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Effectiveness of a *Bacillus megaterium*, as a probiotic in *Salmonella typhimurium* induced infection in rats

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ABSTRACT

Background: Bacterial infections are common despite prophylactic administration of antibiotics. The wide-spread use of antibiotics in patients has contributed to the emergence of multiresistant bacteria. A restricted use of antibiotics must be followed in most clinical situations. In patients there are several reasons for an altered microbial flora in the gut in combination with an altered barrier function leading to an enhanced inflammatory response. Several experimental and clinical studies have shown that probiotics may reduce the number of potentially pathogenic bacteria and restore a deranged barrier function. **Methods:** In this study Wistar albino rats were chosen to evaluate the effectiveness of Probiotic therapy by inducing them with infection using known bacterial pathogen *Salmonella typhimurium* and Treatment with common probiotics such as *Bacillus megaterium.*After the induction and treatment period the samples (Blood, Serum and tissues) were analyzed for haematological parameters, Biochemical markers and histopathological nature by comparing with non-induced and induced controls. **Results and Discussion:** The haematological, biochemical and the histopathological parameters studied were comparatively abnormal in induced animals and also they were very close to normal range in rats induced and treated with probiotics. The effectiveness was good for *Bacillus megaterium*. The reason behind that may be the ways and means how the organisms accommodate and accustom to the intestinal environment. **Conclusion:** The study has clearly indicated that probiotics could effectively be used for various ailments induced by pathogenic microorganisms.

KEY WORDS: *Bacillus megaterium,Salmonella typhimurium,* Probiotics, infection in rats

INTRODUCTION

The definition of "probiotics" evolved over the years and in 2001 a group of experts convened the currentlyFAO/WHO definition of probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Probiotics consist in bacteria or yeast, able to recolonize and restore microflora symbiosis in intestinal tract. Probiotic bacteria usually belong mainly to *Lactobacillus* and *Bifidobacterium* groups, in particular *Lactobacillus acidophilus* and *Bifidobacterium bifidus*, which include different strains (*L. rhamnosus, L. bulgaricus, L. salivarius, L. plantarum, L. casei, B. infantis, B. longum, Streptococcus thermophilus*). Bacillus species such as *Bacillus megaterium* are also gaining importance as a member in Probiotics.Some common probiotics, as *Saccharomyces boulardii* are yeasts[1]**.**Interest has increased in lactic acid bacteria because of its potential to improve cholesterol as well as its anti-carcinogenic, anti-pathogenic and anti-diabetic properties[2]. Daily consumption of these bacteria is probably the best way to maintain their effectiveness[3]. Recent research on the molecular biology and genomics of the probiotic bacteria *lactobacillus* has focused on its interaction with the immune system, anti-cancer potential, its effect on adipocyte cell size and body fat, and its potential as a bio-therapeutic agent[4].

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MATERIALS AND METHODS

In this study 3 groups Wistar albino rats were chosen to evaluate the effectiveness of **Probiotic therapy** by inducing them with infection using known bacterial pathogen *Salmonella typhimurium* and treatment with common probiotics such as ***Bacillus megaterium.***After the induction and treatment period the samples (Blood, Serum and tissues) were analyzed for **haematological parameters**, **Biochemical markers** and **histopathological nature** by comparing with non-induced and induced controls.

Animals:

Animals are divided into five groups, each group containing 6 (Albino rats) animals. Adult male albino rat of Wistar strain weighing around 250 to 300 grams were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of 25±2ºC and 55-65% relative humidity. A 12±1hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the institutional animal ethics committee.

Bacillus megaterium

B. megaterium is a gram positive, aerobic, spore forming bacterium. It is found predominantly in the small intestine.

Salmonella typhimurium

In Gram's staining, the morphological characteristics of the isolated salmonella exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope. Biochemical tests showed MR positive, VP negative, Indole negative and ferments carbohydrates producing acid and gas.

The cultures were obtained from Microlabs, Institute of Research and Technology, Arcot, Vellore Dt, Tamilnadu.

Experimental groups

Group 1- No induction and treatment- Routine food.

Group 2- Induction with 2 drops of *Salmonella typhimurium* given orally to the rat for 30 days.

Group 3- 2 drops of *Salmonella typhimurium* along with 2 drops of *Bacillus megaterium* for 30 days.

The animals were observed for physical activity, feeding, drinking and changes in weight. The rats were sacrificed at the end of the experimental period and the venous blood was collected into clean sample bottles. This was allowed to clot and then centrifuged at 3000 rpm and 5 minutes after which the serum was separated and stored frozen until needed for analysis. Finally blood samples were collected and subjected to haematological analysis. Serum samples were collected from those bloods for biochemical analysis. Organs such as large intestine, and liver were taken and subjected to histopathological analysis.

Statistical analysis

The statistical analysis was done using student's't' test. The results of biochemical analysis are presented as the mean value ± standard error (SE). Differences between the control and test groups were analyzed by the least significant difference at $P < 0.05$ confidence level using SPSS software.

RESULTS

General observations:

All animals were observed daily during the study period. The general condition of animals receiving bacterial inoculation was affected for 2 to7 Days later. Subjective observation indicated reduced activity, hunching, bristling fur, and reduced consumption of food and drink.

Histopathological findings:

Findings in the spleen are shown in Table 1. Sections from saline-treated control animals did not have histopathological changes. On day 7, mild acute congestion was evident in the red pulp. Micro-abscesses or granulomas were not observed, but a few germinal centers were detected in the white pulp.

On day 14, massive congestion was observed in the red pulp. Numerous granulomas were disseminated throughout the red pulp, whereas the white pulp remains unaffected. The granulomas contained central necrotic areas with pyknosis and were infiltrated with and surrounded by macrophages. On day 21, heavily congested red pulp with granulomas was seen. The number and size of the granulomas were reduced, compared with that of day 3. On day 28, the healing process was evident, with only mild congestion of the red pulp and no visible granulomas. Progressive disappearance of the cellular exudate and regeneration of normal tissue structure was observed. Only a few germinal centers were found in the white pulp. Spleen weight, Body weight, and their ratio were observed and tabulated in table 1.

Table 1. Histopathological study from rats used in Probiotic study

Parameters	N	Control*	Day 0	Day 7	Day 14	Day 21	Day 28
Body weight (g)	7	14	21	28	7	14	21
Spleen weight (mg)	237	260	280	312	237	260	280
Spleen/body weight ratio (mg/g)	707	778	802	832	707	778	802

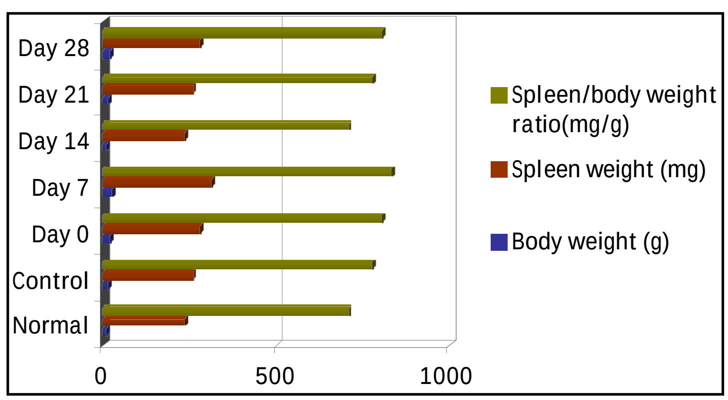

Fig 1. Histopathological study from rats used in Probiotic study

Table 2. Haematological Tests Results from rats used in Probiotic study

Haematological Tests	Control	*S.typhimurium*	*Bacillus megaterium*
Haemoglobingms %	12.0 %	11.7 %	
TRBC millions Cells/cu mm	6.0	6.2	3.1
RBC distribution width %(RDW)	12.7	13.5	35.9
TLC cells/cu mm	7000	13000	12700
Platelets lakhs cells/ cumm	2.9	5.0	1.46
Neutrophil	54 %	62 %	41%
Eosinophil	02%	02 %	02%
Basophil	00%	00 %	00%
Lymphocyte	43%	34 %	55%
Monocyte	01%	02 %	02%
PCV	33%	31 %	09%

BLOOD INDICES

MCV fl	54.2	51.3	54.2
MCH pg	19.9	19.3	21.8
MCHC %	36.9	37.7	40.3

Fig 2. ***Bacillus megaterium*** **growth in Nutrient agar**

Table 3. Biochemical markers analysis- ***Salmonella typhimurium*** **infected and treated with probiotic bacteria**

Microorganisms	Biochemical markers				
	Glucose (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	SGOT (Units/L)	SGPT (Units/L)
Bacillus megaterium	81.5	20.48	0.9	73.41	43.29
S.typhimurium	91.3	50.5	1.4	179.6	136.1
Control	116.0	32.0	1.3	52.3	34.9

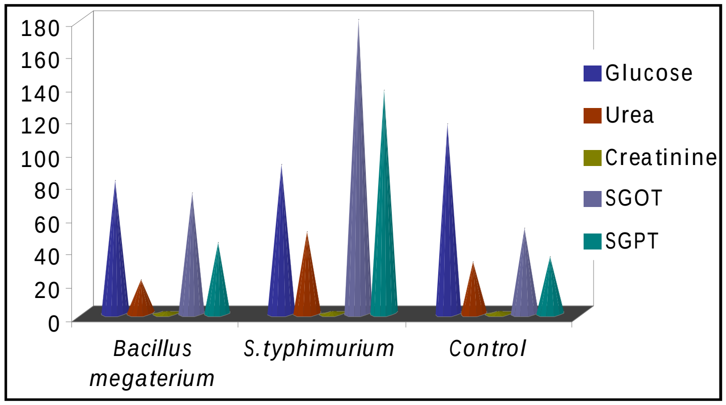

Fig 3. Biochemical markers analysis- ***Salmonella typhimurium*** **infected and treated with probiotic bacteria**

DISCUSSION

The haematological, biochemical and the histopathological parameters studied were comparatively abnormal in induced animals and also they were very close to normal range in rats induced and treated with probiotics.The effectiveness was good for ***Bacillus megaterium***. The reason behind that may be the ways and means how the organisms accommodate and accustom to the intestinal environment. The study has clearly indicated that they could effectively be used for various ailments. Anyway, when using it to human, separate clinical trials should be carefully carried out along with the side effects.

The viability of probiotic strains is considered crucial to ensure optimal functionality. This is explained by the fact that after ingestion these bacteria have to survive the inevitable three biological barriers such as salivary lysozyme, the acidic environment of the stomach and to the bile acids in the duodenum[5]. Therefore to ensure their survival during passage through the gastrointestinal tract, the probiotic strains are tested in terms of resistance to pH and bile acids. These tests were conducted on several strains and the results were different depending on the species[6]. In general resistance in the digestive environment is low as a result currently investigated novel approaches such as those based on mechanisms to stress adaptation of probiotic bacteria[7].

There are few studies in this direction that shows that probiotics have the ability to colonize the intestinal mucosa since they could be isolated by biopsy[8,9]. It is also important to assess the activity of probiotics in situ, in this context, the new techniques of molecular biology open new directions for evaluation[10]. The immune response in these situations is of major importance in combating gastroenteritis in humans. Similar beneficial effects have been detected in bladder and colon cancer[11, 12]. Lactic acid bacteria in general have quite positive history in this regard. Cases of infection have been reported with some strains that are currently abundant in human intestinal mucosa[13]. The gastrointestinal environment contains a wide range of contents ranging from harmless beneficial dietary and microbial flora to harmful pathogenic bacteria[14.] Ruminal bacteria can also produce such bacteriocins which by their presence are able to modify the ruminal ecosystem. Some studies even recommend using ruminal bacteriocins as an alternative to antibiotics in cattle[15].

In vitro studies have shown that strains of lactic acid bacteria are effective in removing or stopping the activity of pathogenic bacteria. Studies in vitro with human cell lines have helped to investigate how probiotics adhere to the intestinal epithelium[16.] Their use has its explanation in the fact that mimics the intestinal barrier that pathogenic microorganisms must pass in order to infect and then systemic circulation to reach various parts of the body[17.] Production of certain metabolites such as lactic acid lowers the pH with a decisive role in inhibiting the development of pathogenic bacteria. But there are also cases where pathogen inhibition (*Shigella*) is due not only to pH but also to some antibacterial substances secreted by lactic acid bacteria[18]. Secretion of hydrogen peroxide is also an important factor and was identified as having inhibitory effect on growth and development of *E. coli* 0157: H7[19.] Competitive exclusion of pathogens can be used efficiently to farm animals after treatment with antibiotics to prevent infection with *Salmonella* during especially because the host microflora is in recovery. This concept involves administration of non-pathogenic bacterial cultures (one or more strains) in order to reduce colonization or presence of pathogenic bacteria in the intestine[20, 21, 22].

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