Leaky cells and the use of *Lactobacillus casei* in the treatment of laboratory animals infected with amoebic dysentery

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Abstract :

In the present study, a total of 45 Albino Sprague-Dawley rats were suffered from watery diarrhoea were examined. All samples of feces had *Entamoeba histolytica* .The studied rats were divided into three groups ; the first one was gave orally 2 ml of *Lactobacillus casei* extraction, the second was gave 2 ml of *L. casei* suspension, and the third was gave 2 ml of normal saline as positive control . The 1st group was appeared 12/15 (80%) of recovery, the 2nd group appeared 15/15(100%) of recovery, and the 3rd group appeared no recovery 0/15 (0.0). Also two ml of vein Blood was collected from rats to determine hemoglobin level (Hb), packed cell volume (PCV), Red Blood Cells Count, and white blood cells differential (WBC's differential). Our results showed that the means of RBC's count (4.7, 5.8, 4.5, 4.7)x 10^6/mm^3, Hb levels (12.26,14.77,12.54,13.2) gm/dl and PCV values (37.8, 42.1, 36.9, 37.6)% for control, infected, treatment with *L.casei* extract and treatment with *L.casei* suspension groups , respectively. Also there were rise in numbers of lymphocytes, monocytes and eosinophils significantly, and decline in the ratio of types heterophils and basophils. This study concluded that the use of *L. casei* is useful in the treatment of diarrhea caused by amoebic dysentery parasite .

أستخدام راشح وخلايا بكتريا *Lactobacillus casei* في علاج الحيوانات المختبرية المصابة بالزحار ألأميبي

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الخلاصة :

في الدراسة الحالية تم فحص 45 جرذ البينو يعانون من اسهال مائي. كانت جميع العينات المفحوصة مصابة بطفيلي الزحار الأميبي . تم تقسيم الجرذان المدروسة الى ثلاث مجاميع،جرعت المجموعة الأولى فمويا ب 2 مل من خلاصة بكتريا عصيات حامض اللاكتيك والثانية بنفس المقدار من عالق نفس البكتريا أما المجموعة الثالثة فجرعت ب 2مل من المحلول الملحي كسيطرة . أشارت النتائج الى شفاء 80% من جرذان المجموعة الأولى وشفاء تام 100% من جرذان المجموعة الثانية أما مجموعة السيطرة الموجبة فلم يلاحظ اي شفاء ونفقت بسبب الجفاف . كما تم سحب 2 ملل من الدم الوريدي من الجرذان للتحري عن مستوى الهيموغلوبين ، حجم خلايا الدم المضغوطة ، تعداد كريات الدم الحمر ، والتعداد التفريقي لكريات الدم البيض . بينت نتائج الدراسة الحالية بأن المتوسط الحسابي لعدد كريات الدم الحمر (4،7 ؛5،8؛ 4،5 ؛ 4،7)×10^6/مم^3 ، معدل الهيموغلوبين (12،26؛ 14،77؛ 12،54؛ 13،2) غم /دسي لتر ، حجم الخلايا المضغوطة (37،8؛ 42،1؛ 36،9؛ 37،6) % لكل من السيطرة ، المصابة، المعالجة بخلاصة البكتريا ، والمعالجة بعالق البكتريا على التوالي. كما وجد زيادة في أعداد الخلايا اللمفية وأحادية

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النواة والحامضية ونقصان في نسبة أنواع الخلايا اللمفاوية المختلفة والقاعدية .أستنتجت هذه الدراسة بأن استخدام بكتريا حامض اللاكتيك يفيد في علاج حالات الاسهال المتسبب عن طفيلي الزحار الاميبي.

Introduction :

Amebiasis is a common worldwide disease in developing countries, caused by infection with the protozoan parasite *Entamoeba histolytica*. About 40,000 people are estimated to die each year from amoebic colitis and amoebic liver abscess (ALA) (Walsh, 1986). A small fraction of *E. histolytica*–infected people present with clinical symptoms of colitis or extraintestinal invasion leading to the diarrhoea (Sharma and Ahuja, 2003).

Traditional medications taken to prevent diarrhoea include bismuth sub-salicylate and prophylactic antibiotics. Bismuth subsalicylate frequently is not effective as a preventive agent because of non-compliance. Prophylactic antibiotics are also not recommended for diarrhoea as the etiologies of diarrhoea varies widely and the concern over antibiotic resistance by overuse of antibiotics overweighs the potential benefits(Yates, 2005). One of the most promising is the use of probiotics (micrbiotherapy) for the prevention of various types of diarrhoea. Use of probiotics microorganisms such as lactic acid bacillus (*Lactobacillus*) lowers dependence on antibiotics,is relatively inexpensive and is well tolerated, even for prolonged use (McFarl, 2000).

Lactobacillus Therapy has been shown to be effective in the treatment of a variety of disorders including gastrointestinal disorders ,vaginal infections, hepatic encephalopathy, hypercholesterolemia and conditions related to deficiency of vitamins B. *Lactobacilli* have proved to be useful in delaying the indication of tumor and have found use in prevention of colon cancer. *Lactobacilli* are thus valuable therapyeutically and are often used as adjuvants to antibiotic therapy. They also find use as growth enhancers for domestic animals and poultry.The therapyeutically used species of *Lactobacillus* include *L.acidophilus* , *L.brevis*, *L.casei*, *L.bulgaricus* and *L.bifidus*. However, the evidence of effectiveness of implantation , survival and proliferation of these organisms in the gut are not impressive (Woods, 1992). The aim of present study is to assess the efficacy of therapeutic use of probiotics for the treatment of diarrhoea due *E. histolytica*.

Materials and Methods :

Samples collection :

Diarrhoeal stool samples were collected from 45 of Albino Sprague-Dawley rats had weight (95-105) grams, aged (8-10) weeks and examined under microscope to observe the parasite of *Entamoeba histolytica*. The studied rats were divided into three groups ; the first one was gave orally 2 ml of bacterial extraction , the second was gave 2 ml of bacterial suspension, and the third was gave 2 ml of normal saline as control. In the sixth day, the stool was examined for presence of *E.histolytica*.

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Preparation of bacterial suspension :

Documentation of diarrhoea is based on clinical assessment and self-report of symptoms and is defined as >3 loose stools/day (Hill, 2000 ; Shaw *et al*., 2004). The *Lactobacilli* suspension ($2.5*10^8$ colony/ ml) by using Macferland tubes method for bacterial counting (Murray *et al*., 2007) .

Preparation of bacterial extraction :

The abstract of *Lactobacilli* was prepared according to Massi *et al.*,(2006) by cultivation the *Lactobacilli* bacteria in 100 ml of Ragosa broth and incubated at 37C for (24-48) hours, then it centrifuged at (6000 round / minute) for 10 minutes. The sediment was taken and determine the moisture rate and excluded for it weight in each time in preparation of abstracted doses (AOAC, 2002).

Blood tests :

A two ml of vein Blood was collected. Hemoglobin concentration was determined by the Sahli's system (Spain) and Hematocrit was measured using heparinized micro-hematocrit tubes and a micro-hematocrit centrifuge(Germany). Thin blood films were fixed and stained for Red Blood Cells Count (DeMaeyerand Adiels-Tegman,1985).

Results :

A total of 45 Albino Sprague-Dawley rats were suffered from watery diarrhoea were examined.Samples of feces were detected by microscopy . All samples of feces had *E. histolytica* . The studied rats were divided into three groups ; the first one was gave orally 2 ml of bacterial extraction extraction , the second was gave 2 ml of bacterial suspension, and the third was gave 2 ml of normal saline as control . The 1st group was appeared 12/15 (80%) of recovery, the 2nd group appeared 15/15(100%) of recovery, and the 3rd group appeared no recovery 0/15 (0.0) (table 1,2).

Table 1. Treatment of infected rats by *Lactobacillus casei*

Name of Parasite	No. of infected rats	Treatment with *L.casei* extract	Treatment with *L.casei* suspension	Control
E.histolytica	**45**	**15**	**15**	**15**

Table 2. The rate of treatment response

Treatment	No. of recovery rats	%	No. of non- recovery rats	%
***L.casei* extract**	**12**	**80**	**3**	**20**
***L.casei* suspension**	**15**	**100**	**0**	**0**
Normal saline	**0**	**0**	**15**	**100**

P-value	C.S
0.936	Significant

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Table 3 shows the means of RBC's count (4.7, 5.8, 4.5, 4.7), Hb levels (12.26,14.77,12.54,13.2) and PCV values (37.8, 42.1, 36.9, 37.6) for control, infected, treatment with *L.casei* extract and treatment with *L.casei* suspension groups , respectively.

Table 3. RBC's count, Hb levels, and PCVmeasurements of group categories

	RBC (x 10^6/mm^3)	Hbgm/dl	PCV%
control	4.7	12.26	37.8
Infected group	5.8	14.77	42.1
Treatment group with *L.casei* extract	4.5	12.54	36.9
Treatment group with *L.casei* susp.	4.7	13.2	37.6

P-value	C.S
0.00	Non Significant

The total count and differential of leukocytes appears in table 4. The large number of WBCs (8.29) was shown in infected group, while the low number was in treatment group with *L.casei* extract (7.7).

Table 4. WBC differential of infected and treatment groups

	Total Leucocytes mm^3x 10^3	Monocytes %	Lymphocytes %	Heterophils %	Eosinophils %	Basophils %
Control	7.8	5.4	59.1	30.5	1.7	4.3
Infected group	8.29	7.0	54.6	34.2	3.8	2.2
Treatment group with *L.casei* extract	7.7	7.2	64.0	27.1	1.5	1.4
Treatment group with *L.casei*	7.8	7.1	63.9	25.6	1.1	2.3

P-value	C.S
0.04	Non Significant

Discussion

Probiotics are viable microorganisms having a beneficial effect on the prevention and treatment of specific pathological conditions. Some probiotics, such as *Lactobacillus* species and others, have been found to induce innate immune mech-anisms, including enhancement of epithelial barrier function in the intestine and cytokine production in monocytes and natural killer cells, and induction of phagocytic activity in neutrophils (Perdigó *et al*., 2002; Gill ,1998). The oral administration of probiotic bacteria may

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generate resistance to infection directly related with the innate immune response (Berczi *et al*., 2000).

Various clinical trials have been conducted using viable lactic acid bacilli against diarrhoea causing organisms. The present study was revealed to the beneficial effect of *Lactobacillus casei* in the treatment of acute infectious diarrhea caused by *Entamoeba histolytica*. It is evident that *L. casei* enhances innate resistance to *Entamoeba histolytica*, as was showed by lower parasitaemia in rats previously stimulated once or twice with lactobacilli than the control, suggesting that parasites were affected by the non-specific mechanism elicited by viable lactobacilli, and that the spleen plays an important role in the elimination of parasites (Federico *et al*., 2006). Similar results showed that *L. casei* was an effective biologic agent in the prevention and treatment of Enterotoxigenic *Escherichia coli* (ETEC) in rats (Jacalne *et al*.,1990). Researchers found that, the addition of *L. casei* decreased the duration of bloody diarrhea and enhanced the elimination of *E. histolytica* cysts (Dinleyici *et al*., 2009; Bercu *et al*., 2007). Research in immunodeficient mice has also suggested that treatment with probiotics can reduce the parasite burden in intestinal epithelium (Alak *et al*., 1999). Putative mechanisms of action of probiotics include production of pathogen-inhibitory substances, inhibition of pathogen attachment, inhibition of the action of microbial toxins, stimulation of immunoglobulin A, and trophic effects on intestinal mucosa (Gary, 2001).

Supplementation of beneficial probiotic bacterial flora, such as *L. acidophilus*, *Bifidobacterium bifidus* and *L. cassei*, preferably in the form of a varied, vigorous and abundant culture, will restore the healthy intestinal ecology and stabilize the mucosal lining of the gut. A supplemental dosage of at least one billion organisms per day is necessary to achieve the critical mass of bacterial restoration and successfully reinvigorate healthy intestinal ecology (Isolauri *et al*.,1999 ; Tannock,1997).

Geeta Shukla *et al*.,(2008) were observed that *L. casei* fed 7 days prior to *Giardia* infection was more effective and efficient in eliminating the infection from mice (Geeta Shukla *et al*., 2008). A previous in vivo study in the prevention of gastrointestinal infection used fermented milk with *L. casei* and *L. acidophilus* as immunobiological agents against *Salmonella typhimurium* infection in mice (Perdigon *et al*., 1990).

A common belief about how probiotics work is that ingestion improves the "balance" of the intestinal and vaginal microflora so that pathogen growth is restricted. Recent studies indicate that this concept is simplistic and that probiotics probably work by multiple mechanisms. Furthermore, each agent may have unique actions (Perdigon *et al*., 1990; Geeta Shukla *et al*., 2008). Probiotics have been also proposed to influence gut microflora and development of immune response. The underlying mechanisms are however not clear, involving stimulation of different subsets of immune system cells to

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produce cytokines, which in turn play a role in the induction and regulation of the immune response, and to enhance intestinal IgA immune responses and increase intestinal mucin production (Gill, 2003).

The use of blood investigations is of great important because they change significantly under any effective (Guton and Hall, 2006). The arrival of the gut bacteria particularly *L.casei*, they can cause changes in blood levels of standards significantly. This comes from the effect of metabolic products enhanced bacterial lactic acid. As well as their ability to compete in the stick on the walls of the small intestine and the removal of the pathogens, as well as for non-production of immature cells from red blood cells in the bone marrow and thus the lack of access standards in the blood picture.Our results have agreed with (Thongsong *et al*., 2008).

The results indicated for the high moral in the preparation of white blood cells may be the cause of this increase, return to the stimulation of certain cells such as T-lymphocytes and that motivates some to increase the number of eosinophils in the blood- stream and works of some to increase the permeability of blood vessels for the purpose of migration of these cells to the site injury. These results in agreement with previous studies (Roitt and Rabson, 2000; Xiao *et al*., 2003).

The rise in numbers of lymphocytes between other types of white blood cells (WBC's) could be due to the influence of lactic acid bacteria reluctance that caused the stimulation of lymp hocytes T and B types that perpetuate their effectiveness in stimulating the lymph nodes in the digestive system and as was mentioned by a researcher (Ko *et al*.,1999) and that this reluctance has led to the lack of need to use white blood cells from a monocyte type in which the work in the phagocytosis of germ cells in cases of infection, and thus prepare the cells did not change in the results obtained as stated by (Rees, 2005) .

The decline in the ratio of types(Heterophils and Basophils**)** of white cells could be due to the increase in lymphocytes of T and B species, which refers to increased humoral and cellular immunity by lymphoid cells start in causing this decline, and as was mentioned by (Shaniko Shini, 2003) **.**

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