DOI: 10.24996/ijs.2024.65.12.10

ISSN: 0067-2904

The Role of Probiotics in Treating Echinococcosis in Mice

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Received: 11/9/2023 Accepted: 27/11/2023 Published: xx

Abstract

This study investigated the role of probiotics in treating echinococcosis, considering three time periods (3, 5, and 7 months) and three bacterial dilutions. Mice were injected subperitoneally before and after primary infection with the rudiments. Before infection, the mice were injected with the Pro-30Max probiotics twice, with 48 hours interval. On the fifth day, they were infected with protoscoleces, and after 48 hours they were treated with the bacteria. The injections continued every 72 hours for all periods. A decrease in the number and weight of hydatid cysts was recorded reaching the value of 0.00 mg. Whereas a decrease in the weight of the liver and spleen and their inflation factors were recorded after 7, 5 and 3 months. The lowest weight of the liver and spleen reached 1.2869 and 0.196 grams, and their lowest inflation factors were 36.106 and 7.118, respectively, when treated with a dilution of 18×10^8 compared with the control group. At the same dilution, the percentage reduction of hydatid cysts increased after 7 months, reaching 100% compared with the control group.

Keywords: ***Lactobacillus plantarum, Bifidobacterium lactis, Streptococcus thermophiles, Echinococcosis***

دور البروبايوتيك في معالجة داء المشوكات في الحيوانات

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الخلاصة

تحرت هذه الدراسة عن دور البروبايوتك في معالجة داء المشوكات ، واستخدم فيها ثلاث فترات زمنية 7,5,3 أشهر وثلاثة تخافيف من البكتريا وحقنت الفئران تحت البريتون قبل وبعد الإصابة بالرؤيسات الأولية . قبل الإصابة حقنت الفئران بالبكتريا مرتين وبفاصل زمني 48 ساعة ، وفي اليوم الخامس تم اصابتها بالطفيلي ، وبعد 48 ساعة عولجت بالبكتريا ، واستمرالحقن كل 72 ساعة ولجميع الفترات . سُجِّل انخفاض في أعداد وأوزان الأكياس العدرية الى 0.00 mg ، وانخفاض في اوزان الكبد والطحال ومعامل تضخمهما بعد 7,5,3

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أشهر إذ بلغ أقل وزن للكبد والطحال 1.2869 و 0.196 غم ، وأقل معامل تضخم لهما 36.106و 7.118على التوالي عند المعاملة بالتخفيف 18× 10^8 بالمقارنة مع مجموعة السيطرة، وعند التخفيف نفسه ازدادت نسبة اختزال الأكياس العدرية بعد 7 أشهر إذ بلغت 100% بالمقارنة مع مجموعة السيطرة.

الكلمات المفتاحية: *Lactobacillus plantarum, Bifidobacterium lactis, Streptococcus thermophiles*, *Echinococcosis*.

1. Introduction

Cystic echinococcosis (CE) is a chronic parasitic disease caused by the tapeworm (*Echinococcus granulosus sensu lato*) which threatens human health and social development worldwide [1, 2, 3]. It is a dangerous zoonotic disease that affects humans and animals and is caused by the larval stage of *Echinococcus granulosus*. Sheep and other herbivores serve as intermediate hosts, while dogs and other carnivores serve as definitive hosts for this tapeworm [4, 5]. Humans can also become an accidental host or the so-called dead-end host by unintentionally consuming food and drink contaminated with the parasite eggs that come out with the feces of the definitive host, paving the way for infection in humans [6]. The eggs hatch in the human alimentary canal into tumor balls (hexacanth embryo) and are transmitted 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%), may also affect other organs and tissues [6, 7]. Most patients do not suffer from any clinical symptoms at the beginning of the disease as the incubation period of the disease is long. The symptoms eventually appear in pear shape due to the growth of abscesses determined by their size and location before becoming chronic infection [8, 9]. The growth stages of the parasite take place slowly inside the human body making the prediction of the infection more difficult, making the clinical diagnosis of the disease focuses on indirect symptoms rather than identifying the parasite directly. Ultrasound is one of the most important diagnostic methods in the United States. as it also usually uses serum assays, especially the enzyme-linked immunosorbent assay (ELISA) based on the antigen of the hydatid cyst fluid of *E. granulosus* [10]. Hydatid cysts in the liver can be treated with both surgical and non-surgical methods such as ultrasound therapy. Nanoparticle therapy such as selenium nanoparticles that have been used as anti-parasitic drugs in recent times, percutaneous therapy and chemotherapy are used in treating this infection. Chemotherapy is quite effective when used on various parasitic diseases such as leishmaniasis and echinococcosis. As for its effect on echinococcosis, it can reduce the size and tension of the cysts and, in some rare cases, may kill the components of the cyst. Albendazole (ABZ) and praziquantel (PZQ) are often used to treat hydatid cysts [11, 12, 13, 14]. PZQ, a protoscolicidal, could also be combined with ABZ to enhance the efficacy. The World Health Organization (WHO) divided hydatid cysts in the liver into five types (CE1 to CE5) and three biological stages (active, transitional and inactive). However, the reasons behind self-healing or maintaining chronic infection are still unknown until now [1, 2, 15]. But there are several reasons, including the lack of effective vaccines and worms' tolerance to drugs meant to kill them has prompted scientists into using alternative options or methods in an attempt to eradicate some parasitic infections in recent years. Probiotics are used as an alternative to the prescription drugs against many parasitic diseases such as leishmaniasis and echinococcosis [16, 17, 18, 19]. Based on the recommendations of the WHO, probiotics are defined as exogenous gram-positive microorganisms that positively affect the gastrointestinal balance and are beneficial to the health of the host when administered through the gastrointestinal tract. In general, probiotics which can be isolated from human intestines, feces, and dairy products, are also found in both fruits and vegetables [20, 21, 22]. Probiotics bacteria have many benefits. They can modify their physiological and chemical environment by strengthening the bonds and changing the peristaltic motion of the intestine, mucus secretion, increasing the acid function, increasing the receptors on the acidic epithelial cells and finally the nutrients. They are also

responsible for the biological production of active molecules such as antibodies, hydrogen peroxide H_2O_2, bacteriocin. All these molecules have antimicrobial properties, have a role in immune modulation, either by stimulating cells producing IgA antibodies accompanying cells producing IgM antibodies and secretory antibodies IgA, or by stimulating the humoral immune response. They can modulate the differentiation of normal lymphocytes into TH2, TH1 and then stimulate cytokines by interacting with dendritic cells. Probiotics bacteria have many characteristics that affect human health, the most important of which is their impact on the development of living microorganisms that inhibit the organism in a way that confirms the appropriate balance between pathogenic and non-pathogenic bacteria which are necessary for the normal function of the organism [23].

Due to the unparalleled advantages of probiotics and their potency curative for the remediation of contagious sicknesses (bacterial, viral, parasitic), this study which was coducted for the first time in Iraq, concentrated on the implementation of PRO-30 Max Probiotics (*Lactobacillus acidophilus, L. plantarum, L. casei, L. reuteri*, *Bifidobacterium bifidum, B. lactis, B. longum,* and *Streptococcus thermophilius),* exploring their biological activities against hydatidosis in experimental animals.

2. Materials and Methods

2.1 Laboratory Animals

Three to four weeks old, BALB/c strain, male Swiss albino mice were used in the current study. All experiments were accomplished at the animal house of the College of Veterinary Medicine, University of Mosul, from 3/1/2022 to 5/2/2023.

2.2 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 primary cephalopod suspension to 20 µl of 0.1% eosin dye on a slide, which was then examined under a light microscope. As the color was adopted as a criterion for examining vitality, the live protoscolices appeared bright green in color due to the exclusion of the eosin dye by the membranes of the protoscolices. The dead protoscolices, however, appeared red in color due to the acceptance of the acquired dye and its calculation according to the approved method [24, 25, 20].

2.3 Bacterial Strains Preparation and Inoculation

PRO-30-Max bacteria were obtained in the form of capsules from Natures Aid Co. in the United Kingdom. Each capsule was 150 mg which was equivalent to 30 billion cells from eight species belonging to three genera, the genus of *Lactobacillus,* including the species *L. acidophilus , L. plantarum, L. casei, L. reuteri,* and the genus *Bifidobacterium* including *B. bifidum, B. lactis, B. longum*, and the genus Streptococcus, including one species, *S. thermophilus* (Figure 1). Bacteria, that were activated in the middle of nutrient broth, were incubated at 37°C for 24 hours. After the incubation period, the bacterial growth was examined by making a slide of activated bacteria in a liquid medium from the nutritional broth and staining it with Gram stain. In order to ensure the growth of gram-positive bacteria, three dilutions of bacteria were prepared by taking 0.1ml of the activated bacterial medium and adding it to the tube. The first tube contained 0.9 ml of normal saline. The preparation of the dilutions continued to the dilution limit of 10^{10} where 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}), reaching 18 colonies in the 10^8 dilution, 14 colonies in the 10^9 dilution, and 12 colonies in the 10^{10} dilution [17, 20, **26**].

Figure 1: PRO-30 Max bacteria

2.4 Experimental Design

Twenty mice at the age of one month were taken and distributed in four cages in the first experiment. Each cage had 5 mice. The mice in the first three cages were injected with the PRO-30 Max bacteria, as the mice in the first cage were injected with a dilution of 18×10^8 /ml CFU. In the second cage, the dilution was 14×10^9 CFU/ml, and in the third cage, the dilution was 12×10^{10} CFU/ml with two consecutive doses 48 hours apart. These mice were then injected with 2000 protoscolices on the fifth day of activation (that is, four days after the mice were given the first dose of bacteria). The injection of bacteria continued every 72 hours, for a period of 80 days. Mice in the fourth cage were injected with live protoscolices as a positive control group. Three months after inducing infection (infection with protoscolices), mice injected with bacteria and mice of the positive control groups were dissected.

In the second experiment, 20 mice at the age of one month, were distributed in four cages, with 5 mice per cage. Mice in the first three cages were injected with the PRO-30 Max bacteria, mice in the first cage were injected with a dilution of 18×10^8 CFU/ml, in the second cage injected with dilution 14 x 10^9 CFU/ml, and in the third cage a dilution of 12 x 10^{10} CFU/ml with two consecutive doses 48 hours apart. Mice were injected with 2000 protoscolices 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 protoscolices as a positive control group. Five months after inducing infection (infection with protoscolices), bacteria-injected mice and mice of the positive control groups were dissected.

And in the third experiment, a month old 20 mice were taken and distributed into four cages, with 5 mice 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 a dilution of 18×10^8 CFU/ml, in the second cage injected with dilution 14 x 10^9 CFU/ml, and in the third cage a dilution of 12 x 10^{10} CFU/ml with two consecutive doses 48 hours apart, then mice were injected with 2000 protoscolices, 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 protoscolices as a positive control group. Seven months after inducing infection (with the protoscolices), bacteria-injected mice and mice of the positive control groups were dissected.

2.5 Mice Dissection

Mice were anesthetized using ether, and then fixed on a special dissection dish and the abdomen was opened using a dissection kit to reveal growing hydatid cysts and their spread in the internal organs such as the liver, spleen, lungs, kidneys and abdominal cavity.

3. Statistical Analysis

The data was analyzed statistically according to the completely random design (CRD) method with five replications for the purpose of testing the effect of each of the five treatments and the three time periods and the overlap between them. The differences between the means were compared according to Duncan's Multiple Range Test. The Statistical Analysis System (SAS) was adopted in the implementation of all statistical analysis [27].

4. Results

Table 1 shows significant differences in the number of cysts. A clear and significant decrease was observed in the number of cysts at a probability level ($p \leq 0.05$) in the treated mice compared with the control group after 3, 5 and 7 months of infection respectively. The positive control group recorded a significant increase in the number of cysts amounting to 11.600, 14.800, 18.000 cysts respectively (Figures 2, 3, 4), compared to dilution 12 x 10^{10}CFU/ml in which the number of cysts reached 10.600, 8.600 and 6.800 cysts respectively after 3, 5, and 7 months of infection (Figures 5, 6, 7). The dilution 14 × 10^9 CFU/ml recorded an average of 7.800, 5.800 and 4.600 cysts respectively after 3, 5 and 7 months of infection (Figures 8, 9, 10). Number of cysts was 0.000 in the dilution 18x10^8 after 3, 5 and 7 months of infection (Figures 11, 12, 13).

Table 1: Changes in the numbers of hydatid cysts in mice infected with primary cephalopods treated with PRO-30 Max bacteria compared to the positive control group for three months, five months, and seven months.

Treatments / Periods	3 Month	5 Month	7 Month	Means of treatments
18×10^8 CFU/ml	0.000h	0.000h	0.000h	0.000d
14×10^9 CFU/ ml	7.800ed	5.800gf	4.600g	6.067c
12×10^{10} CFU/ml	10.600c	8.600d	6.800ef	8.667b
Control+	11.600c	14.800b	18.000a	14.800a
Means of periods	7.5a	7.3c	7.35b	

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

Table 2 shows the highest significant reduction percentage in the numbers of hydatid cysts that reached 100% after 3, 5 and 7 months after injecting 18×10^8/ml CFU dilution (Figures 11, 12, 13). While the percentages of reduction using the dilution 14×10^9 /ml CFU in the third, fifth and seventh month were 50.8%, 60% and 75.4% respectively, (Figures 8, 9, 10). The reduction percentages at dilution 12× 10^{10}C / ml CFU reached 62% in the third month. In the fifth month, the reduction was 68.9% and 86% in the seventh month (Figures 5, 6, 7) .

Table 2: Percentage reduction of cyst numbers in mice infected with protoscolices and treated with PRO-30 Max bacteria.

Treatments /Pperiods	3 Month(%)	5 Month (%)	7 Month (%)
18×10^8 CFU/ml	100%	100%	100%
14×10^9 CFU/ ml	50.8%	60%	75.4%
12×10^{10} CFU/ml	62%	68.9%	86%
Control+	0%	0%	0%

The weights of developing hydatid cysts (Table 3) showed significant differences between control group (Figures 2, 3, 4) and the treatments in the third, fifth, and seventh month of

infection. The dilution of 18×10^8 /ml CFU recorded the lowest rate of cyst weights after the same periods, followed by the two dilutions 14×10^9/ml CFU and 12×10^{10}/ml CFU which recorded 0.54400 and 0.77200 mg respectively three months after infection, (Figures 5, 8), 0.37200 and 0.58200 mg respectively, after five months of infection (Figures 9, 12) and 0.25800 and 0.47600 mg. respectively seven months after infection (Figures 7, 10).

Table 3: Changes in the weights of hydatid cysts in mice infected with protoscolices and treated with PRO-30 Max bacteria.

Treatments / Periods	3 Month	5 Month	7 Month	Means of Treatments
18×10^8 CFU/ml	0.000h	0.000h	0.000h	0.000d
14×10^9 CFU/ ml	0.544ed	0.372f	0.258g	0.391c
12×10^{10} CFU/ml	0.772c	0.582d	0.476e	0.610b
Control+	0.846cb	0.878b	1.196a	0.973a
Means of periods	0.540a	0.458c	0.482b	

Similar letters indicate no significant differences and different letters indicate significant differences.

Table 4 shows that there were significant differences in the changes in liver weights between treated mice and the control groups. Using the Duncan multi-range test, a significant decrease was observed in the rates of liver weights in mice treated with dilution 18×10^8/ml CFU reached 1.3260, 1.3060 and 1.2869 gm respectively, compared to control groups that recorded a significant increase in liver weights 2.3820, 2.7960 and 3.5940 g respectively after 3, 5 and 7 months of infection.

Table 5 shows significant differences in the rates of hepatomegaly. The lowest rate of hepatomegaly was 36.106 after using 18×10^8 CFU/ml dilution. Whereas the control group recorded the highest inflation rate of 77,485, seven months after infection. The weights of spleen (Table 6) showed significant differences in the averages of the treatments. The lowest value recorded was 0.19600 mg using 18×10^8/ml CFU dilution and the highest value of spleen weight in the control group was 0.75400 mg, seven months after infection. Table 7 shows significant differences in the splenomegaly of spleen with the lowest value of 7.118 using 18 x 10^8/ml CFU dilution. The highest coefficient of splenomegaly was 7.118 for the control group 63,644 seven months after infection.

Table 4: Changes in liver weights in mice infected with protoscolices and treated with PRO-30 Max bacteria.

Treatments / Periods	3Month	5 Month	7 Month	Means of Treatments
18×10^8 CFU/ml	1.3260d	1.3060d	1.2869d	1.30600b
14×10^9 CFU/ ml	1.3740d	1.3440d	1.3160d	1.34467b
12×10^{10} CFU/ml	1.4540d	1.4040d	1.3660d	1.40800b
Control+	2.3820c	1.7960b	3.5940a	2.92400a
Means of periods	1.634b	1.4625c	1.890a	

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

Table 5: Changes in the hepatomegaly factor in rats infected with protoscolices and treated with PRO-30 Max bacteria.

Treatments / Periods	3 Month	5 Month	7 Month	Means of Treatments
18×10^8 CFU/ml	56.323dc	46.025dgfe	36.106g	46.121c
14×10^9 CFU/ ml	59.511c	48.112dfe	36.987g	48.204cb
12×10^{10} CFU/ml	65.943c	50.719dfe	37.702g	51.455cb
Control+	45.871gfe	60.452c	77.485a	61.269a
Means of periods	56.912a	51.327b	47.07c	

Similar letters indicate no significant differences. Different letters indicate significant differences.

Table 6: Changes in spleen weights in mice infected with protoscolices and treated with PRO-30 Max bacteria.

Treatments / Periods	3 Month	5 Month	7 Month	Means of Treatments
18×10^8 CFU/ml	0.23600e	0.21600e	0.19600e	0.21600d
14×10^9 CFU/ ml	0.28400ced	0.25400ed	0.22600e	0.25467c
12×10^{10} CFU/ml	0.36200cbd	0.31200ced	0.27600ced	0.31667b
Control+	0.37800cb	0.45200b	0.75400a	0.52800a
Means of periods	0.315c	0.3085b	0.363a	

Similar letters indicate no significant differences. Different letters indicate significant differences.

Table 7: Changes in splenomegaly index in rats infected with protoscolices and treated with PRO-30 Max bacteria.

Treatments / Periods	3 Month	5 Month	7 Month	Means of Treatments
18×10^8 CFU/ml	13.342c	9.949c	7.118c	10.136d
14×10^9 CFU/ ml	16.953c	12.112c	8.304c	12.457c
12×10^{10} CFU/ml	23.824c	15.718c	10.402c	16.648b
Control+	25.309c	47.857b	63.644a	45.603a
Means of periods	19.8572c	21.409b	22.367a	

Similar letters indicate no significant differences. Different letters indicate significant differences.

Figure 2: Mouse infected with protoscoleces after three months (C+).

Figure 3: Mouse infected with protoscoleces after five months (C+).

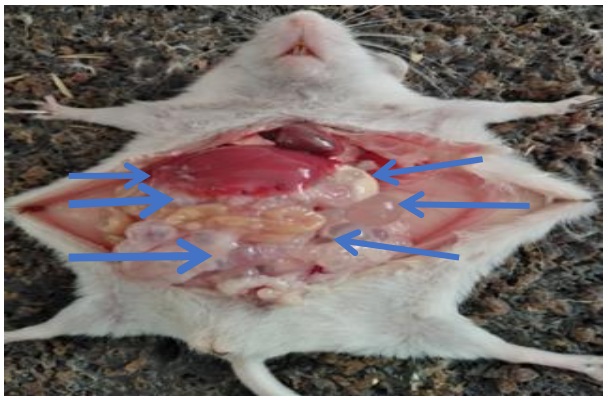

Figure 4: Mouse infected with protoscoleces after seven months (C+).

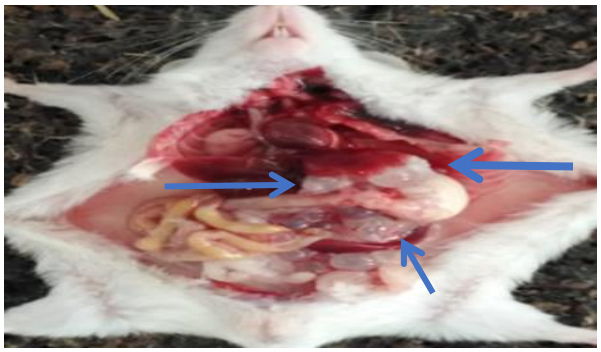

Figure 5: An infected mouse treated with 12×10^{10} /ml CFU dilution after three months.

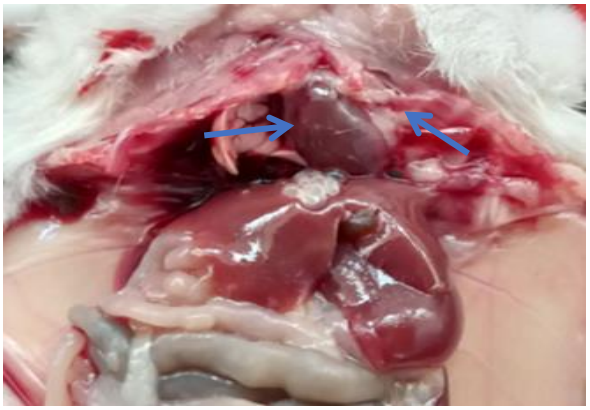

Figure 6: An infected mouse treated with 12×10^{10} /ml CFU dilution after five months.

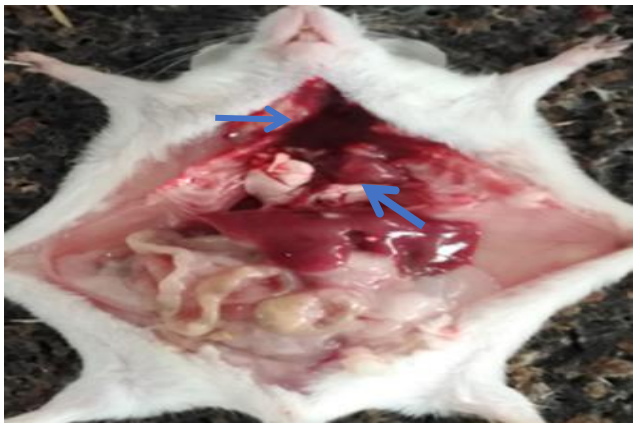

Figure 7: An infected mouse treated with 12×10^{10} /ml CFU dilution after seven months.

Figure 8: An infected mouse treated with 14×10^{9} /ml CFU dilution after three months.

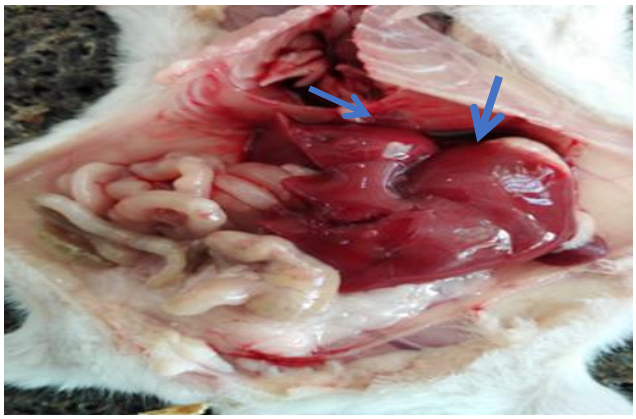

Figure 9: An infected mouse treated with 14×10^{9} /ml CFU dilution after five months.

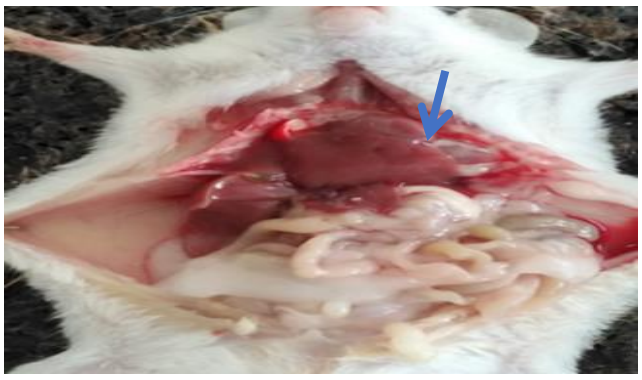

Figure 10: An infected mouse treated with 14×10^{9}/ml CFU dilution after seven months.

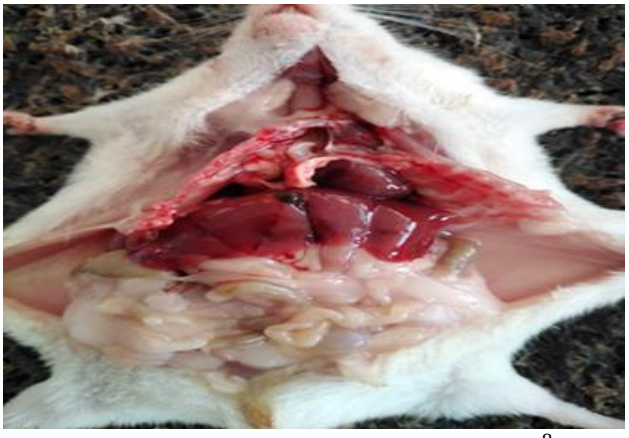

Figure 11: An infected mouse without cysts treated with 18×10^{8}/ml CFU dilution after three months.

Figure 12: An infected mouse without cyst treated with 18 x 10^{8} /ml CFU dilution after five months.

Figure 13: An infected mouse without cyst treated with 18 x 10^{8} /ml CFU dilution after seven months.

5. Discussion

The results of the current study that dealt with the role of probiotics in reducing hydatid disease, showed a decrease in the liver and spleen weights rates and their enlargement factor in the treated mice when compared to the control groups. Probiotics can be defined as live microorganisms which, when taken in sufficient quantities, will have a positive effect on the health of the host by modulating the intestinal flora, maintaining and repairing the intestinal mucosal barrier and intracellular junctions, increasing absorption and metabolism of nutrients

as well as stimulating the immune system [28]. This definition was formulated by the World Health Organization (WHO) in October 2013. Identification of these probiotic microorganisms is always preferred at the strain level, and to be optimal they must be able to positively modulate the host's gut microbiota, stabilize commensal gut microbiota, and restrict colonization by pathogenic microbes [29]. As probiotics can stimulate the production of antimicrobial substances which in turn stimulates the production of antibodies or bacteriocin molecules that have mechanisms to resist harmful organisms. Probiotics can also produce bioactive molecules such as hydrogen peroxide H_2O_2 and modify immunity either by stimulating the humoral immune response by stimulating IgA-secreting cells and their secretory antibodies, or by stimulating dendritic cells that can regulate the differentiation of vital lymphocytes into TH1, TH2 leading to the induction of cytokines. In addition to the ability of probiotics to induce a natural immune response through its effects on Kupffer cells in the liver, thus Kupffer cells will eliminate parasites in the liver, consequently the numbers of parasites and granulomas in the liver and spleen will be reduced, which results in a decrease in liver weight and spleen to normal weight when compared with the control group [19].

The increase in the livers weights of the control groups is attributed to the presence of necrotic areas or granulomas that impede the vitality and activity of tissues. And the increase in the weight of the spleen is attributed to the proliferation of B and T lymphocytes and increase the effectiveness in response to different cell alterations by the parasite [30].

The effect of bacteria occurs through a mechanism in which the efficiency of bacteria positively affects the dependent host. These mechanisms include multiple forms such as improving enzymatic activity, producing antimicrobial substances, modifying the mucosal immune system and changing the gut microflora [31]. The initial activity and effect of probiotics against worms and parasitic protozoa can strengthen the intestinal barrier and regulate the gut microflora [32, 33]. The increase in number of beneficial microorganisms such as Lactobacilli can inhibit the growth of pathogenic organisms by two mechanisms. Organic acid, such as butyric acid, acetic acid and lactic acid, and these compounds are secreted by species of the genus *Lactobacillus* which have a fatal effect on helminth larvae [34]. Gastrointestinal tract has effects on the microbiota by regulating intestinal pH as lactic acid improves the activity of enzymes in the system [35]. Previous studies have revealed that short-chain fatty acids such as lactic, acetic and formic have major roles in regulating microbial balance in the gut and maintaining the morphology and functions of intestinal epithelial cells [36, 2]. The inhibitory effects of probiotics such as *Lactobacillus spp*. and bifidobacteria depends on the pathogenic microbes they combat, as well as the production of antimicrobial peptides (bacteriocins), competition for adhesion to the mucosa, the consumption of nutrients, and modulation of the immune system [37]. Ingestion of probiotics has also been suggested to modulate the composition of the gut microbiota [38]. As recommended by WHO, the selected probiotic strains should possess (i) adhesive properties for mucus and intestinal epithelial cells, (ii) resistance to gastric acid and bile salts and (iii) antimicrobial activities and antagonism against microorganisms thought to be pathogenic. It has also been shown that probiotics have other roles in enhancing the immune system such as modulating or modifying the release of cytokines such as IL-2, IL-12, IL13, IL-15, as these cytokines have a major and important role in maintaining the delicate balance between important defense mechanisms [39].

The current study agrees with studies accomplished by researchers Martínez-Gómez *et al*, Zaiss and Harris [40, 41] who used probiotics to stimulate host immunity against *Trichinella spiralis*, to evaluate the immunomodulating and anthelmintic properties of probiotic strains. Many studies concluded that most of the discovered bacterial species belonging to the genus *Lactobacillus*, including *L. casie*, have a good protective effect against parasitic worms with a

rate ranging between 75-100%. The prevention of the threadworm *T. spiralis* reached about 90% when using *L. plantarum*/P164 which prompted the researchers to consider strains of *Lactobacillus* bacteria as safe when used in the prevention and treatment of *T. spiralis*. Researchers have reported the protective effects of these bacteria is due to their production of nitric oxide and IL-2, IFN-y. A study conducted by Martinez-Gomez *et al*. [42] showed that injecting 200 larvae of *T. spiralis* into the peritoneum of 60 mice with *L. casie shirota* at 1×10^8 CFU dilution for 3 weeks led to a significant decrease in the number of mature worms in the intestines of mice.

The current study also agrees with a study conducted by Sadoon [43] who demonstratde the effects of probiotics on parasitic infections, comprising *Blastocystis hominis*, one of the most widespread parasitic species among humans and all over the world. The study confirmed the role of probiotics in treating many parasitic diseases of the digestive system by stimulating early immunity in children and continued immune improvement in all ages. Same results were obtained in BALB/C mice infected with *B. hominis.*

Schofs *et al.* [44] demonstrated an anti-probiotic effect of *Enterococcus faecalis* CECT7121 (EFCECT7121) on *T. spiralis* infection *in vitro* and *in vivo*. *In vitro* assay showed a decrease in the viability of *T. spiralis* larvae (31.6%) compared to the control group (6.3%) after 48 hours incubation with EFCECT7121 probiotics. However, the isolated antimicrobial peptides AP7121 when inoculated with different concentrations didn't show any larval killing effects. Different therapeutic efficacies of EFCECT7121 probiotics were evaluated in mice *in vivo*. In addition, the protective role of probiotics (EFCECT7121) combined with albendazole (ABZ), the traditional anthelmintic (5 mg/kg), was also evaluated. Oral dose of probiotics to mice pre-infected with *T. spiralis* caused a decrease in larvae per gram (LPG) of muscle tissue in mice ranging from 32.8 – 47.9% on day 28. Whereas ABZ alone resulted in a decrease in LPG of muscle tissue by 60.7%. Infecting mice with the mixture EFCECT7121 + ABZ resulted in a decrease in muscle tissue LPG by 62%, thus supporting that probiotics such as EFCECT7121 have an anthelmintic effect, and their combination with conventional anthelmintic drugs such as ABZ can improve clinical and parasitological outcomes.

Coêlho *et al.* [17] used new preventive methods against infection with hook worms, after noticing the resistance of these worms to traditional medicines, whether in humans or dogs. They noticed that when ten experimental animals were naturally infected with the parasite, with strains of *L. acidophillus* ATCC4536, *L. plantarum* ATCC8014, *L. delbrueckii* H2B20, by 1×10^6 CFU dilution every 48 hours for 28 days, a significant effect appeared on the infection of a hookworm *Ancylostoma caninum*, with an efficiency of approximately 90% in naturally infected dogs. The researchers also noted a significant reduction at the level of probability $p\leq 0.05$ in the number of eggs expelled with stool samples by 82.83% per gram of stool which confirms the immuno-stimulating roles of probiotic, as well as the effective role of *Lactobacillus* species for the prevention and treatment of hookworm in dogs.

6. Conclusions

It can be concluded from the current study that probiotics have several roles that contribute in the prevention, treatment and even elimination of the parasitic disease, hydatidosis, inside the body of the animal model at a certain concentrations by stimulating the immune response against the parasite, through a decrease in the numbers and weights of hydatid cysts, a decrease in liver and spleen weights and their enlargement index in mice injected with protoscolices and treated with probiotic bacteria. These results pointed out that probiotics could serve as an alternative to medication in future consideration.

7. Recommendations

a. Using other strains of probiotic bacteria and finally hydatid cyst disease by stimulating the immune response and thus enhancing immunity.
b. The use of probiotic bacteria strains under study against other parasitic diseases, whether protozoa or helminths, *in vivo*.
c. Study of the synergistic action of probiotic bacterial strains under study with certain types of drugs such as benzimidazole agonists, Praziquantel, and Ivermectine in the treatment of hydatid cyst disease *in vivo*.

8. Ethics Approval

Institutional guidelines for the care and use of animals were followed. All procedures performed in the study and involved animals were in accordance with the ethical standards of the institution or practice at which the study was conducted (date 1/03/2022) .

9. Acknowledgment

Authors thank College of Veterinary Medicine, Education College for Pure Sciences/ University of Mosul for support and all facilities they supplied.

10. Conflict of interest

There is no conflict of interest.

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