EFFICIENCY OF SACCHAROMYCES BOULARDII ON BLASTOCYSTIS HOMINIS IN LABORATORY MICE

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ABSTRACT
The effectiveness of Saccharomyces boulardii yeast as vital probiotics on this parasite was studied in the laboratory mice and compared with metronidazole which used usually in the treatment of intestinal parasites ,included Blastocystis hominis parasite, it was noticed a gradually decreased in the numbers of parasite in stool of mice since the first day of treatment parasite disappeared in the day (12th ) from treatment with Saccharomyces yeast, and disappeared in day (13th ) from treatment with metronidazole, and when efficacy of treatment counted for both of S. boulardii and metronidazole ,founded that this yeast have a higher efficacy for treatment (87.45% ) , while mitronidazole efficacy was (81.36%). The microscopic examination to histological sections of the infected mice in cecum and colon regions showed the ability of the parasite to make histological changes included intense inflammatory-cell infiltration, and mucosal damage. The yeast of Saccharomyces boulardii showed higher ability in the healing and recovering damaged tissues compared with metronidazole which showed less efficacy in healing and recovering damaged tissue.

KEYWORDS: Blastocystis hominis ,Saccharomyces boulardii , probiotic, mitronidazole.

1- INTRODUCTION
Blastocystosis refers to a medical condition caused by infection with a protozoal parasite called Blastocystis, which is an intestinal protozoan that inhabits the gastrointestinal tracts, cecum and colon of humans and other animals .This genus was created by Alexieff in
1911[1], it has a world wide distribution and often the most commonly reported human intestinal protozoan in children and adults.[2] At least (20%) of patients in which the organisms found are asymptomatic. There are many individuals that carry the parasite but are asymptomatic. when symptomatic the usual spectrum of symptoms includes watery diarrhea, abdominal pain, perianal pruritis, excessive flatulence. Other symptoms include anal itching, loss of weight, excess gas and constipation.[3] At present, the first choice of chemotherapeutic agent is metronidazole treatment should be considered in all cases. Metronidazole is the first line of treatment, although resistant organisms are now present in Australia and these may be difficult to clear with treatment[4], as well as using probiotics which are defined as live organisms, that confer benefits to the host. Their efficiency was demonstrated for the treatment of gastrointestinal disorders, respiratory infections, and allergic symptoms.[5] There are different routes by which probiotics may control a pathogen includes: probiotics can modulate their physicochemical environment (nutrients, mucus, receptors availability on epithelial cells, pH, tight junctions, and peristaltism), also it can produce biologically active molecules such as bacteriocins, antibiotics, or oxygen peroxide that possess antimicrobial properties and probiotics can induce immune modulation.[6] Saccharomyces boulardii is a strain of yeast[7], it has been classified as a probiotic agent since the 1950s[8], because of its ability to maintain and restore the natural flora in the large and small intestine and surviving at low pH levels.[9]

This yeast has been prescribed in the past 30 years for prophylaxis and treatment of diarrheal diseases caused by bacteria.[10] There are several clinical trials and experimental studies strongly suggest a place for Saccharomyces boulardii as a biotherapeutic agent for the prevention and treatment of several gastrointestinal diseases, it is also resistant to antibiotics and proteolysis.[11]

2- MATERIALS AND METHODS

2-1 Patients

The present study included (5892) stool sample, were collected from patients of both sexes at different ages, which were carried out during the period from September (2013) till September (2014). All patients were obtained from those who had been admitted to / or attended the laboratories of parasitology of the following health institutions (Central City Teaching Hospital, AL-Imam Ali Hospital, AL-Karaama Hospital and AL-Yarmouk Teaching Hospital).
2-2 Collection of stool sample
Stool specimens were collected in sterile, clean, and dry plastic containers with tight lids specially made for this purpose. Each container was given number representing the patient. Each stool sample after making general stool examination and detection Blastocystis hominis in the slide, this positive sample used for culturing this parasite.

2-3 Culture
A typical sample which contain parasites of B. hominis or more in the field of the slide was taken, and cultured in modified Jones’ medium. Approximately (50 mg) of each fecal specimen was inoculated into a screw-cap autoclaved tube containing modified Jones medium. The tubes were incubated at (37°C) for (24, 48 hr), then after (24 and 48 hr) One drop of sample was examined on direct microscopic examination of the culture and calculated the numbers of cysts (C) or Trophozoite (T). Determination can be done by the numbers of C or T in one ml of sample by using the equation of Al-Iдррисе et al. 2008.12

2-4 Determination of the infective dose
Parasitic dose for infection was prepared according to Robert-Thompson et al.1976.13 parasites were counted and the number were adjusted at ( 1x10^3) cell /0.1 ml and then inoculated orally to cause infection into mice.

2-5 Preparation of yeast Saccharomyces boulardii cells
Lyophilized Saccharomyces boulardii (bioflor - laboratoss- biocodex- France) were obtained from the scientific offices, then reactivated by cultured on sabouraud dextrose broth at pH6, and incubation at mixing incubator for (48 hr) at (30 °C).

At the end of incubation, the yeast were recovered by centrifugation for(10min). at (1000 rpm). The deposit was diluted in (0.05) NaCl and counted under the microscope to obtained (1×10^7 cells/0.1ml).14

2-6 Animals
(24) albino mice males with ages range between (8-10 weeks), were obtained from National Control Center For Drugs and Researches (NCCDR). Mice were put in plastic clean cages, and stool of them was examined before the beginning of the experiment to make sure of clearance mice from any intestinal parasites.
2-6 The experimental design
Immunosuppressed of 24 mice by dexamethazone according to Zhongguo et al. 2006.[15] After (5 days), only18 mice were inoculated orally by micropipette with (0.1 ml) of prepared inoculums of Blastocystis hominis.

In the next day all mice feces was examined to confirm the presence of the parasite in the stool and occurance of the infection ,then the infected mice were divided into 3 groups with 6 mice,. the remaining six mice which has been not infected , kept as negative control group.

Then each group was inoculated as follow

- **Group one**: mice given orally (0.1 ml) contain ( 1x10^3 cell) from Saccharomyces.

- **Group two**: mice given (0.1ml) of metronidazole (30 mg / kg / day) orally as a single dose per day.

- **Group three**: mice were not infective , given orally (0.1 ml ) of normal saline and concidered as a positive group.

- **Group four**: mice were given orally (0.1ml) of (pbs) .This group concidered as a negative control.

2-7 Enumeration of Blastocystis hominis
Parasite in feces were enumerated as Shulka et al. 2008.[16] Briefly, mice feces were collected of the first three groups daily from each mouse. One gram of fecal sample was dissolved in (10 ml) of normal saline, homogenized then counted every day.

2-9 Sufficient treatment calculation
Sufficient treatment for Saccharomyces boulardii and metronidazole were measured according to the method of Xia et al.1996.[17]

2-10 Histological study
Mice were sacrificed (2weeks) post infection. Histological sections prepared from their intestine and were examined with light microscope.
3- RESULTS AND DISCUSSION
The present study evaluated the effect of *Saccharomyces boulardii* as probiotic supplementation on *Blastocystis hominis* in albino mice and compare it with metronidazole. In this study it was found that incubation period of this parasite appeared within (24-48 hr).

It was noticed that orally inoculation of this yeast in infected mice led to reduce the shedding of this parasite in stool of mice since the first day of treatment (2.50 x10^2 cell/gm), and continue to decrease gradually with days till stopped shedding of parasite and became (zero) in day (12th) post inoculation, compare with mitronidazole showed a slight decrease occurred after first five days post inoculation (2.83 , 2.67 , 2.00 , 2.00 , 2.00 x10^2 cell/gm) respectively, then shedding of parasite start decrease gradually till reach to zero at day (13th), when the parasite completely disappear in the mice stool, as seen in table (1). While the infected mice in positive control group maintained on shedding the parasite in stool with continuously increased in number of parasite till day (13th) post inoculation was reached to (11.50 x10^2 cell/gm). There was no significant differences between the mean of the two treatment groups, group treated with.

Table (1): Mean number of *Blastocystis hominis* in treatment and control groups ± SD x 10^2

<table>
<thead>
<tr>
<th>Days</th>
<th>S.boulardii</th>
<th>Mitronidazole</th>
<th>Control</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.50 ± 0.54</td>
<td>2.83 ± 0.75</td>
<td>3.33 ± 0.51</td>
<td>0.756</td>
</tr>
<tr>
<td>2</td>
<td>2.17 ± 0.41</td>
<td>2.67 ± 0.81</td>
<td>4.33 ± 0.51</td>
<td>0.745</td>
</tr>
<tr>
<td>3</td>
<td>1.50 ± 0.54</td>
<td>2.00 ± 0.63</td>
<td>5.00 ± 0.63</td>
<td>0.745</td>
</tr>
<tr>
<td>4</td>
<td>1.33 ± 0.51</td>
<td>2.00 ± 0.63</td>
<td>5.33 ± 0.51</td>
<td>0.686</td>
</tr>
<tr>
<td>5</td>
<td>1.00 ± 0.00</td>
<td>2.00 ± 0.63</td>
<td>6.00 ± 0.63</td>
<td>0.778</td>
</tr>
<tr>
<td>6</td>
<td>0.667 ± 0.21</td>
<td>1.50 ± 0.54</td>
<td>6.50 ± 0.54</td>
<td>0.661</td>
</tr>
<tr>
<td>7</td>
<td>0.667 ± 0.21</td>
<td>1.33 ± 0.05</td>
<td>6.50 ± 0.83</td>
<td>0.789</td>
</tr>
<tr>
<td>8</td>
<td>0.667 ± 0.21</td>
<td>0.833 ± 0.41</td>
<td>7.67 ± 0.81</td>
<td>0.745</td>
</tr>
<tr>
<td>9</td>
<td>0.667 ± 0.21</td>
<td>0.667 ± 0.21</td>
<td>7.83 ± 0.75</td>
<td>0.745</td>
</tr>
<tr>
<td>10</td>
<td>0.333 ± 0.05</td>
<td>0.667 ± 0.21</td>
<td>8.67 ± 0.82</td>
<td>0.778</td>
</tr>
<tr>
<td>11</td>
<td>0.166 ± 0.04</td>
<td>0.500 ± 0.05</td>
<td>10.16 ± 1.16</td>
<td>0.962</td>
</tr>
<tr>
<td>12</td>
<td>0.00 ± 0.00</td>
<td>0.333 ± 0.05</td>
<td>10.16 ± 1.16</td>
<td>0.908</td>
</tr>
<tr>
<td>13</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>11.50 ± 1.04</td>
<td>0.745</td>
</tr>
</tbody>
</table>

when applied the equation of treatment efficacy, found that *Saccharomyces boulardii* efficacy was (87.45%) while mitronidazole efficacy was (81.36%). Table (2).
Table (2): Efficacy treatment for treatment groups.

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>dose / ml</th>
<th>Efficiency of treatment(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharomyces boulardii</td>
<td>0.1</td>
<td>87.45</td>
</tr>
<tr>
<td>Mitronidazole</td>
<td>0.1</td>
<td>81.36</td>
</tr>
</tbody>
</table>

To study the pathogenesis of *Blastocystis hominis* parasite, the positive control group regarded as the best example to study the pathogenesis of parasite in the mice, mice were sacrificed after (2weeks) post infection.

Slides of histological examination of the cecum and colon of positive control group showed intense inflammatory-cell infiltration, mucosal damage, inflammation or increased numbers of infiltration of lymphocytes in the mucosa compared with negative control group, as shown in figure (1), figure (2) and figure (3).

Figure (1): Section of large intestine (colon) in mice of negative control group showing normal structure appearance of colonic mucosa with normal mucus secretion.(H&E), 100 x.
Figure (2): Section of large intestine (colon) in mice of positive control group, showing infiltration of lymphocytes and damage in mucosa tissue. (H&E), 200 x.

Figure (3): Section of large intestine (colon) in mice of positive control group, showing damage in mucosa tissue and hydropic degeneration. (H&E), 400 x.

The results of histopathological study confirm the results of experimental study, the results showed that *Saccharomyces boulardii* led to heal large intestinal tissues which damaged by *Blastocystis*, figure (4). While mitronidazole caused damage in the tissues of large intestine which noticed through the infiltration of lymphocytes occurred, figure (5).
Figure (4): Section of large intestine (colon) in mice treated with *Saccharomyces boulardii*, showing near normal appearance of colonic mucosa. (H&E), 10x.

Figure (5): Section of large intestine (colon) in mice treated with mitronidazole, showing infiltration of lymphocytes and short length of villi. (H&E), 20x.

*S. boulardii* has several different types of mechanisms of action, which may be classified into three main areas: luminal action, trophic action \cite{18}, and the third action in regulation of immune response. \cite{19}
The luminal action of this yeast include antimicrobial activity, The another luminal action achieved by antitoxin effects .The second type of mechanism of action is the trophic action on the intestinal mucosa ,and the third type of action of S. boulardii , is in regulation of immune response. 

There is increasing evidence that the gastrointestinal microflora is a major regulator of the immune system, not only in the gut, but also in other organs. The efficacy and safety yeast Saccharomyces boulardii has been prescribed in the past (30 years) for prophylaxis and treatment of diarrheal diseases caused by bacteria . Importantly, S. boulardii has demonstrated clinical and experimental effectiveness in gastrointestinal diseases with a predominant inflammatory component, indicating that this probiotic might interfere with cellular signaling pathways common in many inflammatory conditions.[10]

REFERENCES


