

Original article:

Efficacy and safety for suspension of bacillus clausii while treating the patient of diarrhoea

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ABSTRACT

Introduction: Diarrhoea is the passage of loose or liquid stools, three or more times a day (or more frequent passage than is normal for the individual). Diarrhoea is usually a symptom of intestinal tract infection, which can be caused by a variety of bacterial, viral and parasitic organisms or it can be antibiotic associated. Bacillus clausii are probiotics when consumed, the entire dose of bacteria reaches the small intestine and inhibits the growth of pathogens causing diarrhoea. This Phase IV study was conducted all across India for evaluating the efficacy and safety of suspension containing 2 billion spores of Bacillus clausii per 5 ml.

Methodology: Out of total 259 patients, 215 completed the study. Efficacy assessment was made by analysing the reduction in stool consistency and frequency. Safety assessment was made by analysing the adverse events during clinical trial.

Results: At baseline mean stool frequency per day was 8.0565 which was reduced to 4.674 at day 3 and reduced to 2.382 at day 5. At visit 1 (baseline) the majority patients were having watery stools and at day 5 majority patients were soft or hard stools and no patient was having watery stools.

Conclusion: Suspension containing 2 billion spores of Bacillus clausii per 5 ml is safe and effective in the treatment of diarrhoea when it is given twice a day.

Keywords: Bacillus clausii, Diarrhoea, Stool Consistency, Stool Frequency, Probiotic

INTRODUCTION:

WHO defines diarrhoea as passage of loose or liquid stools, three or more times a day (or more frequent passage than is normal for the individual). Diarrhoea is usually a symptom of intestinal tract infection, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated drinking water or food, or from person to person. Diarrhoeal disease is the world's second leading cause of death in children under 5 years, and around 5.25 lac children every year dies because of it. Worldwide there are around 1.7 billion cases of childhood diarrhoea annually. Diarrhoea causes to leave the human body without salt and water which is essential

for survival and without that, it can be the dangerous situation for any human being. In most of the people, fluid loss and severe dehydration were the main causes of diarrhoeal deaths. Secondary causes such as septic bacterial infections are likely to account for an increasing proportion of all deaths associated with diarrhoea. Malnourished children or who have impaired immunity as well as people who are HIV positive are more at risk of life-threatening diarrhoea.^[1]

Antibiotic-associated diarrhoea (AAD) is the most common adverse event (AE) of antibiotics treatment. The incidence of AAD varies depending upon its route of antibiotic administration, definition of antibiotic(s) used, and the patient

population. The medical literature reports that 11 to 40% of children and 5 to 39% of adults suffer from diarrhoea when treated with antibiotics. The highest risk was found with the use of cephalosporin, clindamycin, broad-spectrum penicillin, amoxicillin and ampicillin. Antibiotics cause diarrhoea primarily by two mechanisms, first is by eliminating or diminishing bacterial species of the normal microflora, impairing vital microbial functions such as provision of nutrient short-chain fatty acids to colonisation and metabolism of bile acids; the second is by creating a place for the overgrowth of pathogens in the intestine including *Clostridium perfringens* type A, *Clostridium difficile*, *Klebsiella oxytoca*, *Candida albicans* and *Staphylococcus aureus*.^[2]

Normal gut microbiota plays an important role of protecting host against gastrointestinal (GI) tract diseases. During acute diarrhoea, the normal GI microbiota goes through radical changes that enable the overgrowth of unwanted microorganisms. Administration of probiotics (beneficial live microbes) restores the gut microbiota and control the severity of diarrhoea.^[3]

Probiotics are live microbes and when administered in adequate quantities confer a health benefit. *Lactobacillus* are spore forming bacteria, and spore forming bacteria are heat stable, can be stored at room temperature without any loss of viability and also resistant to low pH like acidic conditions of the stomach and hence can survive the transit to reach the intestine. *Bacillus clausii* spores germinate to live cells to adhere to the walls of the bowel and colonize the mucosa. *Bacillus clausii* has great stability at acidic conditions; the entire ingested dose of bacteria reaches the small intestine.^[3]

There are three distinct mechanisms of *Bacillus clausii* by which it inhibits the growth of pathogens. First mechanism is colonization of free

ecological places on surface of intestine, which won't be available for the growth of other microorganisms. This first mechanism is also useful to treat AAD. Second is *Bacillus clausii* competes with other microorganisms for epithelial cell adhesion, which is mainly relevant for intermediate germination phase or spores in the initial phase. Third is production of enzymes and/or antibiotics secreted into the intestine. These enzymes exhibit lytic action against *Pseudomonas aeruginosa*.^[3]

AIMS AND OBJECTIVES

Most of the studies on *Bacillus clausii* are conducted on western population with only one study being done on the Indian sub-continent. This study was conducted in order to substantiate the claims of *Bacillus clausii* in the treatment of diarrhoea in Indian paediatric patients with regards to its safety and efficacy.

MATERIALS AND METHODS

This was a Phase IV Clinical study conducted with 16 Paediatric speciality investigators all across the India from January 2017 to April 2017. Total 259 patients were recruited for the study out of which 215 patients completed trial and 44 patients were lost to follow-up.

Inclusion and Exclusion criteria

Patients of either sex having age more than 1 year and less than 12 years, suffering from acute diarrhoea were shortlisted for the study. Only those patients who were willing to sign informed consent form and ready to adhere to the protocol were selected for the PMS trial.

Patients having hypersensitivity to any of the excipient of study formulation or Patients who cannot adhere to the Protocol (Mentally Ill and Patients with Psychological problem) were excluded from the clinical trial.

Sample size

M. Ratna Sudha et al conducted an open label, Phase II clinical trial to evaluate the anti-diarrhoeal

efficacy of *Bacillus clausii* strain in 27 patients suffering from acute diarrhoea. [4] As this was a phase IV clinical trial so the number of patients were kept 215 at 16 centres all over the India. Initially the study was started with 259 patients but 44 patients were lost to follow-up.

Study Intervention

Each 5 ml of suspension (study medication) contains 2 billion spores of *Bacillus clausii*.

10 vials of study medication were provided to each patient by the sponsor. All the samples were dispensed by the investigator to the patient and advised to consume 2 vials per day in the interval of 12 hours for the study period of 5 days.

Study procedure

The only investigators holding post-graduate paediatric degree were involved as an investigator for conducting the study. The study duration was kept 5 days. Patients of acute diarrhoea who met with the decided exclusion and inclusion criteria were recruited for the clinical study by the investigator. A detailed medical history was obtained from each patient and physical examination was conducted by the investigators. Patients were dispensed with 10 vials of study probiotic suspension; each 5 ml of suspension contains 2 billion spores of *Bacillus clausii* by investigators and asked to consume 2 vials a day in the interval of 12 hrs. All eligible patients would be informed about the nature of the study and consent would be taken. A detailed medical history will be obtained from all enrolled patients, which will be followed by thorough clinical examination. Patient or Patient's guardians will be instructed to keep a diary of daily symptoms.

Three visits were planned for all the patients recruited in this study-the first visit was baseline visit (V1) on day 1 before treating patient with the study probiotic suspension, the second visit was reevaluation visit (V2) on day 3 and third visit was

conclusion visit (V3) on day 5. Adverse events occurring and total symptom score were noted during each visit along with medical history and physical examination. Investigators were asked to discontinue the study drug in case of severe adverse event and with discretion, clinical experience in case of mild or moderate adverse events. In case of any safety-related issues and adverse events or serious adverse events, the investigator by choice can withdraw the patient from the trial and treat according to the severity of the symptoms

Concomitant therapy

No Pharmacological intervention and any medication including any antibiotic or any antidiarrheal drugs were allowed during study duration of 5 days. Diarrhoea causes to leave the human body without salt and water which is essential for survival of human body so as to rehydrate the body taking of ORS powder along with sufficient quantity of water and drinking of water at regular interval was encouraged. If the patient is severely dehydrated then fluids can be given to him by IV route of administration. And patient was suggested to have food having high nutrition value.

Efficacy assessment

Before treating the patient with the study medication stool frequency per day and stool consistency (watery, semisolid, soft or hard) was recorded in the CRF and then the patient was asked to take the study probiotic suspension and the same parameters were recorded at visit 2 and visit 3.

Safety assessment

Throughout the clinical study patients were asked by the investigators for any adverse events and if present noted in the case record form (CRF) during each post-dose visit. Noted adverse events were classified into 2 categories as serious or non-serious adverse events. Adverse event were classified as drug related or nondrug related

adverse events by using Naranjo’s scale of probability. Adverse events observed were followed up and treated if necessary by the investigators till their resolution.

Regulatory matters

All the patients participated in the study have read and signed the ICF.

RESULTS

A total 259 patients were recruited at 16 centres across India, 215 patients completed the clinical trial. Demographic characteristics are mentioned in the table 1.

Table 1: Demographic characteristics of the patients recruited for the study

Mean age of patients (years)	3.5
Males	78
Females	137

Efficacy analysis

The primary assessment was done by analysing the Stool consistency (Hard/ Soft/ Semisolid/ Watery) and reduction in stool frequency. Then the data was analysed, patients having the stool frequency 1 to 3, 4 to 6, 7 to 10 and 11 and above were grouped and named as patients of normal, mild, moderate and severe frequency of stools respectively. And reduction in stool frequency at visit 2 and 3 was analysed as compared to visit 1. Similarly number of patients were analysed having stool consistency of hard, Soft, Semisolid or Watery at visit 1, 2 and 3.

In visit 1, 43 (20 %) patients were having severe stool frequency, 73 (33.95 %) of moderate, 59 (27.44 %) of mild and 40 (18.6 %) of normal stool frequency. In visit 2, 8 (3.72 %) patients were of severe, 33 (16.27 %) of moderate, 82 (38.13 %) of mild and 90 (41.86 %) patients of normal stool frequency. In visit 3, there was no patient of severe, 3 (7.39 %) of moderate intensity, 38 (17.67 %) of mild intensity and 174 (80.93 %) of normal stool frequency. The improvement occurred in the condition of the patient in regards to frequency of the stools is shown in the fig. no. 1.

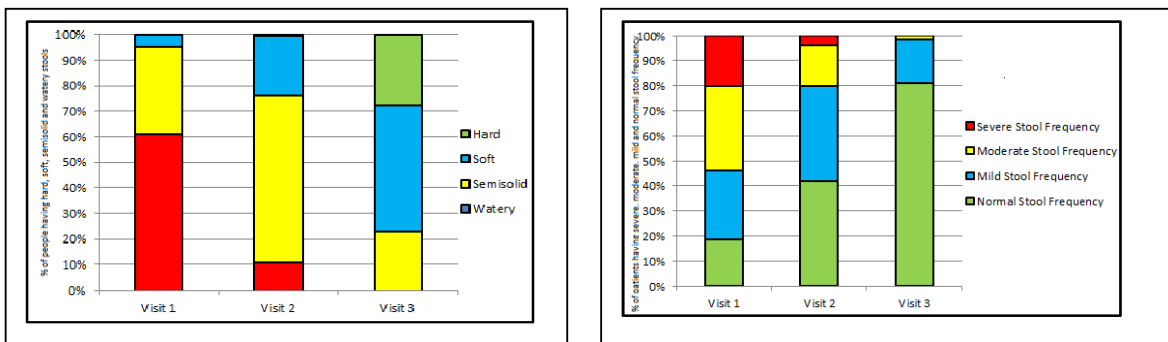


Fig. 1: The improvement occurred in the condition of the patient in regards to frequency of the stools at visit 2 and visit 3 as compared to visit 1.

In visit 1, 131 (60.93 %) of patients were having watery stools, 73 (33.95 %) were having semisolid and 11 (5.11 %) having soft stools and no patient was having hard stools. In visit 2, 23 (10.69 %) patients were having watery stools, 140 (65.11 %) were having semisolid and 51 (23.72 %) having soft stools and 1 (0.46 %) patient was having hard stools. In visit 3, no patient were having watery stools, 49 (22.79 %) were having semisolid and 107 (49.76 %) having soft stools and 59 (27.44 %) patients were having hard stools. The improvement occurred in the condition of the patient in regards to consistency of the stool is shown in the fig. no. 2

Fig. 2 Improvement occurred in the condition of the patient in regards to consistency of the stool at visit 2 and visit 3 as compared to visit 1.

At baseline mean stool frequency per day was 8.0565 which was reduced to 4.674 at day 3 and reduced to 2.382 at day 5.

Safety analysis

The overall incidences of reported study drug related adverse effects were 29 seen in 24 patients. The list of adverse events with the number of episodes is mentioned in Table 2

Table 2: Adverse events, no. of episodes, no. of patients and percentage of patients experienced from total population

Adverse event	No. of event	No of patient	% of patients
Nausea	14	5	2.32
Stomach upset	5	4	1.86
Total	19	8	3.72

DISCUSSION

In visit 1, 43 (20 %) patients had severe stool frequency, 73 (33.95 %) of moderate, 59 (27.44 %) of mild and 40 (18.6 %) of normal stool frequency. In visit 2, 8 (3.72 %) patients were of severe, 33 (16.27 %) of moderate, 82 (38.13 %) of mild and 90 (41.86 %) patients of normal stool frequency. So in visit 2, there was reduction of 16.28 % patients having severe frequency of stool, 17.38 % patients were reduced having moderate frequency of stools and increase of 10.69 % patients having mild intensity and also there was the increase of 23.26 % patients having the normal frequency of stools. In visit 3, there was no patient of severe frequency, 3 (7.39 %) of moderate frequency, 38 (17.67 %) of mild intensity and 174 (80.93 %) patients had normal stool frequency. In visit 3 no patient was of severe frequency of stools. In visit 3 there was reduction of 8.88 % and 20.46 % of patients of

moderate and mild intensity. So at the end there were 80.93 % patients who were having normal frequency of stools. Stool frequency at visit 1 was 8.87 which was reduced to 5.63 at visit 2 and at visit 3 it was reduced to 2.806, which means at visit 3, average frequency of stools is less than 3 which is not covered under diarrhoea condition

In visit 1, 131 (60.93 %) of patients had watery stools, 73 (33.95 %) had semisolid and 11 (5.11 %) had soft stools and no patient had hard stools. In visit 2, 23 (10.69 %) patients had watery stools, 140 (65.11 %) had semisolid and 51 (23.72 %) had soft stools and 1 (0.46 %) patient had hard stools. In visit 2 there was 50.24 % and 31.16 % reduction in patients having watery and semisolid stools whereas 18.61 % and 0.46 % increase in the patients having soft and hard stools. In visit 3, no patient were having watery stools, 49 (22.79 %) had semisolid and 107 (49.76 %) had soft stools

and 59 (27.44 %) patients had hard stools. In visit 3, there was no patient having watery stools and there was reduction of 42.32 % in patients having semisolid stool. There was increase of 26.24 % and 26.98 % in patients having soft and hard stools.

So at visit 2 and visit 3, 52 and 165 patients were completely cured from diarrhoea.

As per WHO, the patients who are having frequency of stools 3 or less times a day and having non-watery stools are cured from diarrhoea. [1] So as per that in visit 2 and visit 3, 52 (24.186 %) and 165 (76.744 %) patients have been cured respectively.

M. Ratna Sudha et al conducted an open label, Phase II clinical trial to evaluate the anti-diarrhoeal efficacy of *Bacillus clausii* strain in 27 patients suffering from acute diarrhoea of average age 35.44 ± 8.08 years. All patients were allotted to take a capsule of *Bacillus clausii* containing 2×10^9 cfu, two times a day for a study period of 10 days. Efficacy assessment included frequency of defecation, duration of diarrhoea, stool consistency and abdominal pain on day 1, 3, 6 and 10. Safety was evaluated by assessing the type and incidence of adverse effects such as increase in pulse rate and blood pressure, clinical laboratory tests (serum glutamic pyruvic transaminase, complete blood count, serum creatinine, and stool examination as well as microscopy, on day 1 (baseline) and at the end of the study at day 10) and physical examination. The results of this study showed that the mean duration of diarrhoea of the patients decreased to 9.26 ± 3.05 from 34.81 ± 4.69 ($P < 0.0001$) min per day, the frequency of defecation decreased to 1.78 ± 0.50 from 6.96 ± 1.05 ($P < 0.0001$) times per day, abdominal pain decreased to 0.74 ± 0.71 (absent) from 3.22 ± 0.93 (severe) ($P < 0.0001$), and stool consistency improved to 1.22 ± 0.42 (soft) from 3.93 ± 0.38 (watery) ($P < 0.0001$). There was no significant

change in safety parameters during treatment. All the results showed that the *B. clausii* potentially be effective in relieving the symptoms of diarrhoea without causing any adverse effects to the patient. [4]

E. C. NISTA et al conducted a clinical study to evaluate the effect of *Bacillus clausii*, a probiotic, on incidence and severity of antibiotic associated side effects during treatment of *H. pylori*. 120 patients infected with *H. pylori* were randomly screened and one group treated with a standard 7 days triple therapy with *B. clausii* three times a day (each preparation containing 2×10^9 spores), Amoxicillin 1 g two times a day, Rabeprazole 20 mg twice a day and clarithromycin 500 mg twice a day for 14 days from the baseline. And second group was treated with Rabeprazole 20 mg twice a day, Amoxicillin 1 g two times a day and clarithromycin 500 mg twice a day and placebo of *B. clausii* for 14 days from the baseline. Side-effects were evaluated using a list of questions and were recorded for 4 weeks from the start of therapy. The incidences of diarrhoea, epigastric pain and nausea in patients treated with *B. clausii* were significantly lower compared to placebo group. Intensity of diarrhoea and nausea in patients treated with *B. clausii* was significantly lower compared to placebo group. In patients of *H. pylori* concomitant therapy of *B. clausii* reduces the most common adverse effects of antibiotic therapy. [5]

Keya Lahiri et al conducted a clinical study on 131 children suffering from acute diarrhoea in Navi Mumbai, India. All the children were divided into 2 groups, children in one were treated with *Bacillus clausii* (Probiotic), Zinc and oral rehydration therapy (ORT) and 2nd group of children was treated with Zinc and ORT. Children below 2 years, 2 to 6 years and 6 to 12 years were 73 (55.7%), 35 (26.7%) and 23 (17.5%) respectively. In Group 1, duration of the study was 22.64 hours and in Group 2 it was 47.05 hours ($p < 0.01$). In Group 1 and

Group 2 the frequency of diarrhoea showed improvement within 24 and 60 hours respectively ($p < 0.01$). Average period of hospitalization was 2.78 days for Group 1 whereas 4.30 days for Group 2. [6] As per WHO, the diarrhoea can be treated by combination of oral rehydration salts (ORS) for treating dehydration, Zinc supplements to reduce duration of a diarrhoea episodes and reduce stool volume, in severe cases IV fluids should be given to patient and child should have nutrient-rich foods.

So in this study we followed the treatment guidelines but Zinc suspension was not allowed to be taken by the patient instead of that suspension of bacillus clausii was given to test its efficacy when patient is treated on monotherapy of bacillus clausii. [1]

CONCLUSION

Suspension of Bacillus clausii provides optimum symptomatic relief and is safe for use in the symptomatic management of acute diarrhoea.

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DISCLOSURE

This study was conducted as a part of Pharmacovigilance activity for Enterolife Aqua marketed by Centaur Pharmaceuticals Pvt Ltd.

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