RESEARCH ARTICLE

Role of *Lactobacillus plantarum* and *Lactobacillus acidophilus* as a Treatment of Cryptosporidiosis in Mice

Israa M. A. AL-Khaliq,[1*] Abdullateef J. Nasser, [2] Moayad M. Ghadban[3]

[1]*Department of Microbiology, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq*
[2]*Consultant Clinic Laboratory, Al-Kindy Teaching Hospital, Baghdad, Iraq*
[3] *Consultant Clinic Laboratory, Medical City Teaching Hospital, Baghdad, Iraq*

Received: 7th April, 2021; Revised: 3rd July, 2021; Accepted: 12th August, 2021; Available Online: 25th September, 2021

ABSTRACT

Objective: To evaluate the therapeutic activity of probiotics mixture *of Lactobacillus plantarum* and *Lactobacillus acidophilus* towards *Cryptosporidium* infection in experimentally infected mice. Oocysts of *Cryptosporidium* were separated from the stool of humans to infect mice.

Methods: Forty male albino mice were split equally into four groups, every group contained 10 mice, the group I (early treated group), were treated from the 1st day from infection to the 11th post-infection, group II (late treated group), were treated from the 4th day from infection to the 15th post-infection, and group (III) (untreated group), were mice considered as a positive control group.

Results: It was showed that daily application of a mixture of *L. plantarum* with *L. acidophilus* could reduce the parasitic infection in mice as compared with the untreated group, and it was confirmed that the using of these probiotics in the early treated group was more efficient than the using of these probiotics in the late treated group.

Conclusion: A mixture of *L. plantarum* and *L. acidophilus* are good therapeutic agents for cryptosporidial infection.

Keywords: *Cryptosporidium parvum*, Cryptosporidiosis, Immunosuppressed mice, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, Probiotics.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.27

How to cite this article: AL-Khaliq IMA, Nasser AJ, Ghadban MM. Role of *Lactobacillus plantarum* and *Lactobacillus acidophilus* as a Treatment of Cryptosporidiosis in Mice. International Journal of Drug Delivery Technology. 2021;11(3):812-816.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cryptosporidium parvum is one of several parasitic species that cause the disease cryptosporidiosis in the intestinal tract of mammals.[1] It can be transmitted via animal-to-human or animal-to-animal contact,[2] infections in humans can occur by contaminated food and contaminated water with *Cryptosporidium*.[3]

Transmission of this parasite in the environment occurs by ingestion of cysts stage and cause infection of the intestinal epithelial layer. Diarrhea is the main symptom of self-limiting in individuals with sound immune systems, while in immunocompromised patients, the symptoms are severe dehydration, malnutrition, electrolyte imbalances, and death.[4] The occurrence of diarrhea may be due to the destruction of the surface area of microvillus, the presence of the toxin, or adhesion factors that induce attachment of this parasite to host cells,[5] it could be acute, watery, and non-bloody diarrhea. Other symptoms may include nausea, vomiting, anorexia, and abdominal pain. This parasite can be spread extra-intestinal to affect the liver, gall bladder, lung where it causes respiratory cryptosporidiosis.[6]

Illness is self-limiting, and symptoms typically resolve completely within (2 to 3) weeks in immunocompetent individuals.[7] Mechanism of defense against *Cryptosporidium* related with cellular immune response and producing gamma interferon (IFN-γ).[8] When cells of epithelial tissue are infected, this will increase the production of cytokines, inflammatory chemokines, and determined the mechanisms of antimicrobial killing,[9] also the presence of intestinal flora has shown resistance to *C. parvum* infection.[10] Till now, there is no active drug for cryptosporidiosis rather than healthy immunity, and for this reason, researchers use other therapies included probiotics, the active organisms, providing health to humans.[11]

Probiotic strain should have specific characters such as protection against pathogens, has immune stimulation, be nonpathogenic, existing in the intestine, ability to attach epithelial gut,[12] from these commonly probiotics are gram-positive lactic acid bacteria (*lactobacillus*) that are delivered orally as probiotics.[13]

**Author for Correspondence: israaalhasan@yahoo.com*

This study aimed to gain the therapeutic activity of composition from *Lactobacillus plantarum* and *Lactobacillus acidophilus* by using an immune-suppressed mice model infected with *C. parvum*.

MATERIALS AND METHODS

Preparation Inoculum of *Cryptosporidium parvum*

Cryptosporidium Oocysts were taken from 20 patients with chronic diarrhea in parasitology labs of AL-Kindy Teaching Hospital and Medical City Teaching Hospital from (January 2018 till July 2019). Stool samples were examined for detection *Cryptosporidium* oocysts by staining with safranin stain.[14] Fresh positive samples were put in saline and concentrated by floatation technique with Sheather's sucrose, then used phosphate buffer saline (PBS) by centrifugation for washing three times method at (700) × for 10 minutes.[15] Oocysts were preserved at 4ºC still used, and its numbers in the suspension were approximately (10^4) oocysts/mL.[16]

Preparation Cells of *Lactobacillus* sp.

A commercial vitalatic B, obtained from Vitane Pharmaceuticals, Inc., USA, was provided as capsules, each one contains ($2x10^9$) CFU of these two bacteria species: *L. plantarum* and *L. acidophilus*. The orally inoculated dose daily for each mouse was prepared and checked to a concentration of ($1×10^8$) CFU in 0.1 mL PBS.

Animals

Forty albino male mice BALB/c, aged (4–6) weeks, weighing (20–25) gm obtained from National Control Center for Drugs and Researches. Their stool was examined before the beginning of the experiment to detect if there are any intestinal parasites.

Experimental Design

Thirty mice were immune-suppressed, and ten mice were left without suppression and infection (negative control group). Immune suppression occurs with an injection of 0.1 mL of dexamethasone (MSD company/mice/day for 5 days),[17] before the inoculation orally by micropipette of this parasite (1×10^4) oocyst/mL, then divided into three groups each one contained (10) mice.

- *Group I (early treated group):* Mice were orally given 0.1 mL of suspension of *L. plantarum* and *L. acidophilus,* which contain (1×10^8) cell/mL (as a single dose/day) from the 1st day of infection till the end of the experiment.
- *Group II (late treated group):* Mice were orally given 0.1 mL of suspension of *L. plantarum* and *L. acidophilus,* which contain ($1x10^8$) cell/mL (as a single dose/day) from the 4th day of infection till the end of the experiment.
- *Group (III) (untreated group):* Mice were orally given 0.1 mL of PBS (as a single dose/day) from the 1st day of infection until the experimental end; it was the positive control group.

Enumeration of *Cryptosporidium* oocysts in Stool

Probiotics effect was estimated by oocysts counting in mice stool from the first infection day and repeated every day till vanishing oocyst. The stool of each mouse was collected every day and mixed with 1-mL normal saline. Slides were stained with safranin stain and oocysts counted by hemocytometer.[18]

Study Histopathologically

After the treatment period, histological sections prepared from mice intestine were examined with a light microscope to detect the histopathological changes after staining with hematoxylin and eosin.

Statistical Analysis

Data were computerized, and statistical comparisons between groups were made. By using SPSS version 22, data were coded and entered. Statistical analysis using standard deviation, arithmetic means, also t-tests were used to compare treatment activity among different groups.

RESULTS

Treatment activity was evaluated by oocysts counting shedding in the stool till the experiment ends.

Daily application of commercial vitalatic B containing a mixture of two probiotic bacteria species (*L. plantarum* and *L. acidophilus)* was able to reduce infection in mice as compared with the untreated group (positive control group) as shown in Figure 1. The early treated group (G.I) developed a significant decrease ($p < 0.05$) in oocysts number compared with the untreated group (G.III), until shedding was completely stopped at 11th day post-infection. Furthermore, the number of oocyst in the late treated group (G.II) until shedding was stopped entirely at 15th days post-infection was significantly ($p < 0.05$) less in the untreated group (G.III) in oocysts number, but significantly ($p < 0.05$) more than in the early treated group as shown in Table 1.

Histological study of the small intestine (ilium) of the untreated group showed colonization of *Cryptosporidium* as small spherical structures in brush borders of the villi, widening, notch sharp top, and shortening of these villi (Figure 2) as compared with the early treated group that showed cure from parasite, and ulceration in the intestinal epithelial cells as shown in Figure 3. While late treated group showed edematous areas of epithelial cells, and shortening of the villi (Figure 4).

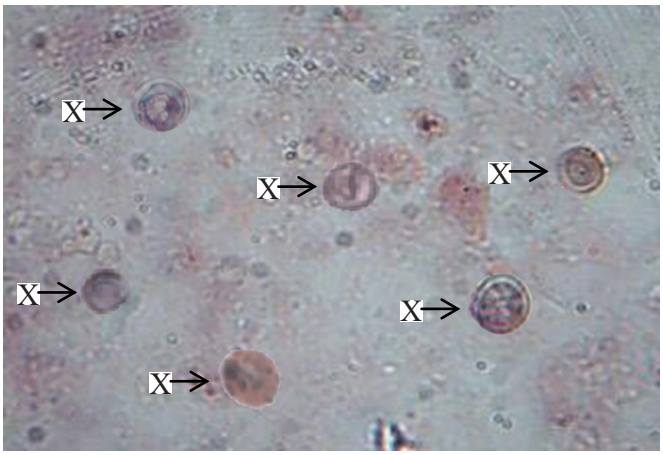

Figure 1: *C. parvum* (oocyst stage) (X) in the stool of untreated group with safranin stain, (400 X).

Table 1: *Cryptosporidium oocyst* means numbers/H.P.F ± S.D. in the stool of treated mice groups and untreated mice group at the days of examination.

	Means ± SD of groups			
Days	*Group (I): Early treated*	*Group (II): Late treated*	*Group (III): Untreated*	*Statistical analysis*
1	112 ± 35.82	NA	118 ± 39.04	p<0.05
2	98 ± 33.51	NA	124 ± 37.16	p<0.05
3	86 ± 31.51	NA	130 ± 35.21	p<0.05
4	76 ± 27.62	135 ± 38.16	135 ± 40.51	p<0.05
5	64 ± 21.28	123 ± 36.75	141 ± 38.63	p<0.05
6	57 ± 14.16	111 ± 31.54	149 ± 33.54	p<0.05
7	45 ± 11.75	98 ± 29.83	156 ± 35.83	p<0.05
8	34 ± 7.13	82 ± 23.52	163 ± 28.15	p<0.05
9	22 ± 4.81	71 ± 8.21	171 ± 19.62	p<0.05
10	12 ±1.45	59 ± 17.16	180 ± 21.04	p<0.05
11	0.00	47 ± 15.04	189 ± 19.75	p<0.05
12	0.00	35 ± 12.63	194 ± 16.58	p<0.05
13	0.00	23± 9.83	202 ± 12.21	p<0.05
14	0.00	11 ± 4.51	210 ± 1.81	p<0.05
15	0.00	0.00	219 ± 1.04	
Mean	60.6 ± 18.90	72.27 ± 20.65	165.4 ± 25.34	

NA : Not Applied
Significance difference (P<0.05)

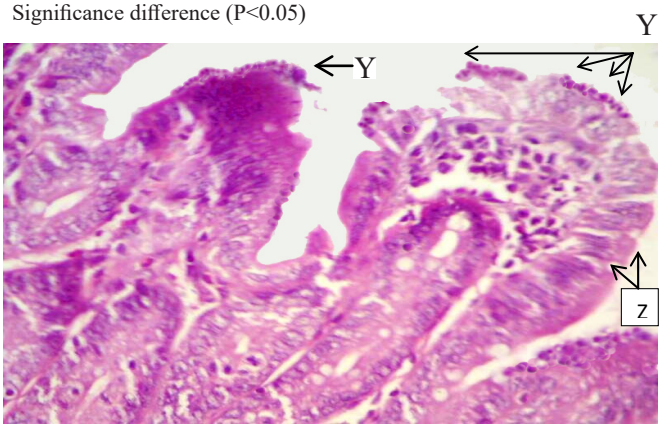

Figure 2: Section of the small intestine of Ileum in mice of untreated group showing colonization of *Cryptosporidium* as small spherical structures in brush borders of the villi (X), widening, notch sharp top (Y), and shortening of these villi (Z) (H&E), 400 X.

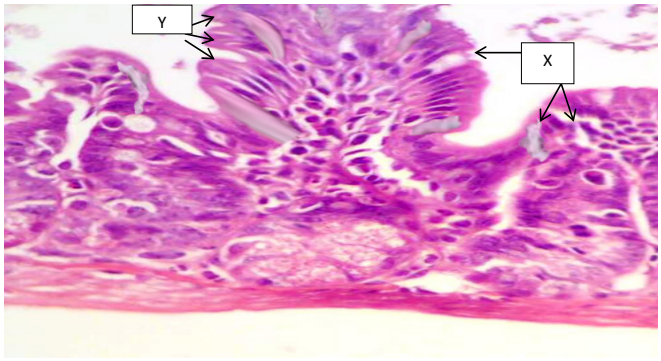

Figure 4: Section of the small intestine of Ileum in mice of late treated group showed edematous areas of epithelial cells (X) and shortening of the villi (Y) (H&E), 400 X.

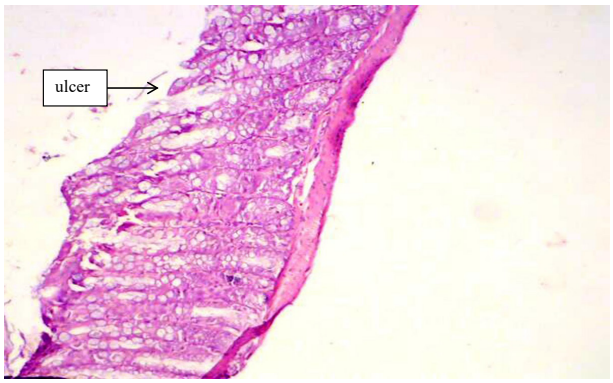

Figure 3: Section of small intestine of Ileum in mice of an early treated group that showed cured of parasite, and ulceration in the intestinal epithelial cells (H&E), 400 X.

The results confirmed the effectivity of a mixture from *L. plantarum* plus *L. acidophilus* as a treatment of infection with *Cryptosporidium* by gut cells modulation to prevent colonization as multiplication of this parasite.

DISCUSSION

The antagonism activity of lactic acid bacteria was investigated against Candida albicans in vitro and gave a higher inhibition average.[19] Till now, cryptosporidiosis infection in the immune-compromised patients cannot treat, besides the multiple side effects that they produced,[20] while immunocompetent individuals can heal from this infection because the ability to eradicate parasite, depends on both innate and acquired immunity.[21] So, new treatment against Cryptosporidium became needed. In this study, mice model

was immunosuppressed experimentally and probiotic treatment was used to reinforce health because of their roles on ecology of gastrointestinal flora and modulation of immunity by balancing pro-inflammatory, anti-inflammatory cytokines.[22] In addition, probiotics are needed to promote mucosal epithelium cells to proliferate and act as the first defense line toward intestine pathogens.[22] Most observed effect of probiotic is used in the treatment of diarrhea. Clinical studies of using probiotics such as *Lactobacillus,* showed the elimination effect to diarrhea caused by rotavirus. Large numbers of studies in mice with low immunity were mentioned that treatment with probiotics can reduce cryptosporidiosis with intestinal epithelium.[23] Other studies reported that, *L. veuteri* and *L.acidophilus* had decreased both duration periods, also numbers of oocyst regarding *Cryptosporidium* in the stool of infected mice.[24,25]

A study done on humans was recorded a 12-years old girl infected with severe diarrhea caused by cryptosporidiosis and treated with a mixture of Lactobacillus rhamnosus (109) units/day *Lactobacillus casei Shiro*ta (6.5×10^9) units/day, for four weeks course of treatment. Examination of stool sample after probiotics treatment was clear from oocysts.[26] In this study, a mixture of *L. plantarum* and *L. acidophilus* was evaluated because they are used widely to prevent and treat intestinal parasites.[27] Oocysts numbers were reduced significantly in stool samples collected from the early treated group compared to the late treated group; in comparison with the untreated group, all these results prove the critical function of these species used in this study.

Histologically, results showed sharp notch top, shortening, and widening of the intestinal villi in mice of untreated group, which agreed with other researchers who reported abnormalities in villi structure.[28]

These two probiotics fixed the damage of mucosa in both mice of the early treated group and mice of the late treated group, compared with the damage in microvillus in mice of the untreated group. These results documented the anti-*Cryptosporidium* activity of these probiotics *in vivo* by modulation of gut cells to inhibit both habitation and reproduction of Cryptosporidium, which reduces cryptosporidiosis intensity.[12] Similar result was reported by Alak *et al.* (1997),[29] who recorded that daily intake of *L. reuteri* was effective to inhibit *C. parvum* from intestinal habitation and lesions of tissue in the immunosuppressed mice. Also, probiotic bacteria regulate cytokine secretion (IL-12, IL-10, TNF, and α IFN-γ), which have an essential role in defense mechanisms,30 where IL-10 and secretory IgA are essential anti-*Cryptosporidium* immune responses, are provoked by some probiotic strains.[31] Other studies reported that probiotic bacteria stimulate immune system cells to produce cytokines, which has an important key of immune response stimulation and organizing, promote intestinal IgA immune response, and excess intestinal mucin production.[32]

CONCLUSION

It can be concluded that *L. plantarum* and *L. acidophilus* are perfect to the mucosal immune system and contain important effects as prophylactic agents against *Cryptosporidium*. It is recommended that more research be done to use these probiotics as complementary medicine in cryptosporidiosis management.

REFERENCES

1. Natapol P, Supawadee P. Cryptosporidiosis: A zoonotic disease concern.VeterinaryWorld. 2018;11:681–686. doi: 10.14202/vetworld.2018.681-686.
2. Casemore DP , Wright SE, Coop RL.Cryptosporidiosis - human and animal epidemiology. In: Fayer R, Xiao L. (Ed.),Cryptosporidium and Cryptosporidiosis. Boca Raton, FL: CRC Press; 1997:66-92.
3. RobertsonLJ.Approachesto detecting Cryptosporidium oocysts in different food matrices, In: Cryptosporidium as a foodborne pathogen. Springer New York, New York; 2014:25-38.
4. Graczyk TK, Fayer R, Knight R, Mhangami-Ruwende B, Trout AJ, Da Silva AJ, Pieniazek NJ. Mechanical transport and transmission of Cryptosporidium parvumoocysts by wild filth flies. American Journal of Tropical Medicine and Hygiene. 2000: 63, 178-183. doi: 10.4269/ajtmh.2000.63.178.
5. Carey CM, Lee H, Trevors JT. Biology, persistence and detection of Cryptosporidium parvumand Cryptosporidium hominisoocysts. Water Research. 2004;38:818-862. doi: 10.1016/j.watres.2003.10.012.
6. Warren C, Guerrant R. Clinical Disease and Pathology. In: Fayer. R., Xiao, L.,ed. Cryptosporidium and Cryptosporidiosis. 2nd ed. Boca Raton, FL: CDC Press.2008:235-254.
7. Hunter PR, Hughes S, Woodhouse S, Raj N, Syed Q, Chalmers R, Verlander NV, Goodacre J. Health sequelae of human cryptosporidiosis in immunocompetent patients. Clinical Infectious Diseases. 2004;39:504-510. doi: 10.1086/422649.
8. Tessema TS, Schwamb B, Lochner M , Förster I, Jakobi V, Petry F. Dynamics of gut mucosal and systemic Th1/Th2 cytokine responses in interferon-gamma and interleukin-12p40 knock out mice during primary and challenge Cryptosporidium parvum infection. Immunobiology. 2009;214:454-466. doi: 10.1016/j.imbio.2008.11.015.
9. McDonald V, Korbel DS, Barakat FM, Choudhry N, Petry F. Innate immune responses against Cryptosporidium parvum infection. Parasite Immunology. 2013;35:55-64. doi: 10.1111/pim.12020
10. Harp JA, Chen W, HarmsenAG. Resistance of severe combined immunodeficient mice to infection with Cryptosporidium parvum: the importance of intestinal microflora. Infection and Immunity; 1992;60:3509-3512.
11. Colin H, Francisco G, Gregor R, Glenn RG, Daniel J, Merenstein B, Bruno P, Lorenzo M, Roberto BC, Harry JF, Seppo S, Philip CC, Mary Ellen S. Expert consensus document: the International Scientific Association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature reviews gastroenterology & hepatology. 2014;11:506-514. doi: 10.1038/nrgastro.2014.66.
12. Gupta V, Garg R. Probiotics. Indian Journal of Medical Microbiology. 2009; 27:202-209. doi: 10.4103/0255-0857.53201.
13. Kraehenbuhl JP, Ricciardi-Castagnoli P. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacterial. Nature Immunology. 2001;2:361-367. doi: 10.1038/86373.
14. Baxby D, Blundell N, Hart CA. The development and performance of a simple, sensitive method for detection of Cryptosporidiumoocysts in faeces. Journal of Hygiene.1984;93: 317-323. doi: 10.1017/s0022172400064858.

15. Reese NC, Current WL, Ernest JV, Bailey WS. Cryptosporidiosis of man and calf: a case report and results of experimental infections in mice and rats. American Journal of Tropical Medicine and Hygiene. 1982;31:226-229.doi: 10.4269/ajtmh.1982.31.226.
16. Pavlasek I. First detection of Cryptosporidium spp. Oocysts in calf faeces by floatation method. Folia parasitology. 1982;29: 115-118.
17. Regh JE. Effect of interferon-gamma in experimental C. parvum infection. Journal of the Infectious Diseases. 1996;174:229-32. doi: 10.1093/infdis/174.1.229.
18. Shukla G, Sidhu RK, Verma A. Restoration of anthropometric, biochemical and histopathological alterations by Lactobacillus casei supplementation in Giardia intestinalis infected renourished BALB/c mice. Antonie van Leeuwenhoek. 2012:102:61-72. doi: 10.1007/s10482-012-9713-3.
19. Khalid AH, Sara MF. The Inhibitory Effect of Lactobacillus acidophilus and Lactobacillus plantarum against Candida albicans Associated with Denture Stomatitis. Baghdad Science Journal. 2015:12:479-484. doi.org/10.21123/bsj.2015.12.3.479-484.
20. Miao YM, Gazzard BG. Management of protozoal diarrhea in HIV disease. HIV Medicine. 2000:1,194-199. doi: 10.1046/j.1468-1293.2000.00028.x.
21. Oelschlaeger TA. Mechanisms of probiotic actions - a review. International Journal of Medical Microbiology . 2010;300:57-62. doi: 10.1016/j.ijmm.2009.08.005.
22. Isolauri E, Sütas Y, Kankaanpää P, Arvilommi H, Salminen S. Probiotics: effects on immunity. The American Journal of Clinical Nutrition. 2001:73 (2 Suppl.):444-450. doi: 10.1093/ajcn/73.2.444s.
23. Szajewska H, Mrukowicz JZ. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: a systematic review of published randomized, double-blind, placebo-controlled trials. The Journal of Pediatric Gastroenterology and Nutrition.2001:33 (2 Suppl):17-25. doi: 10.1097/00005176-200110002-00004.
24. Heyman M. Effectof lactic acid bacteria on diarrheal disease.The Journal of the American College of Nutrition. 2000;(2 Suppl): 137-146. doi: 10.1080/07315724.2000.10718084.
25. Foster JC, Glass MD, Courtney PD, Wood LA. Effect of lactobacillus and Bifidobacteriumon Cryptosporidium parvumOocys Viability. Food Microbiology. 2003;20:351-357.
26. Pickerd N,Tuthil D. Resolution of cryptosporidiosis with probiotic treatment. Postgraduate Medical Journal. 2004:80(940):112-113. doi: 10.1136/pmj.2003.014175.
27. Fang Y, Polk DB. Probiotics: progress toward novel therapies for intestinal diseases. Current opinion in Gastroenterology.2010:26 (2):95-101. doi: 10.1097/MOG.0b013e328335239a.
28. Connor BA, Shlim DR, Scholes JV, Rayburn JL, Reidy J, Rajah R. Pathological changes in the small bowel in nine patients with diarrhea associated with coccidian like body. Annals of Internal Medicine.1993:119(5):377-382. doi: 10.7326/0003-4819-119-5-199309010-00005.
29. Alak JI, Wolf BW, Mdurvwa EG, Pimentel-Smith GE, Adeyemo O. Effect of Lactobacillus reuteri on intestinal resistance to Cryptosporidium parvum infection in a murine model of acquired immuno deficiency syndrome. Journal of the Infectious Diseases. 1997:175(1):218-221. doi: 10.1093/infdis/175.1.218.
30. Arvola T, Laiho K, Torkkeli S, Mykkänen H, Salminen S, Maunula L, Isolauri E. Prophylactic Lactobacillus G Greduces antibiotic-associated diarrhea in children with respiratory infections:a randomized study. Pediatric. 1999;104:e64. doi: 10.1542/peds.104.5.e64.
31. Borchers AT, Selmi C, Meyers FJ, Keen CL, Gershwin ME. Probiotics and immunity. Journal of Gastro enterology. 2009:44(1):26-46. doi: 10.1007/s00535-008-2296-0.
32. Gill HS. Probiotics to enhance anti-infective defences in the gastrointestinal tract. Best Practice & Research Clinical Gastro enterology. 2003;17:755-773. doi: 10.1016/s1521-6918(03)00074-x.