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The Suppressive effects of Human Derived Probiotics Ingestion against *Listeria monocytogenes* in Murine Model

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ABSTRACT

This study investigated the potential *in vivo* antagonistic behavior of human derived probiotics; *Bifidobacterium longum* Bl and *Lactobacillus acidophilus* La against infection with invasive food born *Listeria monocytogenes* Lm in murine model. Mice Pre-feeding with probiotics conferred a significant protection against orally challenged mice with virulent isolate of Lm. A significant reduction in the level of Lm colonization of the gastrointestinal tract in mice was observed in the probiotics pre- colonization in comparison to the control animals, in that the Lm viable count decreased by almost two log cycle, and this reduction was accompanied by a marked elevation in the level of sIgA and serum IgA, particularly in mice pre-colonized with Bl, as the level of sIgA rose about 3.7 folds. Probiotics pre- colonization also stimulated phagocytic activity, the peritoneal macrophages were pronounced a significant ($P< 0.05$) enhancement in the phagocytic function, the phagocytic index (PI) increased to 65.7 ± 3.95 % and 55.2 ± 3.1 % respectively, when Bl and La were orally administrated to mice. The antilisterial activities appear to be non – lactic acid dependent behavior.

Keywords: Probiotic, *Lactobacillus*, *Bifidobacterium*, *L. monocytogenes*, Antagonistic effects, immunomodulatory activity.

1. INTRODUCTION

Listeria monocytogenes is Gram – positive, nonspore-forming, motile, facultative anaerobic bacterium. It can grow and reproduce inside the host's cells. It is one of the major food-borne pathogens and responsible for listeriosis [1], The manifestations of listeriosis include septicemia, meningitis (or meningoencephalitis), corneal ulcer, pneumonia and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (second to third trimester) or stillbirth [2], with 20 to 30 % of clinical infections resulting in death worldwide [3]. The ingestion of products contaminated with this organism may be a potential health threat to high-risk population such as immunocompromised individuals, elderly, and pregnant women and their unborn fetus [4]. Currently antibiotic – based treatment are the most accepted treatment option for listeriosis. As vaccination is unavailable and the use of antibiotic is declining due to an increase in resistance and allergies [5]. Improved treatment are still needed, and alternative therapies or complementary to antibiotic therapy are currently becoming more popular than traditional eradication methods. In this respect the use of probiotic is a potentially promising tool to prevent listeriosis. According to an expert consultation by the food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are live microorganisms which when administrated in adequate amount confer a health benefit on the host [6]. Among probiotics, *Bifidobacterium* and *Lactobacillus* are of the favorite genera in studies, and potential application of these probiotics is continuously widening, with new evidence accumulating to support their therapeutic and prophylactic efficacy of many

diseases [7]. They often used in fermented dairy products or food supplements and because of most these genera members are reported as GRAS (generally regarded as safe), these microorganisms and their metabolites. are novel in human therapeutics [8]. Several antilisterial activities were demonstrated in human origin *Bifidobacterium* and *Lactobacillus*, and a few studies have been conducted in this respect, some of theories have been proposed to explain the antilisterial activities of probiotics, These are mainly due to competitive exclusion of pathogen binding, modulation of host immune system, and production of inhibitory compounds, such as organic acids, oxygen catabolites (e.g., hydrogen peroxide), proteinaceous bacteriocins and bacteriocins – like peptides, and amino acids metabolites [12,13].

The aim of this study was to assess the antilisterial activities of potentially human derived probiotic isolates in murine model (*in vivo*).

2. MATERIALS AND METHODS

2.1. Bacteria and growth conditions

Bifidobacterium longum Bl and *Lactobacillus acidophilus* La were used in the study, both are human origin and previously suggested to be promising probiotic strains with regard antilisterial activity [14,15]. Bacteria cultured in de – Man, Rogosa and Sharpe (MRS) broth for La, and MRS broth supplemented with L- cysteine 0.05% for Bl, cultures were incubated at 37°C under anaerobic conditions (anaerobic jar supplied with Gas Pak, BioMerieux, France). To prepare mice diet, log – phase bacterial cells were collected by centrifugation (4000g /20 min at 4 °C), cells were washed twice with sterile phosphate buffer saline (PBS) and resuspended in PBS, pH 6.5 and adjusted to an approximate concentration of 10^8 CFU/ ml by comparison with MacFarland turbidity standard, 100µl inoculum of each probiotic suspension was used for colonization of mice gut by oral micropipette. The test pathogen *Listeria monocytogenes* Lm, was kindly provided by Biology Dept./ Baghdad university/ Iraq. A fresh culture of Lm was grown in brain heart infusion (BHI) broth medium and adjusted at concentration of 10^5 CFU/ ml. for mice infection.

2.2. Animals

Six weeks old Swiss albino mice weighting 25.2 - 28.6 gm were used in the study, were obtained from National Control Center for Drugs and Researches (NCCDR) / Iraq. Mice were kept in plastic cages and maintained on a 12:12 light : dark cycle at controlled temperature 25 ± 2 °C, during the entire duration of experimentation (2 weeks).

2.3. Experimental design

The experimental animals, mice (n= 40) were assigned randomly to four different groups (A,B,C, D), ten mice per group (Table 1). The group A animals were left uninfected, and fed 100 µl of PBS / day for two weeks, considered as control group. Stable colonization of group B animals gastrointestinal tract (GIT) were achieved by daily two consecutive orally administration with 100 µl (10^8 CFU/ ml) of Bl suspension for two weeks. Same protocol was followed for colonization of group C animals with La suspension. A week later of probiotics colonization, animals in groups B, C, D were orally challenged with 100 µl (10^5 CFU/ml) of Lm suspension three times within a week, with one day interval between infections. The animals weight, illness symptoms, and survival rate (number of alive/total number of mice) was recorded daily throughout the study.

Table 1: Experimental animals groups design

Group A	Control group
Group B	Bl pre –inoculated + Lm infected group
Group C	La pre – inoculated + Lm infected group
Group D	Lm infected group

2.4. Gastrointestinal tract colonization

Probiotics colonization of mice GIT was monitored by viable cell count (CFU) in freshly collected feces samples at time intervals of 2, 4, 6, 8, 10, 12, 14 days of experiment. Whereas Lm mice GIT infections was monitored by re - isolation of bacterium from feces samples in intervals of 8, 10, 12, 14 days. Briefly; fecal samples were collected in Eppendorf tubes, weighted, homogenized and 10- fold serial dilution were prepared, 100 µl aliquot was inoculated on agar plates, MRS agar for probiotics counts and BHI for Lm counts. CFU was expressed as ($\log_{10}$ CFU/ gm feces). Identification of feces recovered probiotics were confirmed by API 50 CHL (BioMerieux, France). All the counting was done triplicates.

2.5 Detection of some immunological parameters

2.5.1 Immunoglobulin A (IgA) titer

The titer of secretory immunoglobulin A (sIgA) in the intestinal fluid and serum IgA levels of experimental animals were determined by Sandwich ELISA using Mouse IgA kit (Cusabio, China), following the manufacturer's protocol. Test were performed in triplicate, read at 450 nm with ELISA reader (Bio – Rad), the titer expressed as ng/ml. The sIgA of mice small intestinal contents were prepared according to

the procedure described by [16]. In brief, the day 15 of experiment mice were anesthetized and sacrificed the small intestine samples of each mouse were flashed with 5ml ice – cold PBS, centrifuged 10000g/20 min at 4°C, the supernatant was taken with ml PBS containing 0.1 mg/ml soy bean trypsin inhibitor, and kept at -20°C until use. The fresh cardiac blood was used for detection of serum IgA, approximately 0.5 ml of cardiac blood was collected from anesthetized mice, blood samples left at room temperature for 1 h, sera collected by microfuge (3000g /15min at 4°C), the carefully obtained sera were kept at -20 °C until use.

2.5.2 Phagocytic activity assay

The phagocytic activity of peritoneal macrophages and peripheral blood leucocytes of mice were assessed, and the obtained data was expressed as Phagocytosis index (PI). The peritoneal macrophages were obtained from sacrificed mice by exsanguination. Peritoneal cells were extracted from the peritoneal cavity with 20 mL of ice-cold sterile PBS. The peritoneal exudate centrifuged (400g / 10 min, at 4°C), cells pellet resuspended gently in cold PBS [17]. 100µl of each peritoneal macrophage suspension and EDTA mice treated blood were opsonized separately with equal volumes of *E.coli* suspension (10^8 CFU/ml) in glass test tube, tubes were incubated for 60 min at 37°C to allow phagocytosis proceed, following tubes were cooled at 4°C, to stop phagocytosis. Smear of mixtures was prepared, fixed with methanol, stained with 10% Giemsa stain for 10min and examine under oil immersion microscope. The number of macrophages containing one or more *E.coli* cell was counted and percentage of phagocytic cells was determined by counting at least 100 cells from randomly chosen fields according to the following formula [18]:

$$\text{Phagocytosis index (PI)} = \frac{\text{No. of phagocytic cells}}{\text{No. of (phagocytic + non phagocytic cells)}} \times 100$$

2.6. Determination of Lactic acid concentration

At the end of experiment (two week), Lactic acid concentration was determined in the mice intestinal contents. A part of the small intestine of anesthetized and sacrificed selected mouse in each experimental group was homogenized in PBS, and pH was measured. The homogenate was centrifuged at 2000g / 10min at 4°C, and the supernatant was collected [19]. The amount of lactic acid was assayed using the Lactate kit (Sigma – Aldrich/ UK), following the manufacturer's protocol, and the concentrations were expressed as mM/ml.

2.7. Statistical analysis

Results are expressed as the mean ± standard and the data subjected to analysis system – SAS program [20]. Least significant difference –LSD test was used to significant compare between means in this study. The level of significance was set at $P < 0.05$

3. RESULTS AND DISCUSSION

3.1 Antagonism and illness symptoms

The study investigated the efficacy of probiotics pre-feeding on the antagonistic defense strategies against test organism, the invasive food born bacteria *L. monocytogenes*, The antagonistic behavior of human derived; *B . longum* Bl and *L. acidophilus* La were analyzed *in vivo*, in murine model. Animals weight were monitored throughout the study (two weeks) as an index of general well – being. It was found non statistically significant difference ($P>0.05$) among the groups animals (Table .2). The slight increase in animals weight of groups B and C may be refer to effectiveness of probiotics administration in limiting Lm infection severity and improving intestinal ecosystem physiology and function. Thereby improve mice digestion and immunity, consequently mice health [21]. The main recorded illness symptoms was associated to group D animals (pathogen challenged group). It was confined to lethargy, hair loss, movement disorders, and shedding of loss feces was observed in the last two days of experiment. The mentioned clinical signs were almost inhibited in group B and C animals, except the lethargic of some animals. The survival rate (number of alive/total number of mice) was recorded daily, there have been no deaths in the animals of groups A, one mortality was recorded in both experimental groups B and C animals over the inoculation sessions. While the survival rate was 40% in group D, as more than half of group animals had died at the end of experiment. It was clear the probiotics pre – fed were able to confer a protection against infection with Lm, as demonstrated by the absence of visible illness symptoms and high survival rate, in groups B and C animals in comparison to group D animals, and may indicated that the used probiotics were able to colonize the digestive tract of the mice and exert antimicrobial activity *in vivo*, which decreased the severity of Lm infection and mortality percentage, and improve general health scores [22].

3.2 Gastrointestinal tract colonization

Experimental animals in the groups B and C, were individually received a daily two consecutive dosage of 10^8 viable Bl and La for two weeks. The colonization of mice GIT was tracked by isolation of viable probiotics from mice feces samples intervals. The analysis of obtained data showed that the followed protocol was sufficient to establish good furnishing of mice GIT. Probiotics were properly achieved GIT colonization and maintained high population levels, and was no significant difference ($P>0.05$) in the number of viable probiotics between the both experimental groups B and C throughout the feces sampling period. Bl and La were reisolated from the mice feces during the administration period at average levels 3.5 ± 0.06 – 7.9 ± 0.41 and 3.70 ± 0.07 - 8.07 ± 0.59 $\log_{10}$ CFU/ gm feces respectively (Fig.1).

Table 2: The initial and final weights of animals

	Animals body weight (gm) Mean ± SD		
Animals groups	Initial Wt*	Final Wt	Wt gain
Group A	26.6±1.42	33.4±1.97	6.8±0.43
Group B	25.0±0.96	33.6±2.05	8.6±0.59
Group C	27.0±1.52	32.8±1.62	5.8±0.37
Group D	26.0±1.07	27.2±1.84	1.2±0.04

N: 8 – 10 animals, (P<0.05). Wt*, body weight.

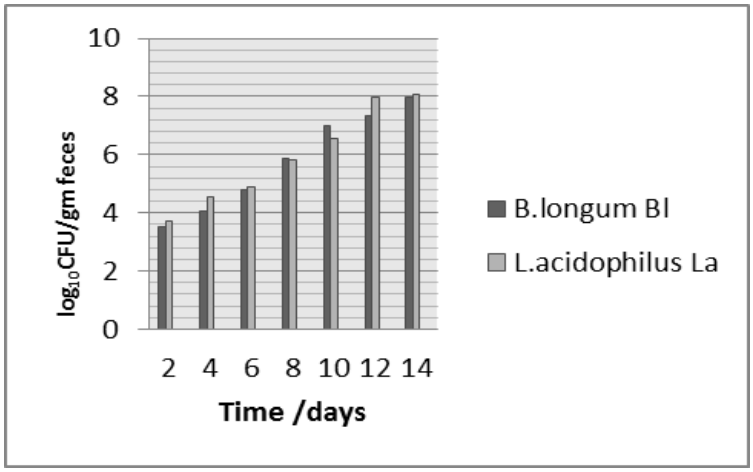

Figure 1: viability (CFU/gm feces) of *B. longum* Bl and *L. acidophilus* La in faces samples of mice in two evaluated groups B and C. (n = 9 -10 / group). Data are presented as mean ±SD. (P<0.05).

Attachment and adherence of live probiotic cells to the GIT mucosa of host after oral administration is generally considered initial events in the successful colonization of probiotics to the mucosal surface of GIT and lead to the direct interaction that can result in modulation of host response. Although adhesion profiles varied widely among members of lactobacilli and bifidobactria strains, but both probiotics have greater ability to adhere to the epithelial cells than pathogens. The proper attachment, adhesion and colonization of the used probiotics in mice GIT may be related to the diversity of cell surface architecture and the bacteria's ability to express certain surface components or secret specific compounds in response to the host environment, consequently favored good furnishing of mice gut, even temporarily, and in addition to the binding to the enterocytes via electrostatic interaction, and steric forces [23]. Transcriptional analysis revealed that *Bifedobacterium* members encode and express pilus – like structures on their cell surface in response to GIT stress, in most cases they possess more than one pilus – encoding locus, for instance *B. dentium* harbors as much as seven gene cluster for sortase - dependent pili. Similarly, expression of two of the three sortase – dependent pili clusters of *B. bifidum* PL2010 is enhanced in the murine GIT and implicated to microorganism persistence [24, 25]. The host gut adhesion factors of *Lactobacillus* members generally grouped into; sortase – dependent [LPXTG] anchor protein motif, S- layer proteins, and fibronectin binding proteins [26].Different moonlighting proteins were detected in the proteomes of different *Bifidobacterium* and *Lactobacillus*, appear to function as minor adhesion factors, but they exhibit specific interactions with carbohydrate moieties of gut lining mucin. Transaldolase, a key enzyme of bifidus shunt , could be identified as a mucin – binding protein and the specificity of this interaction was confirmed by increased mucus binding of recombinant *Bifidobacterium* strains expressing translocase [27,28]. A few moonlighting proteins have been shown to reside on the cell surface of *Lactobacillus*, binding to the cell wall through electrostatic forces. When the extracellular pH becomes weakly alkaline, the elongation factor Tu (EF-Tu) on the surface of *L. reuteri* PLoS specifically bound to mucin's sulfate group, and glyceraldehyde 3-phosphate dehydrogenase (GAPD) of *L. crispatus* are completely released from the cell surface [29]. Surface associated non - protein factors have also been shown to involve in adhesions. Such as LTAs, contributes to the anionic character of cell wall and provide hydrophobicity, which in turn influence

the adhesiveness of cell envelope [30]. The both used probiotics in the study were chosen on the basis of their In vitro antilisterial activities [14,15], and to investigate the impact of precolonization of mice GIT with these probiotics will establish a protective defense against proliferation, colonization, and clearance of Lm, a fecal viable counts of Lm on BHI agar plates were counted. Both probiotic were able to reduce the fecal number of Lm in mice challenged with pathogen. The results showed that pathogen Lm recovery from mice were significantly and gradually reduced in probiotic + Lm inoculated mice. Complete clearance level was not performed, whatever, the Bl pre - fed showed a significant reduction in the count of Lm at last experimental day with a viable count almost about 2.35± 0.08 $\log_{10}$ CFU/ gm of faces in group B animals. Like group B animals, group C animals (La pre - fed) also had shed a lower count of Lm in the animals feces of. In contrast mice infected with Lm alone (group D), the pathogen was aggressively proliferated, as the shedding number of bacteria in the feces samples was significantly ($P< 0.05$) increased, with a viable count increased by almost two log cycle and reached to 7.94 ± 0.44 $\log_{10}$ CFU/gm feces at day 14 (fig.2).

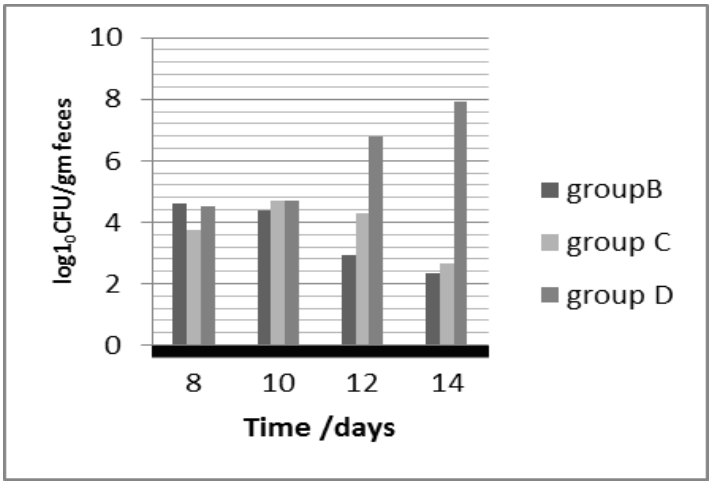

Figure 2: viability (CFU/gm) of *L. monocytogenes* in feces samples of mice in three evaluated groups B ,C, and D.(n = 5 – 10 / group). Data are presented as mean ±SD, ($P< 0.05$).

The obtained data suggesting that, the Bl feeding for two weeks was effective in reduction the proliferation, colonization and in altering the establishment / persistence of Lm infection in mice. The slight less low level of Lm recovery from mice that were inoculated with La indicating that this probiotic was less effective and the former probiotic offered a better protection in animal model. The concept of using probiotics to protect against oral infection with *L. monocytogenes* has been investigated using a few number of model systems. A variety of potentially probiotic strains including *E. coli* Nissle 1917 [31], *L. salivarius*UCC118 and *B. breve* UCC2003, have been shown to inhibit *L. monocytogenes* invasion of GI cell culture line [32]. Oral pre - feeding of live *L. delbrueckii*UFV-H2b20 has been shown to significantly protect against *L. monocytogenes* infection in a mouse model[33]. Similarly *L. sakei* 2a protect gonatobiotic mice against subsequent oral infection with *L. monocytogenes* [34].

Clinical studies claimed the nonimmunological protective mechanisms of probiotics considered to be multifactorial, The mechanism by which the potentially probiotics strains can exclude or reduce the adhesion and colonization of pathogens which are primary and crucial steps in pathogenesis, have been investigated extensively, the steric hindrance and the competitive exclusion of pathogens by probiotics suggested as a possible explanation for displacing pathogens from host cell surface [35]. Probiotics bind to the enterocytes via electrostatic interaction, steric forces or specific surface proteins to enterocytes carbohydrates receptors ,this offer them to bind in high quantities, thereby physically blocking the attachment sites, leaving no space for pathogens to adhere and subsequently cause infection [23]. Besides there are increasing reports indicating that anti – pathogen adhesive activity of *Lactobacillus* and *Bifidobacterium*, mediated by stimulation of mucin production (Mucs) by gut cells, and are able to inhibit the adherence of pathogenic bacteria to intestinal epithelial cells through their ability to increase the production of intestinal mucins. By incubating *L. plantarum* 299v with HT-29 cells, the increased expression levels of mucins MUC2 and MUC3 mRNA was shown to be accompanied by quantitative inhibition of the cell-attachment of EPEC, an effect that could be mimicked by adding purified exogenous MUC2 and MUC3 mucins [36].

3.3 Detection of some immunological parameters

The obtained ELISA results revealed enhancement of sIgA and serum IgA responses. The sIgA levels were significantly ($P<0.05$) increased in the animal probiotic pre – fed before pathogen challenged. Bl pre - fed (group B) was sufficient to enhance sIgA response in the intestinal fluid, sIgA titers was rose up noticeably, with about 3.7 fold higher than that was recorded for control (group A), while the systemic response was relatively less as the titer of serum IgA was elevated about 1.6 fold. Slightly less sIgA responses were noted

in the La pre-fed (group C), as the sIgA titer was elevated about 2.4 fold, while *L. monocytogenes* infection (group D) enhanced the production of sIgA by almost of 1.8 fold (Table.3).

Table 3: The titer of immunoglobulin A ,sIgA and serum IgA in experimental animals

IgA ng/ml	Experimental groups Group A	Group B	Group C	group D
sIgA	213±27.6	793±52.9	521±47.2	399±36.9
serum IgA	298±35.9	489±42.6	402±41.9	422±39.5

(n = 5-10 per group) . Data are presented as mean ±SD. (P<0.05).

The above result suggest that used probiotics can enhance local production of IgA in the mice intestine more than serum IgA. This observation is in line with previous studies showing a significantly higher level of intestinal antibacterial sIgA responses in mice after lactic acid bacteria oral delivery. Following a week pre - fed mix of *L.acidophilus* and *L. casei* the level of anti-*Salmonella enteritidis* sIgA increased about 61% in murine model [37]. In other *in vivo* study, mice fed *Bifidobacteria* for 12 days showed significantly higher levels of fecal IgA compared with that of the control group [38].

There is considerable evidence to support the notion that probiotics displace pathogens in the GIT through stimulation of specific and non-specific immunity. Probiotics are able to stimulate the production of antibodies in the intestinal lumen, specifically sIgA, that represents the first-line defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms through a process known as immune exclusion [39,40]. sIgA promotes the clearance of antigens and pathogenic microorganisms from the intestinal lumen by neutralizing pathogens toxins, blocking their access to epithelial receptors, entrapping them in mucus, and facilitating their removal by peristaltic and mucociliary activities [41]. Interestingly, enhancement of IgA production by orally administered probiotics cannot be generalized for genera, or even species. Indeed, the alteration of sIgA level in intestinal mucosa in response to probiotics is strain dependent. [42].

The phagocytic capacity data was expressed as the percentage of cells showing phagocytic activity PI%. Oral administration of both probiotics raised the phagocytic rate of peritoneal macrophages and peripheral blood leucocytes compared to those from control mice (Table.4). The peritoneal macrophages pronounced significant (P<0.05) greater phagocytic activity than blood leucocytes, However in mice pre - fed with Bl (group B) were exhibited higher macrophages phagocytic capacity but no significant higher (P>0.05) than that was recoded for group C animals. The PI value was elevated 65.7 ± 3.95 % in mice pre - fed with Bl, while PI increased 55.2 ±3.1% in mice pre - fed with La, In contrast relatively lower macrophages phagocytosis was observed in group D, as PI was 47.1±3.36%. The obtained results suggest that the prophylactic effects of used probiotics on *L. monocytogenes* infection may result from the enhancement of the innate immunity of the mice. Phagocytosis constitute the first line of defense against pathogens and are the key players of the innate immune system, they are responsible for early activation of the inflammatory response before antibody production. Phagocytes release toxic agents, eg, reactive oxygen intermediates and lytic enzymes, in various inflammatory reactions, eliminating pathogens by their phagocytic activity. Contact of macrophages with pathogens provokes the expression of proinflammatory cytokines and mediators that orchestrate pathogen killing and further coordinate the immune response [43]. The high phagocytic capacity has been previously reported by other researchers after ingestion of different strains of *Lactobacillus* and *Bifidobacterium* [44, 45]. Oral ingestion of *L. casei* and *L. bulgaricus* activates the production of macrophages [46] and administration of human derived *L. paracasei* L9 activated the phagocyticosis of peritoneal macrophages in mice [47]. Enhanced phagocytic activity was also documented in humans volunteers given *L. acidophilus* and *B. animalis* [48]. The enhanced host resistance to *L. monocytogenes* infection induced by *L. casei* may be mediated by macrophages migrating from the blood stream to the reticuloendothelial system in response to *L. casei* administration before or after infection with *L. monocytogenes* [49].

Table 4: Phagocytic capacity (PI%) of peritoneal macrophages and blood leucocytes in mice pre- fed probiotics and infected with *Listeria monocytogenes*

(PI%)	Experimental groups Group A	Group B	Group C	group D
peritoneal macrophages	40.3±3.67	65.7 ±3.95	55.2 ±3.17	47.1 ±3.36
Blood leucocytes	22.3 ±1.08	41.2 ±3.69	38.4 ±3.05	29.3 ±2.57

(n = 5-10 / group), Data are presented as mean ±SD. (P<0.05).

3. 4 Lactic acid concentration

In this study, even though the group C animals, which were pre - fed with La had a significant (P<0.05) increase in intestinal lactic acid concentration, 41.2 ± 2.67mM/L and pronounced declaim pH4. 5 ±0.2 (Table.5) but the group animals were displayed less inhibitory activity against Lm than those was recorded for BI, as was mentioned in Fig.2, and the concentration of lactic acid was not significantly affected when mice of group B inoculated with BI, but it was achieved higher antilisterial rate. On the basis of this data the antilisterial activities could not be attributed to the lactic acid production. Previous studies have indicated that non-lactic acid antibacterial molecules produced by selected lactic acid bacteria with probiotic potential are active against *L. monocytogenes* [50].

Table 5: Lactic acid concentration (mM/L) In Intestinal contents of mice.

Experimental groups	lactic acid concentration (mM/L)
Group A	25. 4 ±1.37
Group B	32.0 ± 2.09
Group C	41.2 ± 2.67
Group D	29.5 ± 2.14

(n = 5-10 / group). Data are presented as mean ±SD. (P<0.05).

4. CONCLUSION

The present study was shown that orally administered of human derived probiotics, *Bifidobacterium longum* Bl and *Lactobacillus acidophilus* La were able to protect mice against oral *L. monocytogenes* infection. The protection strategies attributed to immunomodulation of mice gut defenses. Furthermore, it is concluded that the enhancement of anti-Listeria activity might not be, lactic acid – dependent activity.

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