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Effect of *Bifidobacterium* Probiotic in the Treatment of Giardiasis Infection in Mice

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Abstract:

Metronidazole therapy is recommended in the treatment of giardiasis,athough some clinical reports mention the resistance to this drug from many pathogens. Many studies were applied to show the effect of probiotic to prevent or to heal diseases of gastrointestine, but only few is known about probiotic activity against infections of protozoa. This study aims to evaluate the efficiency of *Bifidobacterium* against infection with *Giardia lamblia* in experimental mice. It was found that daily application of viable *Bifidobacterium* cells with a single dose (0.1ml⁄mice⁄day) significantly reduced the shedding of *Giardia lamblia* parasite cysts in feces, and infection completely disappeared at the day (15^{th}) post inoculation with this probiotic. Also, it was noticed that *Giardia* cysts were reduced in the group treated with metronidazole, and infection cured at day (17^{th}) from treatment, while the control group showed shedding cysts of this parasite. Histopathologically, the effect of *Bifidobacterium* in vivo by gut cells modulation prevents the colonization of *Giardia*, leading to reduce the infection with this parasite.

Key words: *Bifidobacterium*, Giardiasis,*Giardia lamblia*, Metronidazole, Probiotic.

Introduction:

Giardiasis is a disease caused by flagellated protozoan parasite called *Giardia lamblia* (1). The reported prevalence of this parasite in human in low income countries is 4-43% and 1-7% in high income countries (2). It is the most common intestinal parasite (3), and is the main cause of gastrointestinal disorders (4). The disease is transmitted by fecal oral route (5), its trophozoites encyst and new cysts are found in the host through feces (6), which are resistant to environment and make parasite transmit to other host (7). Cysts are resistant to ozonolysis and chlorination (8), and remain viable for three months in cold water (9).

Giardia is the causative agent of dysentery (10), mostly with other symptoms including nausea, gas, and abdominal cramps (11). This parasite attaches to mucosa but does not invade (12) , the mechanism of causing disease, including a decrease in jejunely water and glucose absorption, leads to : malabsorption of fluids, and osmotic diarrhea (13), immunologic reactions, changed the motility of the gut, destruction of endothelial brush border, and secretion of enterotoxins (14).

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This parasite is generally treated with antibiotics, but the appearance of resistant strains and the side effects of drugs (15), have encouraged researchers to use other therapies including probiotics, which are live microorganisms, and when administered in adequate amounts, provide a health benefit to the host (16).

The idea of using probiotics as a therapy has opened up new angles on the role of gut microflora in disease prevention. *Bifidobacteria* is one of the most common bacteria colonizing the gut and very useful to the host intestine, immune health and metabolism (17).

It was found that the administration of this probiotic, inhibits rotavirus, which is the main cause of diarrhea in infants (18).

Bifidobacterium is considered a conventional treatment of ulcerative colitis (19). Furthermore, a recent study reports the lower incidences of necrotizing enterocolitis in preterm neonates after the routine application of *Bifidobacteria* (20).

The current study aims to gain the effects of *Bifidobacterium* on *Giardia lamblia* infection in immune suppressed mice, and evaluate the activity of this probiotic compared with metronidazole.

Material and Methods:

Sample Collection

This study included (30) samples of stool taken from patients infected by Giardiasis, in Medical City Teaching Hospital in Baghdad between (April, 2017 till October, 2017). A wet slide was prepared by using iodine to demonstrate *Giardia lamblia* (21). The positive samples were kept in cold containers, and transferred to Baghdad University.

Parasite Purification

A method of Bingham and Meyer for purifying the cysts of parasite has been done by suspending the cysts in phosphate buffer saline with using centrifugation method at (700 x) for (10 min), to make the concentration (1×10^6) cysts/ (0.1) ml (22).

Bifidobacteria Cells Preparation

This probiotic was obtained from Biology Department , College of Medicine, University of Baghdad, growth done in MRS broth by being incubated at (37)°C for (24) hours. The supernatant was neglected and the cells of bacteria were washed and suspended to contain (1×10^7)/ml and (0.1) ml was fed orally to experimental animals, using blunt-ended feeding needle (23).

Animals

Thirty two male BALB/c mice, the age ranged between (5-8) weeks old (20-25) gm obtained from animal house, College of Medicine, University of Baghdad. Healthy mice were used in this study and examined for any infection by examination of stool (24)..Mice immunity was inhibited by injection with (0.1) ml of dexamethasone (MSD company) /mice/day for (5) days before the beginning inoculation of the parasite according to Regh (1996) (25), except the mice which were considered as a negative control group (not given inoculum).

Experimental Design

Mice were grouped into (4) groups, each group contained (8) mice.

The first (3) groups were inoculated orally by micropipette with (0.1) ml of prepared inoculum of *Giardia*, and their feces were examined daily to confirm the presence of this parasite in the stool. The last group (IV) were not infected, kept as a negative control group to compare between healthy tissues and infected tissues.

Group I: Animals were given *Bifidobacteria* (0.1) ml containing ($1x10^7$) cell / ml, as a single dose per day.

Group II: Animals were given (0.1) ml of metronidazole (30) mg / kg / day, as a single dose per day.

Group III: Animals were given (0.1) ml of prepared inoculum of *Giardia* and considered as a positive control group.

Group IV: Animals were given (0.1) ml of normal saline. This group was considered as a negative control group.

Enumeration of *Giardia* cysts in feces

Feces of each mouse were collected every day, by taking 0.1 gm of stool sample and mixed with (1) ml normal saline. Slides were stained with Iodine and cysts were counted by haemocytometer (26).

Histopathological Study

Mice were sacrificed (3) weeks post infection by removing small intestine, fixed with (10%) buffered formalin. Slides were examined under the light microscope after staining with haematoxylin and eosin.

Statistical Analysis

Statistical comparisons between groups were done. The data was coded and data entry was done using SPSS version 22. Frequencies, percentages, means and standard deviation were calculated. Inferential statistics such as t- test were used to compare the effect of treatment used between different groups.

Results:

This study investigated the therapeutic effect of probiotic bacteria (*Bifidobacterium*) in treating the infection caused by *Giardia* in immune suppressed mice. The efficacy of treatment was assessed by counting cysts shedding in the feces of mice.

It was found that orally administration of *Giardia* cysts could colonize the gut, and occurrence of infection after (3-8) days of administration in the infected animals. Also, it was noticed that orally administration of *Bifidobacterium* in the infected mice led to reduce the shedding of this parasite in the feces of mice since the first day of treatment , and continue to decrease gradually with days till stopped shedding of parasite and became (zero) in day (15^{th}) post inoculation,compared with metronidazole which showed decrease shedding of parasite after the first day post inoculation ,and gradually disappeared till reaching to zero at day (17^{th}) (Fig. 1).

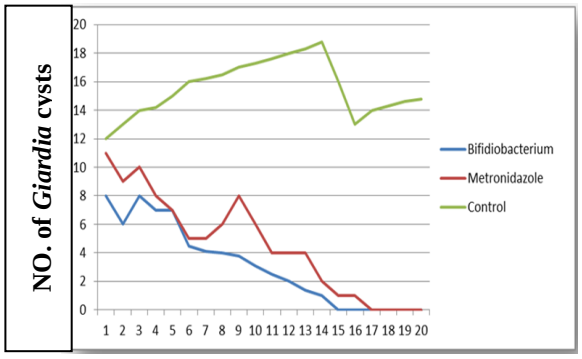

Figure 1. Number of *Giardia lamblia* parasites in treatment groups.

Histological study of the small intestine of negative control group, showed normal stratified appearance of intestinal villi as shown in (Fig.2), compared of the small intestine of positive control group, which noticed marked intestinal villi shrinkage (atrophy) (Fig.3).The histopathological study showed generation of the immune response within the mucosa of small intestine by occurring hyperplasia of pyres patch when using *Bifidobacterium.* (Fig.4), while antiparasitic metronidazole showed shortening of intestinal villi in mice (Fig.5).

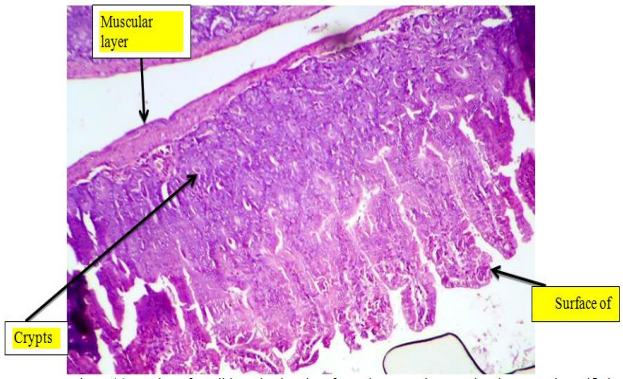

Figure 2. Section of small intestine in mice of negative control group showing normal stratified appearance of intestinal villi .(H&E), 100x .

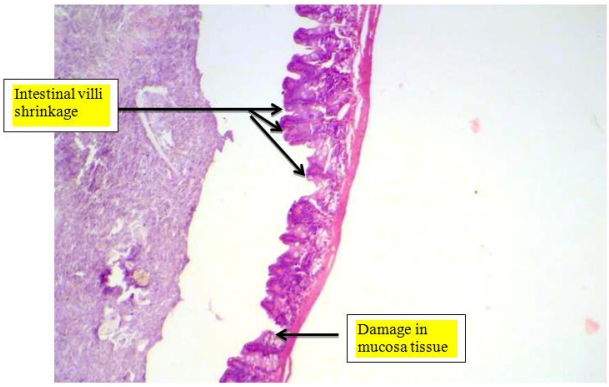

Figure 3. Section of small intestine in mice of positive control group showing marked intestinal villi shrinkage (atrophy). (H&E), 40x.

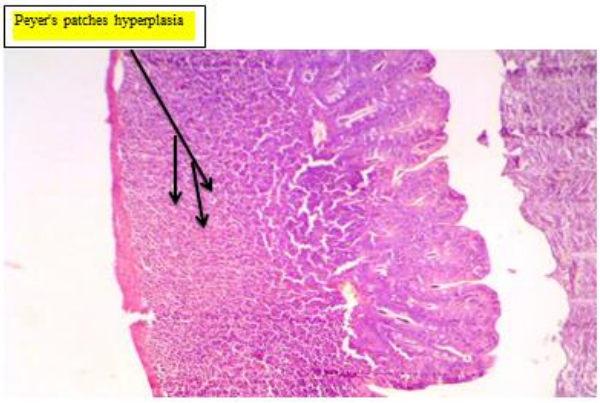

Figure 4. Section of small intestine in mice treated with *Bifidobacterium* showing Peyer's patches hyperplasia. (H&E), 100x.

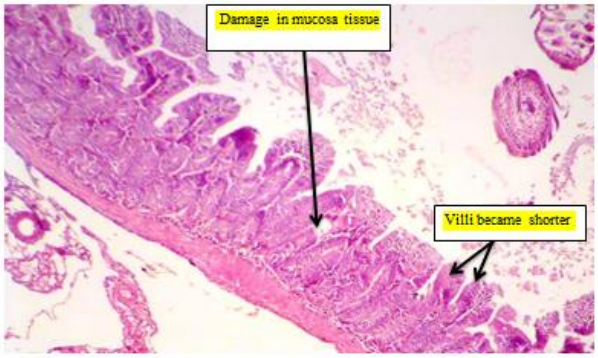

Figure 5. Section of small intestine in mice treated with metronidazole, showing still there is shortening of intestinal villi. (H&E), 100x.

The results showed the activity effect of *Bifidobacterium* in treating *Giardia* infection by modulating cells of the gut to prevent colonization as well as multiplication of this parasite.

Discussion:

Although metronidazole is considered the drug of choice for treatment of giardiasis, there is a limited range of drugs available including nitroimidazoles, quinacrine and furazolidone (25), but some researchers reported the initiation of the resistance to drug (27).

Giardia lamblia has the ability to attach with mucosa of gastrointestinal to produce infection (28), but *Bifidobacterium* might interfere with this adhesion. The structure of intestinal microflora may inhibit the colonization of the mouse gut by *Giardia* parasite (29), also oral application of *Bifidobacterium* may change the structure of the intestinal flor, thus provide protection against pathogens (30) .

There are many important mechanisms that explain the effects of probiotics on pathogens including the ability to adhere with mucosa, modulation of the immune system, and modification of the gut micro flora (31).

Results show that this probiotic can induce immune modulation, by conversion naive T-cell differentiation into Th1, Th2 or by T- regulatory lymphocytes (32).

Mechanisms by which *Bifidobacterium* might improve host health are: including competition for binding sites and available food sources in the intestinal lumen limits *Giardia* survival in this environment (33), raising the immune function through reinforcing function of mucosal barrier, strengthening epithelia integrity and direct antagonism of pathogenic microorganisms (34), reducing mucosal transfer of luminal organisms and metabolites to the host, and increasing mucosal antibody production (34). *Bifidobacterium* has a direct relation with immune response stimulation because they enhance IgA production and secretion through an alteration of the cytokine medium in the gut mucosa, inducing epithelial cell expression IL-10 as well as IL-6, which stimulate production of IgA (35).

Additionally, the results cleared that *Bifidobacterium* was more efficient in treating the infection caused by *Giardia lamblia* than metronidazole, and reducing infection with *Giardia* by the elimination adherence of its trophozoites to the mucosa. It is also works as a coactive factor in the reduction of *Giardia*.

The important activities of this probiotic may play on essential role in the community to prevent spreading of giardiasis.

Conclusion:

Probiotics have a positive action in the treatment of enteric parasite infections. This study provides evidence that *Bifidobacterium* probiotic is a good choice to improve the mucosal immune system and may be implied in the therapy of giardiasis during immunosuppressive states. However, more investigations are needed to move forward in this direction.

Conflicts of Interest: None.

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تأثير المعززBifidobacterium probiotic في علاج الاصابة بالجيارديا في الفئران

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الخلاصة:

ينصح العلاج بالميترونيدازول في معالجة مرض الجيارديسس، على الرغم من أن بعض التقارير السريرية تذكر مقاومة هذا الدواء من العديد من الممرضات. تم تطبيق العديد من الدراسات لإظهار تأثيرالمعززلمنع او لشفاء أمراض الجهاز الهضمي ، لكن قلة قليلة فقط معروفة عن نشاط المعزز ضد اصابات الكائنات الاولية. تهدف هذه الدراسة إلى تقييم كفاءة البيفيدوبكتيريوم ضد الإصابة بالجيارديا لامبليا في الفئران التجريبية. وقد وجد أن التطبيق اليومي لخلايا البيفيدوبكتيريوم الحيوية بجرعة واحدة (0.1 ملغ /فأر/ يوم) قد خفض بشكل ملحوظ من طرح الطور المتكيس لطفيلي الجيارديا لامبليا في البراز، واختفت الاصابة تمامًا في اليوم (15) بعد التطعيم بهذا المعزز. كما لوحظ أن اكياس الجيارديا قد انخفضت في المجموعة المعاملة بالميترونيدازول، وشفيت الاصابة في اليوم (17) من العلاج ، في حين اظهرت مجموعة السيطرة طرح مستمر لاكياس هذا الطفيلي. تشريحيا ، تأثيرا لبيفيدوبكتيريوم في الجسم الحي عن طريق تحويرخلايا الأمعاء يمنع استعمار الجيارديا ، مما يؤدي إلى الحد من الإصابة بهذا الطفيلي.

الكلمات المفتاحية: البيفيدوبكتيريوم، مرض الجيارديسس، جيارديا لامبليا، ميترونيدازول، المعزز.