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EFFECT OF IMMUNIZATION ON SOME ASPECTS OF IMMUNE RESPONSE IN INFECTED MICE WITH ECHINOCOCCUS GRANULOSUS

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Abstract
The present study was conducted as an attempt to chemically treat secondary hydatid disease in white mice of Mus musculus strain Balb/c by Immunity Boost used Immunomodulators, including antioxidant by used immunological test for example Delayed type hypersensitivty reaction (DTH), Phagocytosis, Micronucleus assay, Agar gel immune diffusion assay.

Keyword: - Echinococcus granulosus, Immunization, Immunlogical test.

Introduction

Hydatid cyst disease spreads throughout the body, and only the hair and nails survive. Therefore, the infection with hydatid cyst is one of the dangers to human health, and the economic losses it causes to the poultry animals infected with hydatid cysts and the accompanying deficiency in Proteins, vitamins, milk and wool production for sheep and cows, as well as delayed growth and fertility, and damage to diseased organs such as liver and lungs and its effect on meat production (Gangadhar et al., 2012). Given the importance of hydatid cyst disease in terms of health And veterinary medicine, and the disease in Iraq is limited to E. granulosus, it has been approved experimentally in infecting mice as it is a good model similar to humans to study the growth and development of the parasite as well as its role in clarifying the relationship between the parasite and the host (Fotiadis et al., 1999).

The host's immune system may fail to control some parasitic diseases, which is why the importance of using vaccines for the purpose of immunizing the host using the killed parts of the parasite or its living parts (Gemmall and Lawson, 1986) as the parasites possess acomplex chemical and biological composition, and a large number of antigenic components that can be used in the immunization process, or used in immunological diagnosis, including hydatid cyst fluid antigens (HCFAg) to stimulate Cellular and humoral immune response (Hernandez- Pomi et al., 1997). Researchers have tried to use immunomodulators, including antioxidants, to strengthen immunity (Walker et al., 2004; Rigano et al., 2001).

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Material and Method

Immunological Test

1- Delayed type hypersensitivty reaction(DTH)

For the purpose of examining the cellular immune response of experimental mice according to the method of Ohta et al. (1983), the left foot of the experimental animals was injected with 0.1 ml of HCFAg after sterilization of the injection site, while the right foot was injected with the same volume of physiological phosphate buffer Sterile pH 7.2, 1 ml wine medical syringes were used for this purpose. Skin reaction was measured 3, 24 and 48 hours after injection using Vernea to measure the difference in thickness of the palm of the foot before and after antigen injection

2- Phagocytosis

The method of Hudson and Hay (1980) was followed to study the phagocytic activity in macrophages taken from the peritone al area as follows:-

1- After killing the experimental mice, an incision was made in the skin and from the ventral side, and the skin was pulled out to reveal the peritoneum membrane. The mice were injected with 3 ml of sterile PBS buffer in the peritoneal area, and massaged the abdominal area for 3 minutes. Then the cells were collected in a sterile plastic test tube.

2-The tubes were discarded at a speed of 2000 rpm for 5 minutes. The sear was discarded and the precipitate representing macrophages was washed three times with Hanks' ready-made solution HBSS pH 7.2, cells were suspended in 1 ml of the same solution and the concentration of cells was adjusted to 610 cells/ml

3- Mix 0.25 ml of the macrophage suspension prepared according to the above method with 0.25 ml of human serum of blood type AB with 0.25 ml of the killed yeast suspension.

Yeast was prepared to study phagocytosis by the method of Metcalf et al. (1986) as follows:-

1-Suspend 50 g of dry bread yeast Saccharaomyces cervisiae in 150 ml of sterile normal saline.

2-Put the suspension in a boiling water bath for an hour

3- Filter the stuck after cooling with double gauze cloths

4-Suspended cells were counted using a cell counting slide and the concentration was set at 610 x 1 cells/ml after staining them with trypan blue dye to ensure their viability.

5- Distribute the suspension in small tubes of a capacity of 5 ml and then keep in the freezer at -20° C. When using, dissolve the suspension in a water bath at 37° C for an hour, and wash twice before use using sterile physiological saline.

4-A drop of the mixture was taken and placed on a cell counting slide. After the cells were stabilized, at least 100 phagocytic and non-phagocytic cells were randomly counted under a light microscope with a power of 10 X. Then the phagocytic coefficient was extracted according to the following equation:-

phagocytosis factor = phagocytosis cells number/ total phagocytic cells number x 100%

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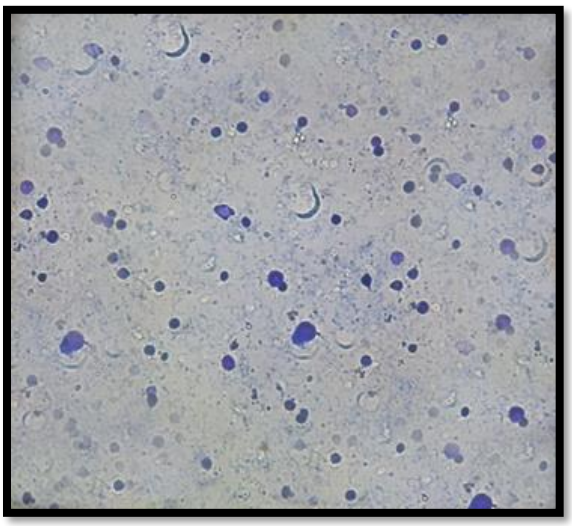
3- Micronucleus assay

The experiment was conducted according to Schimd (1975) method after killing the experimental mice and fixing them in the autopsy dish. The skin and muscle surrounding the femur were removed, and the end of the bone was cut off to be slowly injected with 2 ml of human serum to wash the bone marrow and collect it in clean tubes and sterile. Tubes containing bone marrow cells were centrifuged at 1000 rpm for 10 minutes. The precipitate was taken and the float was neglected, then a small drop of it was placed on the edge of a glass slide and a smear was made. The slide was left to dry at room temperature. The cells were fixed with methyl alcohol for 5 minutes, and the slides were stained after drying with Kamza dye for 15 minutes It was examined microscopically with a lens of100 X power, and five slices were made for each mouse. The percentage of small nuclei was calculated in 500 polychromatic erythrocyte cells with 100 cells per slide (Kirsch- Volders et al., 1997) according to the following equation: Small nucleus percentage =small nuclei number/total number of cells x 100



Picture 1: Micronuclei in the bone marrow of treated mice (100X).

4- Agar gel immune diffusion assay

The examination was performed according to the method of Bombarderi et al. (1974) with some modifications to measure humoral immunity in experimental mice, where agarose gel was prepared at a concentration of 1% by dissolving 1 gm of agarose in 100 ml of sterile normal saline, then pouring 20 ml of The agarose was placed in Petri dishes with a diameter of 10 cm, and after complete hardening, a central hole and 6 holes were made around the site. And put into the peripheral pits serum 50 µl/hole for experimental rats after sequential ascending dilutions (2:1,4:1, 8:1, 16:1, 32:1, 64:1) each time, and put into the hole Central hydatid fluid antigens (HCFAg) at a concentration of 3.36 mg/ml each time, the dishes were placed in a humid chamber in the refrigerator for 24-48 hours and the results were recorded

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Results:

Effect of immunization on some aspects of immune response in mice

1- Delayed type hypersensitivity (DTH)

Table 4-2 shows the rate of delayed hypersensitivity (DTH) in immunized mice and in mice immunized and dosed with antioxidant. By measuring the thickness difference in the left foot pad of mice, the study group showed the largest increase in thickness after 24 hours of HCFAg antigen injection, and the average increase in the immunized group of mice was 0.42 mm It increased with a high significant difference at the level of 0.001 ≥p in the immunized and dosed groups with antioxidants to 0.82 mm, and the thickness difference decreased after 48 hours of antigen injection, and it reached 0.2 mm and 0.6 mm.

Table 1: The rate of delayed hypersensitivity in experimental mice

Average footbed thickness (mm) after specified time intervals ± standard deviation			groups
48h	24h	3h	
0.2±0.01	0.42±0.02	0.27±0.01	Immunized group
0.6±0.04	0.82±0.06	0.62±0.03	Antioxidants added to the group
0	0	0	Negative control group
0.001≤	0.001≤	0.001≤	

2- Phagocytosis parameter

The rate of phagocytosis coefficient, as shown in Table 3-4, increased with statistical significance at the level of p≤0.001 in the group of immunized mice dosed with antioxidants by 74.0% compared to the group of immunized mice and by 65.50%, which also showed significant differences at the level of p≤0.05 compared to the control group.

Table 2: The rate of phagocytosis coefficient in experi

Phagocytosis coefficient ± standard deviation(%)	GROUPS
65.5 a *± 8.9	Immunized group
74.0 a **± 10.2	Antioxidants added to the group
53.75±6.33	Negative control group

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a Comparison with the negative control group
*There is a statistically significant difference (p≤0.05)
**There is a significant statistical difference (p≤0.001)

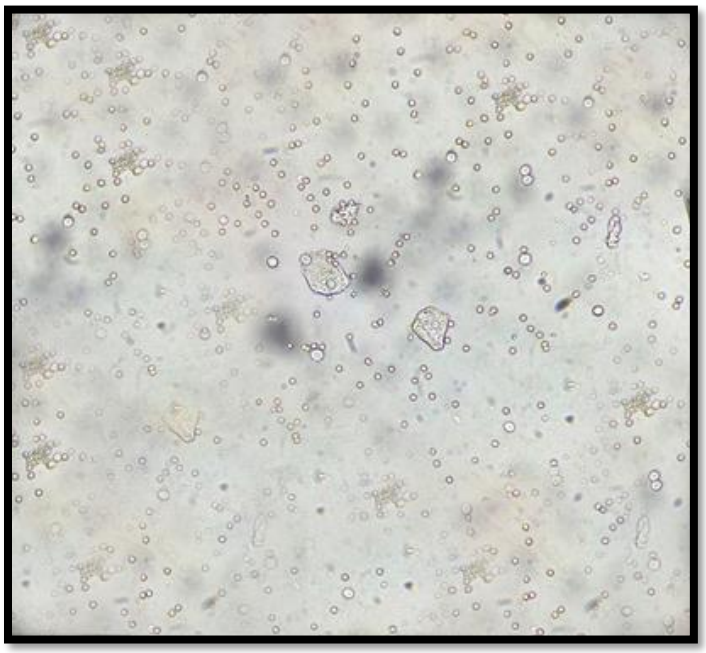

Figure 2: Phagocytosis in the interstitial cavity of treated mice (X10)

The results of the hematological and immunological tests of the experimental mice showed that the group of immunized mice dosed with antioxidants is the best, as well as the gross changes to that group, and then it was positively reflected on the external appearance, movement of mice, their weight and their ability to feed. Naturally with hydatid cysts and treated OFZ, OFZ + PZQ, OFZ + ABZ and ABZ + PZQ to study the hematological and immunological tests as well as the histological study.

Discussion

Many studies have been conducted globally and locally on the infection with hydatid cysts, some of which are related to its life aspects and others are related to the hematological and immunological aspects accompanying the infection, as well as studies that focused attention towards immunization against infection or its treatment.

Some aspect of immune response in mice

1-delayed type hypersensitivity reaction

When studying the specialized cellular immune response represented by measuring the delayed type hypersensitivity reaction in the foot pad, the results of the current study showed an increase in the average thickness of the foot pad in the immunized mice dosed with antioxidants and the treated mice,

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where the highest thickness was(0.82 mm) after 24 hours of antigen injection Then it gradually decreased after 48 hours (Table 1).

The increase in the thickness of the foot pad in experimental mice may who pointed out that the hallmark of hypersensitivity reactions is the accumulation of eosinophils in tissues preceded by the accumulation of Th2 lymphocytes, and concluded that delayed hypersensitivity reactions stimulate the secretion of chemical kinetics and reticulo-endothelial cells that are essential for eosinophil migration and inhibition of eosinophil migration, Th2 cells or using Anti-IFN, and it is clear the vital role they play be due to the ability of immunization and antioxidants to stimulate cellular immunity by stimulating the secretion of cellular kinetics, which in turn work in the transfer and recruitment of white blood cells selectively in allergic reactions, as occurs in the migration of eosinophils to sites of inflammation Late stages of lymphocyte accumulation, and this was confirmed by Teixeira et al. (2001) who pointed out that the hallmark of hypersensitivity reactions is the accumulation of eosinophils in tissues preceded by the accumulation of Th2 lymphocytes, and concluded that delayed hypersensitivity reactions stimulate the secretion of chemical kinetics and reticulo-endothelial cells that are essential for eosinophil migration and inhibition of eosinophil migration, The cells or using Anti-IFN, and it is clear the vital role they play Mast cells to determine neutrophil accumulation in T-dependent neutrophil cells in delayed hypersensitivity reactions mediated by the secretion of two types of TNF and Macrophage-Inflammatory Protein-1 (MIP-1). (Biedermann et al., 2000) The increase in the thickness of the foot pad may also be due to the ability of immunization and antioxidants to stimulate the secretion of TNF-α and IFN-γ, as Willey et al. (2008) indicated that IL-2, TNF-α and IFN-γ secreted by Th1 are responsible. For future hypersensitivity reactions.

These results were in agreement with the findings of Ptak and Asherson (1969) when studying delayed type hypersensitivity in mice, where mice injected with antigen mixed with the complete Freund's aide showed more swelling 24 hours after antigen injection into the left foot pad than after 4 hours of antigen injection. .

excessive Deferred sensitivity, which is an indication of cellular immunity, may be due to the infiltration of neutrophils, macrophages, mononuclear cells, and lymphocytes. Secondary hydatid cysts in albino mice.

This is consistent with what was observed by Ali-Khan (1974) in mice infected with the parasite E. multilocularis. As for the delayed hypersensitivity test in mice treated for four months, the increase in the thickness of the foot pad after 24 hours compared with the negative control group and the increase in the rate of delayed hypersensitivity reactions in the mice treated with OFZ and OFZ + PZQ compared to the other groups and compared to the positive control group (Table 4-). 4 It can be attributed to the efficiency of the therapeutic method used in the current study (immunization + antioxidants + chemotherapy) in reducing hydatid cysts, And the absence of hydatid cysts and for the efficiency of the treatment, and immunization and treatment may play an important role at the beginning of the infection with primary prostatitis.

This was in agreement with Tabar and Borji (2010). As for the positive control group, it showed a severe skin reaction when measuring the thickening difference, and this reaction is caused by the appearance

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of antibodies to the antigens of the primary primates and the production of immune globulins in the early infection.

As a result of dependent mechanical stimulation in T cells (Baz et al., 2006). Alazawi and Abdul Majeed (2014) suggested that the thickening that occurs in the foot pad due to cell-mediated immunity is mediated by the secretion of many cytokines from mononuclear cells, T lymphocytes and macrophages that are activated by antigens.

And then there is a depletion of these T cells, which are involved with a decrease in the thickness of the foot pad as a result of the decrease in the antigen that stimulates the migration of lymphocytes.

2- Phagocytosis coefficient

The activity of phagocytosis is an indicator of the activity of a non-specific cellular immune response, the immunized mice and the antioxidants dosed showed a high phagocytic coefficient compared to the negative and positive control mice (Table 2).

The reason for this may be that the HCFAg immunization and the antioxidants act as an integral part of the regulatory action of phagocytic cells, The parasite may have a role in the production of lymphatic kinetochores that inhibit the killing of the echinococcosis granulocytic worms (Steers et al., 2001).

The researchers pointed out (Steers et al. 1997; Steers et al). And the different parts isolated from it have an effect on chelated macrophage cells in mice, and they also proved that this fluid has an effect on macrophages, Jenkins et al. (1990) The role of macrophages in experimental hydatid cyst injury is their activation to kill Echinococcus significantly in the first two weeks after infection, and Kanazawa et al. (1993) The ex vivo killing of protozoans is increased by IFN-γ, and decreased by IL-10. (Jenkins et al., 1990).

Regarding the phagocytic coefficient of the treated groups, it led to a significant increase in the rate of phagocytosis coefficient (Table 2).

The results indicate that the combination of immunization and therapeutic substances in treated mice leads to an increase in the rate of phagocytosis coefficient at higher rates compared to positive control mice and may be due to the stimulation of components Complement system, especially C3b, which participates in opsonization and attracts neutrophils to infection sites. When using different immune rates Against hydatid cyst disease in albino mice. Jenkis et al. (1990).

The production of IL-2 and INF-γ lymphocytes is produced during the immune response to TB vaccine that activates phagocytic cells, which is similar to HCFAg immunization.

The immune response requires an interaction between phagocytes and Th1 cells, as the phagocytes present antigens to the Th1 cells. IFN-γ stimulates phagocytic cells to remove pathogens within the cell, and that 95% of Parasites are killed one week after infection by activating phagocytic cells that kill the protoscolex (Jenkins et al., 1990). 3 small cores test.

There is an increasing need to assess the toxic effects of the large number of chemicals at the cellular level.

Examination of the micronucleus in treated mice is a sensitive indicator of chromosomal damage in the bone marrow, assessment of cytogenetic damage, and knowledge of the effect of chemotherapy by

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micronuclei testing (Kotova et al. , 2015). Groups of mice treated with OFZ, OFZ + PZQ, OFZ + ABZ and ABZ + PZQ showed significant differences compared with the positive and negative control groups (Table (4-6) The group of mice treated with OFZ and OFZ + PZQ recorded a significant decrease in the rate of micronuclei and the lack of chromosomal changes compared to with.

3- Micronucleus test

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Exposure to mutagenic factors (treatment) is likely to lead to an increase in small nuclei with a decrease in the number of white cells that suppress the immune system.

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