Trop J Nat Prod Res, September 2022; 6(9):1443-1445 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Experimental Trial of Probiotics as a Treatment for *Blastocystis hominis* in Mice

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ARTICLE INFO

Article history:
Received 23 March 2022
Revised 05 September 2022
Accepted 19 September 2022
Published online 01 October 2022

ABSTRACT

Blastocystis is one of the most prevalent parasitic infections in humans, with a worldwide distribution. It is a tiny parasite that lives in the gastrointestinal tract. Probiotic bacteria have been found in the feces of some people who have diarrhea, stomach pain, or other gastrointestinal issues. Probiotic bacteria have proven to be effective in treating many digestive diseases, and they stimulate early immunity in children and improve immunity at all ages. The present study aimed to confirm the probiotic role in parasitic diseases, if any. To do so, Blastocystis hominis was used as a model to confirm probiotic effectiveness in parasitic infections. Children with recurrent diarrhoea had their stools sampled as part of an experimental study. After microscopic inspection of all stool samples for Blastocystis hominis and other parasites, isolated Blastocystis hominis was used to infect BALB/C mice. In experimentally infected mice, the effectiveness of metronidazole coupled with probiotics was compared to that of metronidazole alone and probiotics alone. Compared to the infected group treated with metronidazole and the infected group treated with probiotics, the mean number of cysts in the stools of infected mice significantly decreased in the metronidazole combination probiotics treatment group.

***Keywords*:** Metronidazole, Probiotics, *Blastocystis hominis*.

Introduction

The most common gastrointestinal parasite in humans, *Blastocystis spp*., is a "zoonotic micro-eukaryote parasite" that has historically caused major health problems in developing countries; however, infection is uncommon but serious when it does occur, particularly in children, who experience chronic diarrhoea, malnutrition, nutrition-related physiological abnormalities, and mental biological process disorders.[2] In developing countries, the infection rate is significant and has remained consistent over the last ten years, ranging from 30% to 50%.[2] According to epidemiological studies, *Blastocystis hominis* has been seen in children as well as adults in tropical and sub-tropical settings. The incidence is higher in underdeveloped countries (30–60%) than in developed countries (1.5–10%).[3,4] The saline smear technique, formalin-ether technique, and Trichrome stain are all used to diagnose *Blastocystis spp*.[5] Abdominal discomfort, vomiting, and diarrhoea are gastrointestinal symptoms brought on by *Blastocystis spp*. But there were also reports of irritable bowel syndrome and urticaria sensitization.[6] The most effective and extensively used antibiotic for treating Blastocystis infections is metronidazole. It's given to adults in doses of 250-750 mg, depending on the patient's weight. [7] Probiotic bacteria are exogenous micro-organisms that positively influence the gastrointestinal balance and are beneficial to the health of the host when given orally.[8] Recent experiments have been conducted on probiotic bacteria to investigate the preventive and curative effects of reducing the pathogenicity of many parasites.[9] Common probiotics are yeasts and spore-forming Bacillus spp. They are capable of adhering and generating bioactivated substances that activate the immune system. [10]

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Citation: Sadoon HS. Experimental Trial of Probiotics as a Treatment for *Blastocystis hominis* in Mice. Trop J Nat Prod Res. 2022; 6(9):1443-1445. http://www.doi.org/10.26538/tjnpr/v6i9.18

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Materials and Methods

In this experimental study, stool samples were collected from patients and children suffering from chronic diarrhea. Samples were collected in May, June and July 2021from the private and governmental hospitals in Nineveh Governorate, Probiotic bacteria were obtained from (9 Nutral Pharmma.com). To detect *B. hominis*, the stool samples were examined using microscopy before and after formalin ethyl acetate concentration and permanent trichrome staining, using the iodine wet mount technique. Isolated *B. hominis* was used for animal experiments, Swiss white mice of the type *Mus musculus* were used. Mice were orally inoculated with a 10^4 Blastocystis cyst that had been prepared previously according to Yoshikawa's method.[11] Laboratory bred Swiss mice, 4 weeks old, weighing 25–30g, were used and divided into two groups, the control group (Cg) and the tested group (Tg), The control group (Cg) consisted of three sub-groups of 10 mice each: the negative control Consisting of non-infected , non-treated mice (Cga), the positive control Consisting of infected and non-treatedmice (Cgb), as well as drug control (Cgc) Infected with parasite and treated with metronidazole as 750 mg/kg[7] Once a day for 30 days after 8 days of demonstrating Blastocystis infection .Tested group (Tg) which divided into two subgroups containing of 10 mice each; Tested group (Tga) *Blastocystis spp*. infected, treated with single daily dose of 10 billion probiotic lactobacilli for 21 days[12] and Tested group (Tgb) *Blastocystis spp*. infected, treated with single daily dose of 10 billion probiotic *lactobacilli* for seven days combined with metronidazole as 750 mg/kg .To assure infection establishment, all infected mice were treated 21 days after infection.[13] The anti-Blastocystis action of probiotics and metronidazole when primed mice were sacrificed after cessation of therapy was assessed by "parasitological examination" of their stool. Fresh faecal pellets from the large intestine of each mouse were collected separately and examined daily. The mean numbers of cysts were calculated at the 21st day PI (to assess infection establishment) and the 28th day PI (one-week post-treatment) before mouse scarification, according to each group. Each faecal sample was suspended in 7.0% formalin and homogenized; 100 microns of the solution were examined microscopically, and the number of Blastocystis cysts was counted.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Ethical approval
The study was approved by the Medical Research Ethics Committee at the University of Mosul, Mosul, Iraq with approval number UM.VET.2021.052.

Statistical analysis
The results were statistically analysed by using one-way ANOVA with the help of the statistical package for the Social Sciences (SPSS). According to SPSS, a P value was considered significant when it was less than 0.05, $p < 0.05$.[14]

Results and Discussion

Blastocystis hominis is a single-celled parasite that infects humans and some animals. Blastocystis has drawn a lot of criticism. It has varyingly been viewed as a pathogen and commensal. [15] In parasitological surveys, it is frequently the most frequently isolated protozoan.[5] The current study showed that The mean number of cysts in the positive control non-treated subgroup (Cgb) was 3500 cysts with SD = 36.88, while the mean count in the infected group treated with metronidazole (Cgc) was 2812 cysts with SD = 33.92. The mean number of cysts detected in the infected subgroup treated by probiotics (*Lactobacillus*) (Tga) was 800 cysts with an SD of 28.39. The mean number of cysts detected in infected subgroup treated by combined metronidazole and probiotics (*Lactobacillus*) (Tgb) 520 cyst with SD= 18.4 As shown in Tables (1,2) and Figures (1). There was a decrease in mean parasitic count in all infected treated subgroups compared to positive infected non-treated control subgroup. This was marked and statistically significant in the infected group treated by combined metronidazole and probiotics (Tgb) with ($p < 0.001$) as compared to infected group treated with metronidazole (Cgc) and infected group treated with probiotics (*Lactobacillus*). That is, all infected groups had a lower Blastocystis hominis count in their stool when compared to the control positive group. as illustrated in Table (3) and Figure (2).
The most effective treatment for Blastocystis hominis is metronidazole.[16] In the current investigation in experimentally infected mice, probiotics and metronidazole were compared, and they showed a reduction in mean parasitic count in all infected and treated groups compared to infected, non-treated, and positive control groups. This was more pronounced and robust in the infected probiotic-treated subgroups compared to the infected metronidazole-treated subgroups ($p < 0.001$). This was consistent with the findings of Mohamad *et al* (2016) who proved Several strains of probiotics have demonstrated antihelminthic effects, such as Lactobacillus sporogenes on *Schistosoma mansoni*. Probiotics have a significant effect on worms in the larval stage and egg stage, and at the same time reduce the number of worms and eggs.[17] Many recent experiments have been conducted on probiotic bacteria to investigate the preventive and therapeutic effects of reducing the pathogenicity of many parasites.[18] Several studies have been able to estimate the protective effects of probiotic bacteria against the nematode worm *Toxocara canis* in mice studies. Basualdo *et al.* (2007) confirmed that mice infected with *Toxocara canis* and treated with *Enterococcus faecalis* significantly reduced infection.[19] In addition, different doses of *Enterococcus faecalis* bacteria given to mice had a lethal effect on the larval stages inside and outside the body.[20] The most often used medication to treat an infection caused by Blastocystis sp. is metronidazole. Adult dosages range from 250 to 800 mg, administered three times a day for 10 days, or combined with another medication like paramomycin.[21] Investigations have widely documented metronidazole resistance and treatment failure. Even though it's the initial line of defense against Blastocystis sp. The effectiveness of metronidazole as a treatment for infection varies from 0% to 100%, with an effective dosage ranging from 250-750mg.[22] Antibiotic resistance or variation in the different strains of Blastocystis may be the cause of variable treatment outcomes. A study conducted of metronidazole resistance and subtype-based differences in Blastocystis drug sensitivities noticed that subtypes 7 and 4 are resistant to metronidazole and also have to pass resistance to tinidazole, suggesting that previously unknown pathways of activation and/or resistance are entangled.[23] In the present study, metronidazole and lactobacillus combination were more effective at treating the signs and symptoms of blastocystis hominis than were probiotics This agrees with EL-Askary *et al*.[24]They reported that metronidazole alone was not as effective at treating *B. hominis* as silver nanoparticles, probiotics, and metronidazole together.[24]

Table 1: Demonstration of the mean number of "*Blastocystis hominis*" cysts in stool, the standard deviation in groups, and p-value

Blastocystis hominis	**(Cgb)**		**(Cgc)**		**(Tga)**		**(Tgb)**		
in stool	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***P* Value**
	3500.00	36,88	2812.00	33.92	800.00	28.39	520	18,4	< 0.001*

*****Significant (p<0 05)

Table 2: Comparison of *"Blastocystis hominis* cyst" count in stool between groups (Pair-wise post hoc comparisons)

	(Cgb)	**(Cgc)**	**(Tga)**	**(Tgb)**
(Cgb)		<0.001	< 0.001	< 0.001
(Cgc)	< 0.001		< 0.001	< 0.001
(Tga)	< 0.001	<0.001		< 0.001
(Tgb)	< 0.001	< 0.001	< 0.001	

Table 3: *Blastocystis hominis* reduction count in stool of infected treated groups in all infected groups compared to control positive group

Variables	**(Cgc)**	**(Tga)**	**(Tgb)**
Number of *Blastocystis hominis* cysts in stool	21.21%	65.09%	77,2%

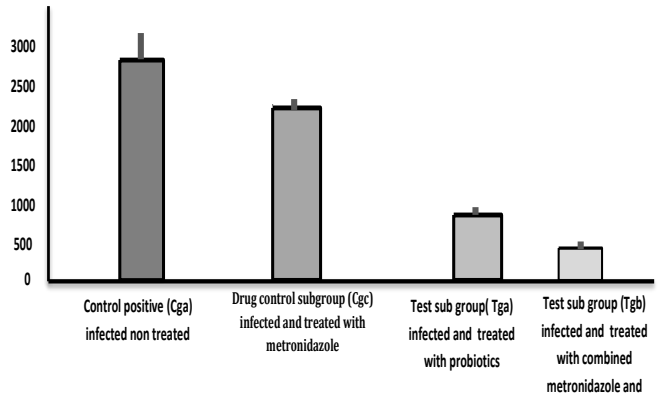

Figure 1: Number of *Blastocystis hominis* in stool of all

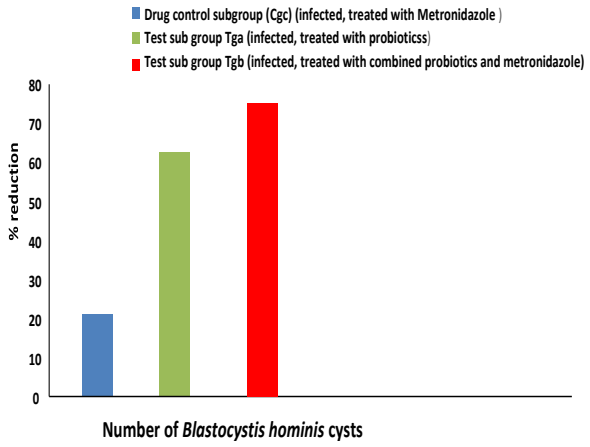

Figure 2: Reduction in *B. hominis* count in stool of infected treated groups

Conclusion

Blastocystis hominis Its detection in the stools of children who complain of diarrhea is crucial since it is crucial to the pathophysiology of the condition. Probiotics were discovered to be more effective than metronidazole in the treatment of Blastocystis hominis.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The author wishes to express his gratitude to the University of Mosul, College of Education for Pure Science, Department of Biology, for their assistance in improving the quality of this work.

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