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Measurement of Interleukins in Mice Treated with Pro-30 Max Probiotics and Injected with Protoscoleces of *Echinococcus granulosus*

Arkan S. Sheet
Department of Biology/ College of Education for Pure Sciences/ University of Tikrit
Asmaa A. Ali
Department of Biology/ College of Education for Pure Sciences/University of Mosul
Abdulkhaliq A. Mohaimeed
College of Science/ University of Tikrit/ Tikrit
Luay Rashan
Dhofar University Research Center/ Salalah/ Sultanate of Oman

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corresponding author:
Arkan S. Sheet
arkan.s.sheet@st.tu.edu.iq
Asmaa A. Ali
dr.asmaa_abdulaziz@uomosul.edu.iq
Abdulkhaliq A. Mohaimeed
Raheem_aljuboorie@tu.edu.iq
Luay Rashan
lrashan@du.edu.om

ABSTRACT

Echinococcus granulosus is a causative agent of cystic echinococcosis, with a cosmopolitan allocation. The current study investigated levels of interleukins IL-2, 12, 13 and IL-15 in the serum of mice treated with PRO-30 Max probiotics, comprising (*Lactobacillus acidophilus, L. plantarum, L. casei, L.reuteri, Bifidobacterium bifidum, B. lactis, B. longum, and Streptococcus thermophilus*), then infected with the protoscoleces of *E. granulosus*. Three dilutions of probiotics, 18×10^{-8}/1 ml, 14×10^{-9} / 1 ml, 12 x 10^{-10}/ ml (CFU), and three periods 3, 5, and 7 months were applied, 0.1 ml of each dilution was injected in mice intraperitoneally, before and after infection with the protoscoleces. On the fifth day of infection, bacterial infection was continued every 72 hours for all periods. Blood was drawn from the ophthalmic venous plexus; serum samples were kept at -20° until use. Interleukins were estimated using ELISA technique. The highest level of IL-2 was at dilution 18×10^{-8}, 195.29 pg/ml, compared with the C+, 30.212 pg/ml after seven months of infection. The highest level of IL-12 was at dilution 18×10^{-8}, 195.19 pg/ml, compared with the C+, 35.955 pg/ml after seven months of infection. The lowest level of IL-13 in mice treated with the dilution 18×10^{-8}, was 31.755 pg/ml, after three months of infection, compared with the C+, 190.919 pg/ml after the seventh month of infection. An increase in the level of IL-15 was observed at the dilution 18×10^{-8}, 193.012 pg / ml, compared with the C+, 30.022 pg/ ml after seven months of infection.

Keywords: *E. granulosus*, interleukins, probiotics, protoscoleces.

INTRODUCTION

Cystic echinococcosis is a chronic parasitic disease caused by the tapeworm *Echinococcus granulosus*. Echinococcosis is a common parasitic disease in humans and ruminants, it is considered an economic and health problem that threatens human health and social development in most parts of the world (Li *et al*., 2019; Zhang *et al*., 2021; Al-Arabi *et al*., 2022). The zoonotic disease is caused by the larval stage of *E. granulosum* (Darwesh and El-Sayed, 2022; Eckert and Thompson, 2017; Al-Taie *et al*., 2022). Sheep and other herbivores act as intermediate hosts, while dogs and other carnivores serve as definitive hosts for this tapeworm (Tamarozzi *et al*., 2016). Humans can become an accidental host or so-called dead-end host by unintentionally consuming food and drink contaminated with the parasite's eggs which are excreted with the definitive host's feces (Regassa, 2019). The eggs hatch in the human alimentary canal into tumor balls (six- spine embryos) and travel through the circulatory system to various organs in the body. These embryos develop into hydatid cysts, most of which are in the liver (69-75%), and lungs (17-22%), but they may affect other organs and tissues (Regassa, 2019; Ghasemirad *et al*., 2022). The incubation period of the disease is long; therefore, most patients do not suffer from any clinical symptoms at the beginning of the disease. The symptoms appear due to the growth of hydatid cysts and abscesses according to their size and location, and then the patients become chronically infected (Khoshnaw and Al-Sakee, 2022; Yadegari *et al*., 2022). The growth stages of the parasite take place slowly inside the human body, which makes predicting infection more difficult, therefore the clinical diagnosis of the disease will focus on indirect symptoms rather than identifying the parasite directly. The most important diagnostic methods in the United States are the ultrasound and serological assays which are usually used, especially the enzyme-linked immunosorbent assay (ELISA) based on the hydatid cyst fluid antigen of *E. granulosus* (Gessese, 2020).

Hydatid cysts in the liver can be treated with surgical or non-surgical methods such as ultrasound therapy (Ramdan and Ali, 2020), nanoparticle therapy such as selenium nanoparticles that have been used as anti-parasitic alternatives in recent years, percutaneous treatment and chemotherapy (Arif *et al*., 2011; Mohammed and Ali, 2022). Chemotherapy can reduce the size and tension of cysts, in other cases may kill the components of the cyst (Kandil *et al*., 2021). Albendazole and praziquantel are often used to treat hydatid cysts (Roos *et al*., 1993). Praziquantel was an exterminator of the protoscoleces and can be combined with Albendazole to enhance efficacy (Sharafi *et al*., 2017). The World Health Organization (WHO) has divided liver hydatid cysts into five types (CE1 to CE5) and three biological stages (active, transitional, and inactive).

Parasitic diseases can be controlled and prevented through hygiene or getting rid of worms using worm-repelling pills as well as vaccines. Because of the side effects of these drugs and vaccines on human health, as well as the resistance of the worms to these drugs, it became necessary to resort to new strategies to combat parasitic infections, as a substitute for medication. Probiotics are aerobic or anaerobic microorganisms that are found naturally in various dairy and non-dairy products, can be easily isolated (Plaza-Diaz *et al*., 2019), and are beneficial to the host when taken in sufficient quantity. The most common species used are *Lactobacillus enterica* and *Enterococci* (Darwesh and El-Sayed, 2022). The protective effect of probiotics comes through several mechanisms, including the ability to produce anti-microorganisms, such as bacteriocins or H_2O_2 and lactic acid, or through colonization and competition between probiotics and preventing the adhesion of pathogens inside the intestine, their ability to competitively inhibit the reproduction of causative bacterial diseases by altering pH and reducing oxygen availability leading to less favorable intestinal conditions, and non-competitive inhibition by bacterial production (Azad *et al*., 2018; Terpou *et al*., 2019), in addition, their products may expel parasitic worms, as well as the possibility of reducing their ferocity. Among the important roles that these probiotics play in the host's body are the modification of the gut microbiota, the enhancement of immunity, the reduction of diseases and stress, nutritional assistance, and the modulation of the immune system by the production of immunoglobulin antibodies (Marrez *et al*., 2018; Sadek *et al*., 2018; Chugh and Kamal-Eldin, 2020; Yousif and Ali, 2020).

Probiotics interact with intestinal epithelium, immune cells in Peyer's patches and the secretory IgA antibody that plays an important role in mucosal immunity. Together these factors act as a barrier against harmful microorganisms. Probiotics also affect dendritic cells that have the function of devouring antigens from the intestine and presenting them to normal T cells, leading to their differentiation into T helper cells (Th1, Th2) or regulated lymphocytes (Liu and Yin, 2022). It has also been shown that probiotics have other roles in enhancing the immune system, such as modifying the release of cytokines such as IL-2, IL-12, IL13, and IL-15, these cytokines have a major role in maintaining the delicate balance between defense techniques (Hayes *et al*., 2010). Cytokines are low molecular weight proteins with multidirectional activities produced by TH1 and TH2 immune cells (Martinović *et al*., 2020; Mohamed and Altaii, 2020), playing an important role in the immune response and regulating inflammation, stimulating the natural and acquired immune system such as cell growth, tissue repair and blood formation. As a mediator between cells, these cytokines perform an important function in determining the level of immune response. Cytokines can stimulate similar signaling pathways in cells or affect different signaling pathways in different cells (Noronha *et al*.,1995; Ismael *et al*., 2022). Echinococcosis can stimulate or suppress the immune response that develops for a long time in hosts, in addition to inducing TH1 and TH2 cell cytokines to different immune pathways to counter parasitic infection, TH1 cell cytokines such as IL-2, IL-12 and IL-15 coordinate responses cellular immune responses, while TH2 cell cytokines such as IL-13 coordinate humoral immune responses (Abo-Aziza *et al*., 2017).

Cytokines act as immune regulators, and their average half-life is short. For this reason, we find it difficult to measure them sometimes in serum. Many factors affect the level of cytokines in the blood, including the type of anticoagulant used when collecting a blood sample, stress, dietary habits, and the patient's condition (his physical activity), duration and method of storage. Immune cells can release inflammatory factors during the blood clotting process, which lead to changes in the level of cytokines (Zhou *et al*., 2010). The interaction between the host's immune system and *E. granulosus* occurs through cytokines and immune cells (Zhang *et al*., 2016).

T-helper cells are efficient immune cells responsible for the secretion of TH1, TH2 immune mediators. The host immune system organizes defensive strategies depending on infectious agents. Echinococcosis can stimulate or suppress an immune response that continues and develops for a long time in its host. Echinococcosis can stimulate or suppress the immune response that develops for a long time in the host, rather than inducing TH1, TH2 cell cytokines to different immune pathways to counter parasitic infection, TH1 cell cytokines coordinate cellular immune responses, while TH2 cell cytokines coordinate humoral immune responses. It was found that the cellular immune response in infected animals is characterized by the penetration of inflammatory cells, and the dominance of cytokines of TH1 cells, compared to uninfected animals, which show an increase in the humoral immune response with high levels of IgG, high levels of IL-6 were found in naturally infected animals with Lower levels of IL-4, IL-10 compared to their uninfected counterparts (Noronha *et al*.,1995). This presence of TH1 and TH2 cytokines, which is a feature of *E. granulosus*, may be due to the presence of different echinococcosis antigens with distinct antigenic determinants for both types of T cells (Hidalgo *et al*., 2019).

In studies concerning experimental infection of mice with echinococcosis after injection with live protoscoleces, researchers have found that the cytokine response exhibits biphasic effects, such as an early, dominant induction of Th1 interleukins, IFN-γ, IL-2, and IL-15, followed by a shift towards Th2 interleukins, IL-4, IL-5, IL-6, IL-10, and IL-13. Mostly, it was demonstrated that the Th2 response will establish the parasite, while Th1 response will be lethal to the parasite, this complex integration may be due to the interaction of regulatory effectors, leading to mixed TH2/TH1 responses. A recent study found dynamic patterns that support the shift of the immune response from TH1 to TH2 (Li *et al*., 2020).

A study concerning peripheral blood mononuclear cells, exhibited that increased concentrations of IL-4 and IL-10 were found to be consistent with TH2 cell activation, while γ-IFN production indicates involvement of a subset of TH1 cells (Rigano *et al*., 1995). IL-4 was found to

be the most increased cytokine (68.7%) during infection by echinococcosis (Bayraktar *et al*., 2005). In addition, an increase of IL-2, 46.8% and IL-10, 40.6% was identified. The coexistence of TH1 and TH2 cytokines was demonstrated in echinococcosis, while cytokines of TH2 cells were more dominant. The clinical status of infection, whether primary infection or relapse, affects the immune response to the parasite as well (Hernandez-Pomi *et al*.,1997). Another study by Al-Mayah *et al*. (2018) revealed that patients infected with hydatid cysts in active stages displayed elevated levels of IL-10, IL-4 and IL-13, thereby enhancing parasite survival by reducing protozoan mortality. Biranvand *et al*. (2020) detected those levels of IL-13 and IL-4 in peripheral blood mononuclear cells cultured *vivo*, *in vitro* decreased significantly after surgical treatment, while the increase in IFN-γ, IL-2, IL-12, and IL-15 was observed after treatment. Zhang and McManus (2006) confirmed the response of TH1 cytokines after surgical treatment, and the response of TH2 cytokines was dominant in the active and transitional phase of the disease, which contributes to the immune response to parasitic agents, as the increase in the level of TH2 cytokines after surgical removal of cysts indicates that surgery stimulates a series of infections such as elevated body temperature, erythrocyte sedimentation rate, leukocyte sedimentation rate, and increased acute phase reactions (Ali and Hussain, 2016). Echinococcosis cytokines may be associated with disease outcomes after clinical interventions (Naik *et al*., 2016).

This study was conducted, for the first time, to determine the lethal effectiveness of Pro-30 Max probiotics on protoscoleces *in vitro* and against hydatid disease *in vivo*.

MATERIALS AND METHODS

Laboratory Animals

In the current study, male Swiss albino mice, BALB/c strain, 3-4 weeks old, were obtained from the animal house, College of Veterinary Medicine, University of Mosul, were used.

Cysts Samples

Hydatid cysts were obtained under sterile conditions from the livers of infected sheep containing live protoscolices. The vitality of the primates was tested by adding 20 µl of the protoscoleces suspension to 20 µl of 0.1% eosin, then examined under a light microscope. The live protoscolices appeared in a bright green color due to the exclusion of the pigment by the membranes of the protoscolices, while the dead protoscolices appeared in a red color due to the acceptance of the pigment (Smyth and Barrett, 1980).

Bacterial Strain Preparation and Inoculation

PRO-30-Max bacteria were obtained from (Natures Aid Co) in the United Kingdom, in the form of capsules, and each capsule contained 150 mg, equivalent to 30 billion cells from eight species belonging to three genera, *Lactobacillus*, including species *L. acidophilus*, *L. plantarum*, *L. casei*, *L. reuteri*, *Bifidobacterium*, including *B. bifidum*, *B. lactis*, *B. longum*, and *Streptococcus*, includes S. *thermophilus*. Bacteria were activated in Nutrient broth, incubated at 37°C for 24 hours. The growth of probiotics was examined by preparing a slide of activated bacteria from nutrient broth and stained with Gram stain, to assure the growth of Gram positive bacteria, three dilutions of bacteria were prepared by adding 0.1ml of the activated bacteria to the tube, The first one contained 0.9 ml of normal saline, and the preparation of the dilutions continued to the dilution 10^{-10}, 0.1 ml was taken from each of the dilutions 18×10^{-8}, 14×10^{-9}, and 12×10^{-10}, and cultured on Nutrient agar medium with three replications for each dilution. The plates were incubated at 37°C for 24 hours. Colonies were counted for each of the three dilutions 18×10^{-8}, 14×10^{-9}, and 12×10^{-10}, recording 18 colonies in the 10^{-8} dilution, 14 colonies in the 10^{-9} dilution, and 12 colonies in the 10^{-10} dilution (Goyal *et al*., 2011; Coêlho *et al*., 2013).

Experimental Design

The first experiment: 20 mice at the age of one month were taken and divided into four cages, 5 mice were placed in each cage, mice in the first three cages were injected with the PRO-30 Max bacteria, mice in the first cage were injected with the dilution 18×10^{-8} CFU/ml, in the second cage with the dilution 14×10^{-9} CFU/ml, and in the third cage, with the dilution 12×10^{-10} CFU/ml, with

two consecutive doses 48 hours apart, then mice were injected with 2000 protoscoleces on the fifth day of activation (that is, four days after the mice were given the first dose of bacteria). Injection of bacteria continued every 72 hours, for a period of 80 days, mice in the fourth cage were injected with live protoscoleces as a positive control group. After three months, mice were anesthetized, blood was drawn, and serum was obtained.

The second experiment: 20 mice aged one month were divided in four cages, 5 mice for each, mice in the first three cages were injected with the PRO-30 Max bacteria, mice in the first cage were injected with a dilution 18×10^{-8} CFU/ml, in the second cage with dilution 14×10^{-9} CFU/ml, and in the third cage with the dilution 12×10^{-10} CFU/ml with two consecutive doses 48 hours apart, then mice were injected with 2000 protoscoleces on the fifth day of activation, injection of bacteria continued every 72 hours, for a period of 140 days. Mice in the fourth cage were injected with live protoscoleces as a positive control group. After five months, all mice were anesthetized, blood was drawn and serum obtained.

The third experiment: 20 mice at age one month were divided in four cages, 5 mice were placed in each cage, mice in the first three cages were injected with the PRO-30 Max bacteria, mice in the first cage were injected with the dilution 18×10^{-8} CFU/ml, in the second cage injected with dilution 14×10^{9} CFU/ml, and in the third cage with dilution 12×10^{-10} CFU/ml, with two consecutive doses 48 hours apart, then mice were injected with 2000 protoscoleces, on the fifth day of activation, injection with bacteria continued, every 72 hours, for a period of 200 days. Mice in the fourth cage were injected with live protoscoleces as a positive control group. After seven months, all the mice were anesthetized, blood was drawn, serum obtained.

Measurement of Cytokines Level by ELISA Technique

Blood Serum

Mice were anesthetized with a solution of diethyl ether, and blood was drawn from ophthalmic venous plexus according to Waynforth and Flecknell (1980), blood was collected in gel tube, left in the tubes for 10 minutes, then centrifuged for 15 minutes, serum was kept in Eppendorf tubes in the freezer -20℃ until use.

Determination of Cytokines Concentration

Sandwich ELISA technique was used to determine the level of cytokines IL-13, IL-12, IL-2, IL-15 in the serum of mice infected with hydatid cysts, according to the instructions of the Chinese manufacturer Sunlong Biotech Co. Ltd. The principle of the Sandwich ELISA technique is based on the interaction between antibodies Stabilized in the bottom of the well's pits with cytokines in the sample of serum, if any, when the enzyme-tagged antibodies were added, the final immune complex (antibody-antigen complex) was formed, which was detected by the color change occurred when adding the substrate, as it is proportional to the intensity of the color change which is directly proportional to the cytokine concentration in the sample. Stop solution was used as a final stage to pause the reaction at a certain point. The intensity of the color change was measured using a plate reader at a wavelength of 450 nm and using the standard curve.

METHOD

The samples and reagents were left to reach the laboratory temperature.

1. Preparation of Standard Protein for Interleukins:

- A series of serial dilutions was prepared from the standard solution at a concentration of 270 pg/ml in the test kit. Five tubes were prepared, numbered 1-5.
- 300 µl of standard solution was added to tube No. 1 (270 pg/ml in the test kit), 150 µl of standard diluents was added, and mixed well, resulting in a concentration of 180 pg/ml.
- (300µl) was withdrawn from tube 1, added to tube 2, in addition to 150µl of standard diluents, mixed well, resulting in a concentration of 120pg/ml

- (150µl) was withdrawn from tube 2, added to tube 3, in addition to 150µl of standard diluents, mixed well, a concentration of 60pg/ml
- (150µl) was withdrawn from tube No. 3 and added to tube No. 4 in addition to (150µl) of standard diluents and mixed well, yielding a concentration 30pg/ml, (150µl) was withdrawn from tube 4, added to tube 5, in addition to (150µl) of standard diluents, mixed well, resulting a concentration 15pg/ml Fig. (1).

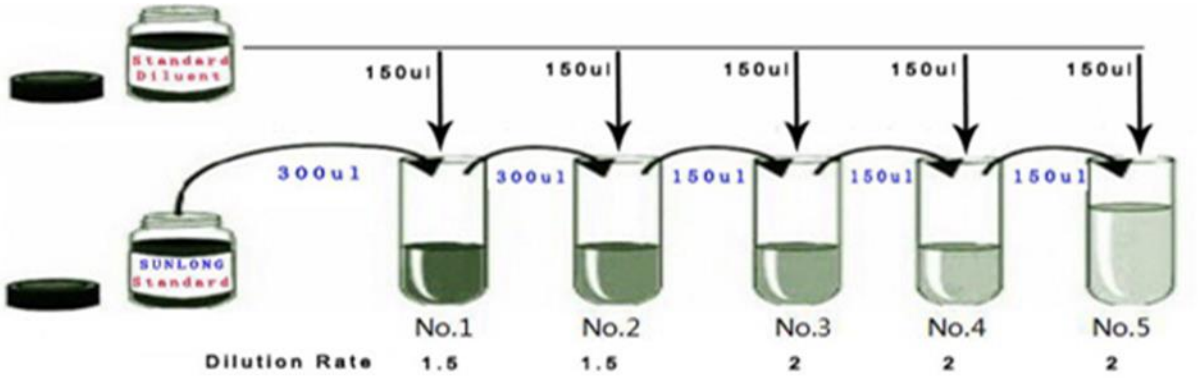

Fig. 1: Steps for preparing a series of dilutions of a standard solution of interleukins

2. Six empty Blank meter wells were left in ELISA plate, 40µl of sample diluent and 10µl of serum (with a dilution factor 5) were added into the sample wells, mixed well with careful stirring.

3. Incubation: The plate is incubated after being covered with a Plate Sealer for 30 minutes at 37 ℃.

4. Dilution: The washing solution diluted 30 times in 1 liter of distilled water. 5. Washing: The plate cover was carefully removed, the plate was washed, adding 300 µl of washing solution in each hole, left for 30 seconds, and the washing process was repeated.

5. Washing: The plate cover was carefully removed, the plate was washed, adding 300 µL of wash solution into each hole and left for 30 seconds, and the washing process was repeated 5 times with the scrubber. The plate was inverted to remove the remaining solution on a paper towel.

6. 50µl of HRP conjugate reagent was added to each hole in the plate except the blank control one, then covered with a plate cover.

7. Incubation: The plate is incubated after being covered with a Plate Sealer for 30 minutes at 37°C.

8. Washing: The plate cover was carefully removed, the plate was washed, adding 300 µL of wash solution into each hole and left for 30 seconds, and the washing process was repeated 5 times with the scrubber, the plate was inverted to remove the remaining solution on a paper towel.

9. Staining by adding Substrate: 50µl of (Chromogen A) solution and 50µl of (Chromogen B) solution was added to each hole, mixed with stirring, incubated at 37°C For 15 minutes.

10. Ending the reaction: 50µl of stop solution (sulfuric acid H_2SO_4) was added to each well to finish the reaction. The color in the pits should change from blue to yellow.

11. Reading the absorbance OD at a wavelength of 450 nm using a Microtiter Plate Reader, the test should be carried out within 15 minutes after adding the reaction termination solution, finally the standard curve was drawn by computer Fig. (2, 3, 4, 5).

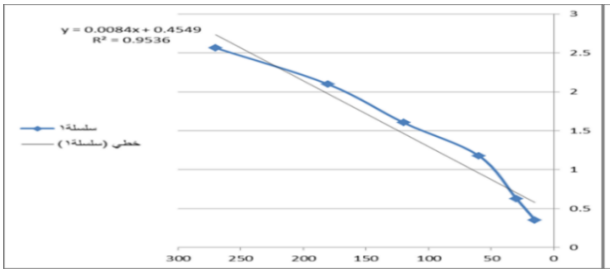

Fig. 2: Standard curve of IL-2

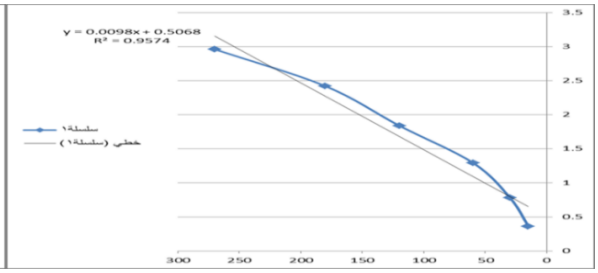

Fig. 3: Standard curve of IL-12

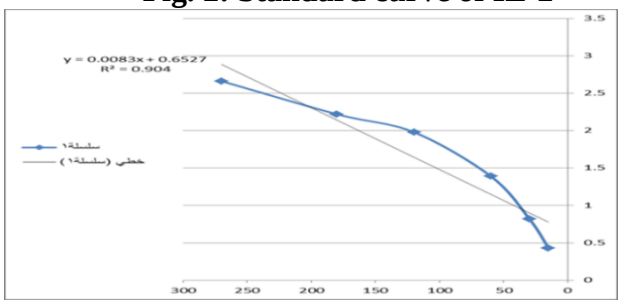

Fig. 4: Standard curve of IL-13

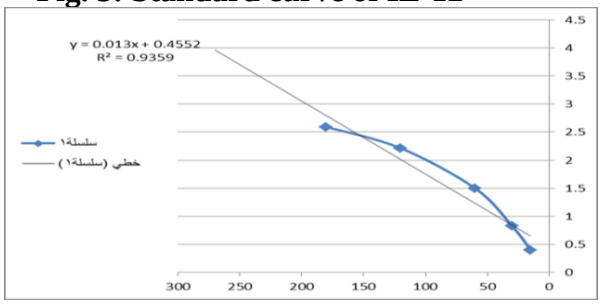

Fig. 5: Standard curve of IL-15

X: concentration of interleukins, Y: absorbance at wavelength 450 nm

Statistical Analysis

The data were analyzed statistically according to the Completely Random Design (CRD) to obtain the effect of each of the five treatments and the three periods and the interfere between them, the differences between means were compared according to Duncan's Multiple Range Test. Statistical Analysis System (SAS) program was applied (Al-Zubaidy and Al-Falahy, 2016).

RESULTS

Level of Cytokines in the Treated Mice Compared to the Control Group

The current study showed significant differences at a potential level with $P \leq 0.001$ of interleukins 2, 12, 13, 15 for mice infected with hydatid cysts treated with bacteria compared with the positive control group, (Table 1) showed the highest level of IL-2 was at dilution of 18×10^{-8}, 195.29 pg/ml, compared with the positive control group, 30.212 pg/ml, seven months post infection. (Table 2) showed that there were highly significant differences in the level of the cytokine IL-12, at dilution 18×10^{-8}, 195.19 pg/ml, compared with the positive control group, 35.955 pg/ml, after seven months of infection. Table (3) revealed significant differences in the level IL-13, the lowest level was 31.77pg/ml at 18×10^{-8} after three months of infection, compared with the positive control group, 190,919 pg/ml after seventh month of infection. Table (4) showed high significant differences of IL-15 at 18×10^{-8}, 193,012 pg/ml, compared with the positive control group, 30,022 pg/ml seven months after infection.

Table 1: level of IL-2 in mice infected with protoscoleces and treated with PRO-30 Max probiotics by Duncan's test

Treatments / Eriods	3 Months	5 Months	7 Months	Means of treatments
18×10^{8} CFU/ml	132.102e	147.311c	195.29a	158.234a
14×10^{9} CFU/ ml	126.223f	135.411d	170.701b	144.111b
12×10^{10} CFU/ml	95.088i	102.132h	112.303g	103.174c
Control+	51.463j	39.501k	30.212m	40.392d
Means of periods	101.219c	106.08875b	127.1265a	

Similar letters indicate no significant difference, different letters indicate significant differences.

Table 2: level of IL-12 in mice infected with protoscoleces and treated with PRO-30 Max probiotics by Duncan's test

Treatments / Periods	3 Months	5 Months	7 Months	Means of treatments
18×10^8 CFU/ml	163.402d	177.492c	195.19a	178.6947a
14×10^9 CFU/ ml	135.802f	145.637e	183.431b	154.9567b
12×10^{10} CFU/ml	105.574i	115.602h	124.745g	115.307c
Control+	56.088j	45.183k	35.955l	45.742d
Means of periods	115.2165c	120.9785b	134.83025a	

Similar letters indicate no significant difference, different letters indicate significant differences.

Table 3: level of IL-13 in mice infected with protoscoleces and treated with PRO-30 Max probiotics by Duncan's test

Treatments / Periods	3 Months	5 Months	7 Months	Means of treatments
18×10^8 CFU/ml	31.755k	40.583j	50.088i	40.80867d
14×10^9 CFU/ ml	100.575h	109.602g	119.745f	109.974c
12×10^{10} CFU/ml	130.802e	140.636d	175.831b	149.0897b
Control+	155.802c	172.299b	190.919a	173.0067a
Means of periods	104.7335c	115.78b	134.14575a	

Similar letters indicate no significant difference, different letters indicate significant differences.

Table 4: level of IL-15 in mice infected with protoscoleces and treated with PRO-30 Max probiotics by Duncan's test

Treatments / Periods	3 Months	5 Months	7 Months	Means of treatments
18×10^8 CFU/ml	170.606b	177.578b	193.012a	180.398a
14×10^9 CFU/ ml	128.444e	137.121d	151.411c	138.992b
12×10^{10} CFU/ml	100.613h	105.184g	112.933f	106.243c
Control+	51.271i	42.315j	30.022k	41.202d
Means of periods	112.7335c	115.5495b	121.8445a	

Similar letters indicate no significant difference, different letters indicate significant differences.

DISCUSSION

The results of the current study, which investigated the measurement of the level of interleukins IL-2, IL-12, IL-13 and IL-15 in the sera of mice treated with PRO-30 Max bacteria, showed an increase and decrease in the level of cytokines secreted by T helper cells of both types TH1 and TH2 when compared with the positive control group, cytokines are proteins that play an important role in the immune response, regulating inflammation, and stimulating the natural and acquired immune system such as cell growth, tissue repair, and blood formation. By playing the role of mediators between cells, these cytokines will perform an important function in determining the level of immune response and have multidirectional activities produced by TH1 and TH2 immune cells. (Rigano *et al*., 1995; Martinović *et al*., 2020).

The present study agreed with the study of (Beyhan *et al*., 2022) who conducted studies on 142 patients aged 18-95 years. The serum of 51 patients with echinococcosis was diagnosed, as well as the serum of 53 patients with fascioliasis and 38 patients as a control group. Then serum samples were taken from the patients and the levels of cytokines IL-4, IL-10, TNFα and IFNγ were examined using ELISA method. The results were evaluated by spectrophotometer and an increase in immunogenic factors was noted in each infection. The cytokine scores were also evaluated according to the age groups and sex of the patients. IL-4, IL-10, TNF-α, and IFN-γ responses were detected in 50.9%, 44.2%, 43.3%, and 43.3% of patients with echinococcosis and in 43%, 39.2%,

34.4%, and 40.6% of patients with fascioliasis, respectively. TH1 and TH2 responses were observed in both infections. There was a significant relationship between fascioliasis and the IL-4 response and between echinococcosis and the responses to IL-4, IL-10 and TNF-α.

The current study agreed with a study conducted by (Ali, 2021), the study dealt with measuring the level of two pro-inflammatory cytokines such as IL-12 and IL-8 in the blood serum of females infected with the *Trichomonas vaginalis*, a single-celled parasite presents in the female and male reproductive tract, transmitted through sexual intercourse. The results showed that the level of IL-12 was significantly elevated in the acute (early) phase, but the level decreased in the chronic (late) phase of the disease. While the level of IL-8 was clearly higher along the acute and chronic disease stages in female patients, when compared to the control group. The results indicated that the level of the studied inflammatory cytokines during infection may change according to the localized infection of *T. vaginalis*, and this supports our current study that IL-12 is a pro-inflammatory interleukin and is increased in the acute (early) phase of the parasitic disease.

Siracusano *et al.* (2012) research, explained an increase in TH1 cell cytokines such as IFN-γ, showed that IFN-γ improves the ability of macrophages to kill protoscolces, while TH1 cell cytokines such as IL-4 and IL-10 reduce this activity. IFN-γ is the key that stimulates and activates the function of macrophages, through the production of nitric oxide (NO). Inhibiting the growth and function of helminths, in addition to other pathogenic factors, plays a role in establishing protective immunity by TH1 during *E. granulomas* infestation, and the destructive role of nitric oxide associated with the production of TH1 response works to reduce the vitality of the parasite. Elevated levels of IFN-γ and NO are found both *in vitro* and *in vivo* during infections in humans, while no detectable levels appear upon reinfection. Most investigators concerning human and mouse studies dealt with the dominance of TH1 response, which is characterized by IFN-γ release after being produced by dendritic cells (DCs) with IL-12, as both were shown to be effective in eliminating the parasite at the early stage of infestation. It was also found that the parasite, through excretory/secretory products, can influence the host's immune response. TH2 cells and parasite survival, which are specifically related to IL-4, IL-5, IL-10, and TGF, are generally related to the parasite's receptive capacity, which leads to chronic infection (Amri *et al*., 2009; Grubor *et al*., 2017).

The current study differed from a recent study conducted by (Li *et al*., 2020) on patients with different stages of hydatid cysts, who noticed an increase in the level of TH1 cytokines and a number of inflammatory cytokines before chemotherapy and in the active stages of the disease during the first year, while TH1 cytokines decreased after. Treatment in the chronic stages after the second and third year of the disease. The differences may be attributed to the stimulation of antibody-mediated TH2 immunity with a parallel increase in the level of TH1-mediated inflammatory responses as an important mechanism of host defense against the parasite (Kakkos *et al*., 2001).

The present study diverged in comparison to a study conducted by (Rostami-Rad *et al*., 2018), who observed a shift in the type of cytokine secreted by TH1-Type cells to TH2-Type, as there was an increase in IFN-γ after the first month of infection, and TH2 cell responses prevailed. Three months after the injury, it was also found that the cytokines IFN-γ, TNF, and IL-2 were prevalent in the early post-injury stage, that is, in the first three months of the injury. However, a decrease in IL-4 appeared after the fourth week of the injury. This shift in cytokine types explains that echinococcosis is a persistent disease and confirms the TH1 reaction in combating *E. granulosus* infection. The shift is the result of activation of immunosuppressive cells, in addition to resistance to the parasite. This discrepancy may be due to the vitality and size of the initial dose injected in the experimental model, as low doses stimulate TH1 responses while high doses stimulate TH2 responses (Stoore *et al*., 2018).

CONCLUSION

Consequences of the current study detected that probiotic had an effective role in regulating the immune response of the host through many of the integrated mechanisms, which had a positive effect on controlling the level of interleukins produced by immune cells such as T-helper cells of both types TH1 and TH2, an increase in the interleukins produced by both types was noticed. TH1 cells in the early stage of infection, but they decrease when responding to treatment with probiotics, on the contrary, an increase in interleukins produced by TH2 cells was noticed in the late stages of infection, not responding to treatment compared to positive control groups in all concentrations and periods.

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قياس الإنترلوكينات في الفئران المحقونة بالرؤيسات الاولية للمشوكة الحبيبية والمعالجة بالبروبيوتيك Pro-30 Max

اركان سعيد شيت

قسم علوم الحياة/ كلية التربية للعلوم الصرفة/ جامعة تكريت/ تكريت/ العراق

أسماء عبد العزيز علي

قسم علوم الحياة/ كلية التربية للعلوم الصرفة/ جامعة الموصل

عبد الخالق علوان محيميد

كلية العلوم/ جامعة تكريت

لؤي رشان

مركز بحوث جامعة ظفار/ صلالة/ سلطنة عمان

الملخص

تعد المشوكة الحبيبية *Echinococcus granulosus* العامل المسبب لداء المشوكات الكيسي cystic echinococcosis عالمي الانتشار. هدفت الدراسة الحالية لقياس مستوى الانترلوكينات IL-2 و IL-12 و IL-13 وIL-15 في أمصال الفئران المعالجة بالمعزز الحيوي البكتيري PRO-30 Max bacteria الذي يضم (*Lactobacillus acidophilus, L. plantarum, L. casei, L.reuteri, Bifidobacterium bifidum, B. lactis, B. longum, and Streptococcus thermophilus*)، والمصابة بالرؤيسات الاولية للمشوكة الحبيبية *E. granulosus*، استخدمت ثلاث تخافيف من البكتريا 18×10^{-8}/ 14×10^{-9} 1 مل / 1 مل، و $1/12\times10^{-10}$ مل (CFU) وثلاث فترات زمنية 3،5، و 7 أشهر. اخذ 0.1 مل من كل تخفيف وحقن تحت البريتون للفئران قبل وبعد الإصابة بالرؤيسات الأولية، في اليوم الخامس من الاصابة استمر الحقن بالبكتريا كل 72 ساعة ولجميع الفترات. سحب الدم من الضفيرة البصرية الوريدية، بعدها حفظ المصل في درجة حرارة −20 درجة مئوية لحين استخدامه. قيست الانترلوكينات بتقنية الاليزا، إذ سجل أعلى ارتفاع لمستوى IL-2 عند التخفيف 18×10^{-8} والذي بلغ 195.29 بيكوغرام/ مل، مقارنة مع مجموعة السيطرة الموجبة، 30.212 بيكوغرام/ مل بعد سبعة أشهر من الاصابة. ولوحظ أعلى ارتفاع لمستوى IL-12 عند التخفيف 18×10^{-8}، 195.19 بيكوغرام/ مل، مقارنة مع مجموعة السيطرة الموجبة، 35.955 بيكوغرام/ مل بعد سبعة أشهر من الاصابة، وسجِّل أدنى مستوى لـ IL-13 في مصل الفئران المعاملة عند التخفيف 18×10^{-8} ، 31.755بيكوغرام/ مل بعد ثلاثة أشهر من الاصابة مقارنة مع السيطرة الموجبة، 190.919 بيكوغرام/ مل بعد الشهر السابع من الإصابة، لوحظ ارتفاع لمستوى IL-15 عند التخفيف 18×10^{-8} بلغ 193.012 بيكوغرام/ مل، مقارنة مع السيطرة الموجبة، 30.022 بيكوغرام/ مل بعد سبعة أشهر من الاصابة.

الكلمات الدالة: المشوكة الحبيبية، انترلوكينات، بروبايوتيك، رؤيسات اولية.