-control, included 14 male BALB/c mice with an age range of 6-8 weeks, which were obtained from the animal house of Baqiyatallah University of Medical Science, these mice they were kept under standard conditions of water, food, temperature and sufficient light. After 2 weeks of time to adapt the mice, the animals were randomly divided into two groups as follows:

Treatment group: The mice of this group were subcutaneously injected with $1x10^9$ sheep red blood cell emulsion (SRBC) and Freund's complete and incomplete Freund's adjuvant (second injection) in a volume of 0.1 ml on the day of the start of the experiment and one week later. Also, mice in the treatment group received oral antidote (200 µl) from the beginning of the study for two weeks. Control group: The mice of this group were challenged with the mentioned emulsion in the same way as the previous group. From the day of immunization, these mice received 200 µl of PBS orally daily.

Evaluation of acquired humoral immunity

To check the humoral immune response, the titer of agglutinating antibodies produced against sheep red blood cell antigen was measured in the animal's body. After anesthetizing the mice, blood was collected from the heart and its head was separated. Serial dilution (from 1.2 to 1.1280) of serum in 96 round well plates prepared (duplicate) and 5% suspension of sheep red blood cells were added to all wells and 3 hours of incubation was done at room temperature. Then the occurrence of agglutination in the wells was studied and the titer of each sample was calculated based on the dilution of the last well in which agglutination was observed.

Assessment of acquired cellular immunity

For this purpose, the intensity of the delayed hypersensitivity reaction and also the proliferation rate of lymphocytes in the spleen cell population were investigated.

A) Measurement of delayed hypersensitivity reaction:

48 hours before blood collection, 0.1 ml of SRBC was injected into the sole of the left foot of the animals, and 0.1 ml of PBS was injected into the right foot of the mice at the same time. After 48 hours and before blood sampling, the thickness of the mice's feet was measured using a caliper, and the level of cellular immunity was calculated according to the following equation:

Cellular immune response index = swelling of the left leg - swelling of the right leg divided by the swelling of the right leg

B) Investigating the amount of proliferation of lymphocytes in the population of spleen cells by MTT method:

after blood sampling from mice, their spleens were removed under sterile conditions and crushed in 1640-RPMI culture medium containing 10% FBS. The resulting tissue was passed through a wire net with a diameter of 0.2 mm to prepare cell suspension. After centrifugation for 10 minutes at 200 g in order to remove RBCs, 5 cc of

lysing buffer was added to the obtained cell sediment. After 5 minutes, while adding 10 cc of the culture medium, it was centrifuged again for 10 minutes at 200g. Then, the cell sediment in RPMI culture medium containing 10% FBS was made into a suspension containing 1 x 10^6 cells per ml. Then 100 µl of it were poured into each of the wells of the 96-well plate at the bottom of the bed. For each sample, three replicates in the presence of 50 microliters of phytohemagglutinin solution (1 mg/ml) and three replicates without the presence of phytohemagglutinin were considered. Empty RPMI medium was also used as a blank in three wells. After 72 hours of incubation in an incubator containing 5% CO2, 25 µl of MTT solution (5 mg/ml in PBS) were added to each well and incubated for another 4 hours. During this period, the regeneration of MTT by living and proliferating cells caused the formation of formazon crystals, which were dissolved by adding 100 µl of DMSO. Then the color intensity was determined at the wavelength of 492 nm.

Investigating the assessment of innate cellular immune activity

For this purpose, the intensity of respiratory burst and the amount of nitric oxide production in the population of spleen phagocytic cells were measured. Measurement of respiratory burst ability in the population of spleen phagocytic cells was prepared in a cell suspension with a number of 2 x 10^6 cells per ml. These cells were incubated in 24-well culture plates for two hours at 37°C and 5% carbon dioxide, then the cells were washed with Hank's culture medium to remove lymphocytes. The remaining cells were incubated with opsonized yeast for one hour, then 100 µl of zymosan and NBT solution were added to each of the houses and incubated for another hour. Finally, 400 µl of N-N dimethylformamide was added to each house. And it was centrifuged at a speed of 3000 rpm for 10 minutes at a temperature of 4 degrees Celsius. 200 µl of the supernatant from each house were poured into a 96-well microplate and the result was read with an ELISA reader at a wavelength of 492 nm.

Measurement of nitric oxide

The amount of nitric oxide production was determined by grease colorimetric method and using the sodium nitrite standard curve. Briefly, spleen cells were incubated with phytohemagglutinin in the same manner as described in the MTT assay. Then 100 µl of spleen cell culture supernatant were poured in pairs into the wells of the 96-well plate at the bottom of the bed. Then 100 µl of 1% sulfanilamide solution were added to the wells. The plate was kept in the dark at room temperature for 10 minutes. Then, 100 microliters of 1% 1-1-N naphthylethylene diamine dihydrochloride solution was added to all the wells and kept again for 10 minutes in the dark at room temperature. Finally, the optical absorption of the sample at a wavelength of 492 nm was read by an ELISA device.

statistical analysis

Mann Whitney-U test was used for comparison. A probability value of $P<0.05$ was considered as a significant level. All statistical analyzes were performed in the SPSS version 19 software environment and the data were reported as mean ± standard deviation.

Results

1-Humoral immunity

Examining the level of anti-SRBC antibody production showed that the oral administration of antidote water increases the antibody titer compared to the control group (Figure 1).

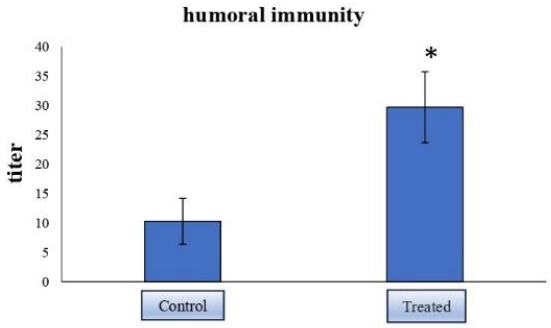

Figure 1: Antibody titer in mice of the studied group

2- Cellular immunity

Examining the thickness of the soles of the mice in the studied groups showed that the oral administration of antidote water after injecting SRBC into the soles of the mice reduces swelling and in other words, reduces delayed hypersensitivity (Figure 2).

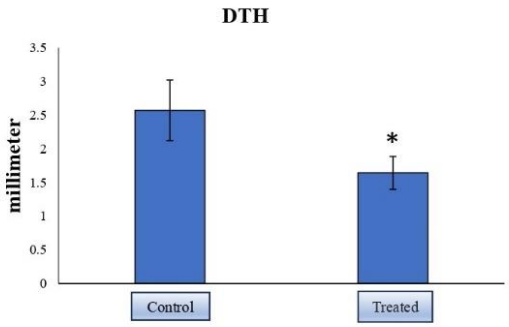

Figure 2: Sole of the foot thickness in mice of the studied groups

3- Proliferation of spleen lymphocytes

After the cultivation of spleen cells, it was found that the oral consumption of antidote water increases the proliferation of cells compared to the control group (Figure 3).

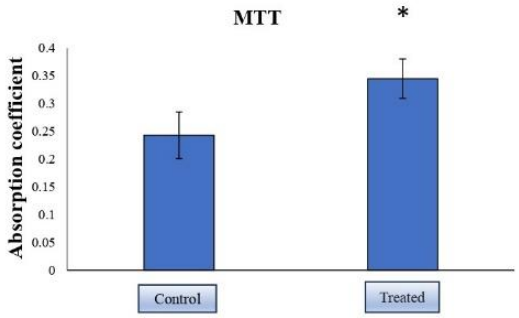

Figure 3: The amount of spleen cells in mice of the studied groups

4- Capability of respiratory explosion and production of nitric oxide

The results of the study showed that the rate of respiratory burst and nitric oxide production in the population of phagocytic cells in the spleen of mice treated with antidote water was significantly reduced compared to the control mice (Figure 4).

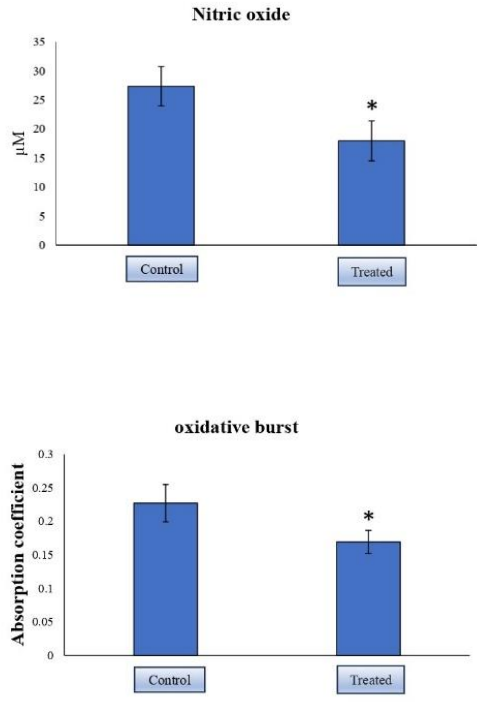

Figure 4: The rate of production of oxidative burst and nitric oxide in rats of the studied groups

Discussion and Conclusion

The living organism's immune system is responsible for identifying cells and self-molecules from foreign ones, destroying them or making them harmless, as well as maintaining the homeostasis of the body. Modulating immune reactions plays an important role in improving the body's performance following immunological challenges. When the host's immune system needs to be inhibited or, on the contrary, to strengthen its function, plants with immunomodulatory properties can be used as an alternative to conventional chemotherapy drugs[9]. Among the different components of the immune system, the spleen as a secondary lymphoid organ plays an important role in removing pathogens from the blood and creating general immune responses. Therefore, the present study was focused on the investigation of humoral immune response and cellular immunity of this tissue. Most of the natural compounds have antioxidant properties that prevent to produced active oxygen species(ROS), free radicals(hydroxyl radical, OH and superoxide radicals) or active oxygen non-free radicals(peroxide, H_2O_2) in metabolism[10]. Studies in this field are very limited. In a study conducted by Heydari Mahmoodreza et al, pre-treatment with antelope antivenom increased survival time and reduced pathological symptoms (including bleeding in the peritoneum, chest and central nervous system, as well as pulmonary vascular congestion) caused by Parsley snake venom was found in mice[11]. In a study conducted by Jokar et al, mineral antidote reduced the severity of the signs and symptoms of Covid-19, but compared to the control group during hospitalization, it did not show a statistically significant difference. In the follow-up two weeks later, the antidote group was in a better condition and the remaining symptoms were less in them[12]. In the present study, the decrease in respiratory burst ability and nitric oxide production among the phagocytic cells in the population of spleen cells in the treatment group mice may be caused by the antioxidant property of the compounds in the water obtained from the antidote. Cellular immune reactions such as DTH are the result of the cooperation of Th1 T cells and macrophage cells[13]. Although the proliferation rate of splenic lymphocytes was significantly increased compared to the current study group, probably the decrease in the activity of macrophages (reduction in the intensity of respiratory burst and production of nitric oxide in the control group) can justify the decrease in the intensity of the DTH reaction. It is also possible that the increased proliferation of T lymphocytes observed in this study is due to their polarization to a direction other than Th1 cells. The

humoral immune response in the present study was evaluated by hem agglutination test[14]. The results related to the humoral immune response show a significant difference between the group consuming water obtained from antidote and the control group in the production of agglutinating antibody, so that the agglutinating antibody titer in the treatment group is more than the control group. In the neutral pH condition of red blood cells, the negative charge on the surface of these cells prevents these cells from connecting to each other. This inhibitory force is called zeta potential because of the size and nature of the five-unit IgM molecule of this antibody. The IgG molecule has a smaller size and only double valency. Therefore, this antibody molecule is not able to neutralize the electric charge between red blood cells[15]. It seems that due to the short duration of the period, the study of produced antibody is more than IgM class. In previous studies, the increase in the number of white blood cells and spleen cells, as well as the ability to harvest microorganisms from monocyte cells, following the use of extracts of different natural compounds, have been mentioned. Of course, in the process of effective removal of a pathogen, the basic step after the removal of the pathogen is to start the mechanisms of respiratory explosion and production of nitric oxide for the effective destruction of the removed agent[16]. Since the intensity of the respiratory burst and the ability to produce nitric oxide in phagocytic cells are significantly reduced. In some studies, antibacterial, antiviral properties against the AIDS virus and anticancer properties of various natural compounds have been reported. Of course, many of these studies were conducted in vitro and examined the direct effect of compounds on infectious agents or cancer cells[17]. Naturally that none of the mentioned cases can indicate the strengthening effect of the immune system or in vivo conditions. Also, although some studies have only mentioned the immune-stimulating aspect of these compounds, some other studies have emphasized the immune-inhibiting effects of natural compounds[14, 18]. With all these interpretations, based on the present study, it seems that the water obtained from the antidote causes the deviation of immune responses from cellular to humoral responses. Therefore, it may be considered as a natural compound that modulates the immune system. It is certain that this study is only a preliminary investigation and it is necessary to conduct more additional tests to evaluate the state of polarization of T lymphocytes and the amount of production of other immunoglobulin classes as well as other indicators of the activity of innate immune components such as macrophages.

Research Highlights

What Is Already Known?

The immune system is the body's defense mechanism against various diseases and infections. Naturally, the deficiency of the immune system causes a long list of immune system disorders. Natural compounds with medicinal properties have been of special interest to people since the distant past due to their use in curing diseases.

What Does This Study Add?

Since the intensity of respiratory explosion and the ability to produce nitric oxide in phagocytic cells are significantly reduced, it seems that padzahr water can be considered as a natural compound that modulates the immune system.

Authors' Contributions

All authors had an equal role in conducting the study and all authors read and approved the final manuscript.

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Conflicts of Interest Disclosures

The authors report no conflicts of interest.

Consent For Publication

not applicable

Ethics approval

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