Role of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* as a treatment of cryptosporidiosis in mice

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**Article History:**
Received on: 12 Jul 2020
Revised on: 25 Aug 2020
Accepted on: 09 Sep 2020

**Keywords:**
Cryptosporidium parvum, Cryptosporidiosis, immunosuppressed mice, Lactobacillus Plantarum, Lactobacillus acidophilus, probiotics

**ABSTRACT**
Cryptosporidiosis is a disease caused by a protozoan parasite called *Cryptosporidium* which infects gastrointestinal epithelium of mammals and produce diarrhea that is self-limited in immunocompetent individuals but life-threatening in immunocompromised patients, especially those with acquired immunodeficiency syndrome (AIDS). Till now, there is no effective therapy other than a healthy immune system. The current study was designed to evaluate the therapeutic efficacy of probiotics mixture of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* against *Cryptosporidium* infection in experimentally infected mice. Oocysts of *Cryptosporidium* were isolated from the human stool and were used to infect mice. Forty male albino mice were divided equally into four groups, each group contained 10 mouse, a 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. The results showed that daily application of a mixture of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* was able to decrease the parasitic infection in mice as compared with the untreated group. It was observed 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. A mixture of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* are good therapeutic agents for cryptosporidial infection.
tom is self-limiting diarrhea in individuals with intact immune systems, while in immuno compromised patients, such as AIDS patients, the symptoms are severe dehydration, malnutrition, electrolyte imbalances, and death (Gracyzk et al., 2000). The occurring of diarrhea may be due to the destruction of the surface area of microvillus, presence of the toxin, or adhesion factors that induce attachment of this parasite to host cells (Carey et al., 2004). 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 liver, gall bladder, lung where it causes respiratory cryptosporidiosis (Warren et al., 2008).

Illness is self-limiting, and symptoms typically resolve completely within (2–3) weeks in immuno-competent individuals (Hunter et al., 2004). Mechanism of defense against Cryptosporidium is produced by cell-mediated immune response and producing interferon-gamma (IFN-γ) (Tessema et al., 2009). When epithelial cells infected, this will increase the production of cytokines, inflammatory chemokines, and determined the mechanisms of antimicrobial killing (McDonald et al., 2013). In addition to the role of immunity, there is obvious evidence that resistance to *C. parvum* infection can be done with the presence of intestinal flora, and colonization of the intestine by *Cryptosporidium* depends on the intestinal microflora (Alak et al., 1997). Till now, there is no effective therapy for cryptosporidiosis other than a healthy immune system, and for this reason, researchers use other therapies included probiotics, which are live microorganisms, when used in adequate amounts, provide a health benefit to the host (Hill et al., 2014).

The probiotic strain should have specific characters such as providing protection against pathogens, has immune stimulation, be nonpathogenic, persisting in the intestine, and has the ability to adhere to the gut epithelium (Gupta and Garg, 2009). The most commonly of these probiotics are Gram-positive lactic acid bacteria (*lactobacillus*) that are delivered orally as probiotics (Rescigno et al., 2001). The aim of this study was to gain the therapeutic efficacy of consumption a mixture of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* by using immuno suppressed mice model infected with *C. parvum*.

**MATERIALS AND METHODS**

**Preparation inoculum of Cryptosporidium parvum**

Oocysts of *Cryptosporidium* were obtained 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 (Baxby et al., 1984). Fresh collected positive samples were suspended in saline and concentrated by using Sheather’s sucrose floatation technique, then washed (3) times in phosphate buffer saline (PBS) by centrifugation method at (700) x for (10) min (Reese et al., 1982). Oocysts were preserved at (4)ºC till used, and its numbers in the suspension were approximately (10^5) oocysts/ml (Pavlasek, 1982).

**Preparation cells of Lactobacillus sp.**

A commercial vitalatic B, obtained from Vitane Pharmaceuticals, Inc., USA, was provided as capsules each one contains (2x10^8) CFU of a mixture of two probiotic bacteria species: *Lactobacillus Plantarum* and *Lactobacillus acidophilus*. The orally inoculated dose daily for each mouse was prepared and checked to a concentration of (1x10^8) 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 immune-competent and not infected (negative control group). Mice were immune suppressed by injection with (0.1) ml of dexamethasone (MSD company /mice/day for 5 days) (Regh, 1996), before the inoculation orally by micropipette of this parasite (1x10^8) 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 a suspension of *Lactobacillus Plantarum* and *Lactobacillus acidophilus*, which contain (1x10^8) 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 a suspension of *Lactobacillus Plantarum* and *Lactobacillus 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 till the end.
of the experimental period. This group was positive control group.

**Enumeration of Cryptosporidium oocysts in stool**

Probiotics effect was estimated by counting Cryptosporidium oocysts in the stool of mice, and the counting started from the first day of infection and repeated daily until clearance of stool from oocyst. Stool of each mouse were collected every day and mixed with (1) ml normal saline. Slides were stained with safranin stain and oocysts counted by hemocytometer (Shukla et al., 2012).

**Histopathological Study**

At the end of probiotics 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 done. By using SPSS version 22, data were coded and entered. Frequencies, percentages, means and standard deviation were calculated. Statistical analyzed using the arithmetic mean and standard deviation; t-test was used to compare the effect of treatment used between different groups.

**RESULTS AND DISCUSSION**

The therapeutic effect of Lactobacillus Plantarum and Lactobacillus acidophilus on the development of Cryptosporidium infection in immune-suppressed mice was investigated. The efficacy of treatment was evaluated by counting oocysts shedding in the mice stool till the end of the experiment.

The results showed that daily administration of commercial vitalatic B containing a mixture of two probiotic bacteria species: Lactobacillus Plantarum and Lactobacillus acidophilus, was able to decrease the parasitic infection in mice as compared with the untreated group (positive control group), as shown in Figure 1. Early treated group (G.I) showed a significant decrease (P<0.05) in the number of oocysts shedding compared with the untreated group (G.III), until shedding was completely stopped at 11th days post-infection, and the number of oocyst in the late treated group (G.II) until shedding was completely stopped at 15th days post-infection, was significantly (P<0.05) less than that in the untreated group (G.III) in the number of oocysts, but significantly (P<0.05) more than in the early treated group, as display in Table 1.

Histological study of the small intestine (ilium), of 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, as shown in Figure 2, as compared with the early treated group that showed cured from parasite, and ulceration in the intestinal epithelial cells, as seen in Figure 3. While late treated group showed edematous areas of epithelial cells, and shortening of the villi, as shown in Figure 4.

The results confirmed the effective effect of Lactobacillus Plantarum and Lactobacillus acidophilus in the treatment of Cryptosporidium infection by gut cells modulation to prevent colonization as well as multiplication of this parasite.

A study done on a human was recorded 12-years old girl infected with severe diarrhea caused by cryptosporidiosis, and treated with a mixture of Lacto-
Table 1: Mean numbers of Cryptosporidium oocyst/H.P.F ± Standard deviation in the stool of treated mice groups and untreated mice group at the days of examination

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

NA: Not Applied; Significance difference (P<0.05)

bacillus rhamnosus ($10^9$) units/day and Lactobacillus casei Shirota ($6.5 \times 10^9$) units/day, for four weeks course of treatment. Examination of stool sample after starting treatment with probiotics was clear from oocysts (Pickerd and Tuthil, 2004). In this study, a mixture of Lactobacillus Plantarum and Lactobacillus acidophilus was evaluated because they used widely to prevent and treat intestinal parasites (Yan and Polk, 2010). 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 confirm the important role of these species used in the present study against Cryp-
Histologically, results showed notch sharp top, shortening and widening of the intestinal villi in mice of untreated group, which agreed with other researchers who reported abnormalities in the structure of villi (Connor, 1993).

The using of these two probiotics fixed the mucosal damage in both mice of early treated group and mice of the later treated group, compared with the damage in microvillus in mice of the untreated group. These results documented the anti-Cryptosporidium effect of these probiotics in vivo by gut cells modulation for inhibition the colonization and multiplication of Cryptosporidium, and this reduces the severity of cryptosporidiosis (Gupta and Garg, 2009). A similar result was reported by Alak et al. (1997), who mentioned that daily ingestion of L. reuteri was effective to prevent C. parvum from intestinal colonization and tissue lesions in the immunosuppressed mice. Also, probiotic bacteria regulate cytokines secretion (IL-12, IL-10, TNF, and α IFN-γ) which have an essential role in defense mechanisms (Arvola et al., 1999), where IL-10 and secretory IgA, which are an important anti-Cryptosporidium immune response, have been shown to be induced by some probiotic strains (Borchers et al., 2009). Other study reported that probiotic bacteria stimulate cells of the immune system to produce cytokines, that has an important role in the induction and regulation of the immune response, also enhance the immune response of intestinal IgA, and increase the production of intestinal mucin (Gill, 2003).

CONCLUSIONS

It can be concluded that Lactobacillus Plantarum and Lactobacillus acidophilus are a good choice to improve the mucosal immune system, and have important effect as prophylactic agents and good promising therapeutic agents against Cryptosporidium. It is recommended that further investigations should be carried out on the applications of these probiotics as complementary medicine in the management of cryptosporidiosis.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES


