Efficacy and Safety of *Bacillus coagulans* LBSC in Drug Induced Constipation Associated With Functional Gastrointestinal Disorder: A Double-Blind, Randomized, Interventional, Parallel, Controlled Trial a Clinical Study on *Bacillus coagulans* LBSC for Drug Induced Constipation Associated With FGIDs

Ankit Rathi, BE, MBS[1] [ID] **and Ravikiran Pagare, MSc, PhD**[1]

Abstract

Background: Active drugs and nutraceutical supplements commonly induce various gastrointestinal illnesses, and constipation is a major gastrointestinal symptom accompanied with functional gastrointestinal disorders. Drug-induced imbalance in gut microbiota may play critical role in such physiological disturbances. Probiotics have been known for resuming normal and healthy gut microbiome.
Objective: To investigate the clinical efficacy and safety of *Bacillus coagulans* LBSC in the treatment of drug induced constipation associated with functional gastrointestinal disorder (FGID) symptoms.
Methods: A prospective, interventional, randomized, double-blind, parallel, multi-arm, controlled trial with 168 patients experiencing drug induced constipation associated with FGID symptoms (DICAWFGID) screened through Rome IV criteria were randomized into 2 arms, i.e. placebo arm (n = 28) and atorvastatin, atenolol, metformin, amitriptyline, and calcium in test arm (n = 28/arm). Patients in both arms received similar dosages (1 g sachet, 3 times a day) for 35 days. The occurrence of constipation using Bristol Stool Form Scale, assessment of degree of constipation on 4-point Likert scale, occurrence of hard stool and degree of stool expulsion on 3-point scale, and defecation frequency were primary endpoints. While, secondary outcomes consisted of the changes in severity of FGID symptoms, visual analogue scale and tolerance to IP, along with reports of adverse events (AEs) and severe adverse events (SAEs).
Results: There was a significant reduction in occurrence of constipation (≥98.6% and *P*-value <0.05) in test arm over the placebo arm. Assessment of co-primary endpoints showed significant improvements in degree of stool consistency (*P*-value 0.0232; CI: 0.1870, 1.1629), borderline significantly superior in degree of stool expulsion (*P*-value 0.0553; CI: 0.0378, −0.4939), while the other co-primary efficacy endpoints displayed considerably improved advancement (non-significant, *P*-value ≥0.05). The intra group analysis of symptoms at start of treatment (SOT) and end of treatment (EOT) revealed a significant reduction in scores for occurrence of constipation and degree of constipation, whereas significant improvement in the scores for degree of

[1]Department of Biological Sciences, School of Science, Sandip University, Nashik, Maharashtra, India

Corresponding Author:
Ankit Rathi, BE, MBS, Department of Biological Sciences, School of Science, Sandip University, Trimbak road, Nashik, Maharashtra 422213, India.
Email: rathiak2@gmail.com

stool consistency and degree of stool expulsion (*P*-value <0.001) after the intervention period. In secondary endpoints, the processed responses clearly signified a considerable positive improvement (non-significant, *P*-value ≥0.05) in other symptoms of constipation associated with FGIDs as determined by the changes in the EOT-SOT score. The study data also highlighted the safety of *Bacillus coagulans* LBSC at the studied dose. No AEs and/or SAEs were documented during the investigation.
Conclusion: At the studied dose, *Bacillus coagulans* LBSC was safe for oral consumption and effective in the management of the drug induced constipation associated with FGIDs symptoms.

Introduction

In present times, majority of the population across the globe is facing one or more chronic disease/s, and certain medications are prescribed for their treatment/management, and to meet the health care needs for maintaining proper functioning of body. For example, statins (rosuvastatin, atorvastatin, simvastatin) are recommended for preliminary prevention of atherosclerotic cardiovascular disease,[1] β-blockers (acebutolol, atenolol, bisoprolol, carvedilol, metoprolol) are prescribed for lowering the blood pressure and heart rate,[2] antidiabetic drugs (metformin) for treatment of type 2 diabetes mellitus,[3] tricyclic antidepressant (amitriptyline, imipramine, desipramine, doxepin) for fibromyalgia, chronic pain, depression, and some type of anxiety,[4] mineral supplements (calcium, phosphorus, iron, sodium, sulfur) for precise growth and functioning of body, amongst many other. These medications and their active formulations when administered in a defined dose provide beneficial therapeutic effects against different physiological conditions. But, along with the desired pharmacological effect, several side effects are also induced in the body after a long term administration.[5] Amongst different side effects, the gastrointestinal ailments like constipation are the most recurrent ones, and the probable reasons could be: (i) Water secretion and pharmacological impact on intestinal motility,[1] and slight antimicrobial activities possessed by statins[6]; (ii) Blockade of β-receptors present all over the body with β-blockers[2]; (iii) Changes in the bile acid pool inside the intestine, and alterations in gut microbiome by metformin[3]; (iv) Inhibition of transient receptor potential channel canonical type 4 responsible for Ca^{+2} permeability and GI excitability by tricyclic antidepressant[4]; (iv) Change in neuromuscular excitability by high calcium levels[7]; (v) Alteration in gastrointestinal physiology,[5] and many others.

Constipation is a symptoms based clinically common gastrointestinal disorder, prevalent in 15%–20% adult population, and having a negative impact on the physical and psychological functions of body.[8,9] The symptoms include hard or lumpy stools, straining during defecation, anorectal obstruction or sensation of incomplete evacuation, manual manoeuvres to facilitate defecation, and/or painful passage of stool, and less than 3 defecations per week due to infrequent, persistent, or incomplete bowel movements.[9,10] These characteristic symptoms of constipation are also associated with functional gastrointestinal disorders (FGIDs), and have a profound impact on the quality of life of patient and also influence the health care system across the globe.[11] FGIDs are prominently designated as ailments of gut-brain interactions, and are very common in clinical practice and community due to nature of associated patho-physiology and the fear accompanied with the term functional.[12]

The current strategies prescribed for treatment/management of constipation are modifications of lifestyle and dietary habits, interventional manipulation of dietary and gut microbiome including transplantation of faecal microbiome, use of antispasmodics, prokinetics, stimulant or osmotic laxatives, bulking agents, and neuromodulators acting on central and peripheral nervous system.[13,14] But the treatment pattern specifically targeting the symptoms based on patho-physiological mechanisms are very less, and more than 50% patients are not satisfied in terms of improvements of associated symptoms.[14] Further, the therapies currently practised for treating constipation and motility disorders are not only deficient in long-term efficacy, but also have safety issues.[15] Nowadays, an alternate approach using probiotics has gained tremendous importance in treatment and management of several gastrointestinal disorders which include constipation, IBS, IBD, indigestion, diarrhoea, nutrient malabsorption, ulcerative colitis, pouchitis, small intestine bacterial overgrowth, and Crohn's disease.[16-19]

Probiotics are beneficial live microorganisms, which on consumption in adequate quantities help build healthy microflora in gut, and confer prominent health benefits to host.[20,21] The health benefits include anti-pathogenic, anti-diabetic, anti-obesity, anti-inflammatory, anti-allergic, and anti-cancer activities, along with positive impacts on urogenital health, brain and central nervous system.[18] Amongst the numerous probiotics, *Bacillus* Spp. are in tremendous focus, and *Bacillus coagulans* has primarily

attracted the attention of scientific community due to their stability against osmolarity, temperature, and desiccation. Further, the spore forming ability makes them resistant to gastric acid, ions, pancreatin, pepsin, bile, digestive enzymes, and mucin of gastro-intestinal phase.[22] *B coagulans* are also known for their abilities to modulate gut microbiome, perform immune-modulations, produce antimicrobials and perform intestinal digestion by secretion of different digestive enzymes such as coagulin and lactosporin.[23] The therapeutic potential of *Bacillus coagulans* to exhibit the anticipated health benefits differs with type and nature of strain, amount used per dosage in the formulation, and extent of patho-physiological condition of patient.[24] Earlier clinical studies have already established the therapeutic potential of *Bacillus coagulans* against ailments of functional constipation. Majeed et al[11] and Majeed et al[25] and Kalman et al[26] have not only reported a positive impact of *Bacillus coagulans* in reducing bloating, functional gases and flatulence issues in adults, and modulation of gut microbiome, but also confirmed its safety after consumption. Further, the efficacy and safety of *Bacillus coagulans* in alleviating the patho-physiological symptoms of IBS and treatment of acute diarrhoea with abdominal discomfort were intervened in a double blind clinical studies.[17,27] In addition, Madempudi et al[22] also reported a significant reduction in symptoms of functional constipation after treatment with *B coagulans* for 4 weeks over the placebo.

Although there are numerous studies supporting the clinical efficacy and safety of *B coagulans* against constipation and associated symptoms. But, the data regarding clinical efficacy of *Bacillus coagulans* LBSC on drug induced constipation associated with FGID symptoms (ICAWFGID) after prolonged consumptions of regularly prescribed drugs is very limited or negligible. And this lack of clinical data associated with constipations induced by consumption of drugs (atorvastatin, atenolol, metformin, amitriptyline, calcium) prompted us to undertake the current investigation, which was a prospective, interventional, randomized, double-blind, parallel, multi-arm, controlled clinical study using *Bacillus coagulans* LBSC. Along with the safety and tolerance of *Bacillus coagulans* LBSC, the degree of constipation, stool consistency, stool expulsion, occurrence of hard stool and defecation frequency in the control group and the interventional group were also evaluated as outcome variables.

Materials and methods

Formulation

The investigational product (IP) contained active probiotic, *Bacillus coagulans* LBSC mixed with excipient. The strength of the active probiotic formulation was 2 billion spores per gram per sachet, which was supplied by Advanced Enzyme Technologies Ltd, Thane, India. The placebo contained only excipient, maltodextrin, 1 gram per sachet. The packaging, labelling and physical appearance of both the products were same except the coded batch numbers used for differentiation.

The Institutional Ethical Committee [S2J INDEPENDENT ETHICS COMMITTEE (S2J IEC)] reviewed and provided the approval before the commencement of the trial (Registration No: ECR/284/Indt/AP/2017). The registration of the trial was done in the Clinical Trial Registry, India (CTRI/2021/08/035889), before the enrolments of patients for the said clinical study. An approved protocol was designed as per the pertinent requirements of ICH - GCP E6 (R2), Declaration of Helsinki (2013),[28] New Drugs and Clinical Trial Rules (2019)[29] and ICMR Guidelines for Biomedical and Health Research involving Human Participants (2017),[30] and FSSAI guidelines and strictly followed for amendments (without any changes) during the clinical study. All the participants were made aware about the study, and necessary information was provided orally and in written format in the language understandable and familiar to them. After understanding the explained information including the associated objectives, possible health benefits and risks, every participant submitted a written informed consent to the investigator.

Clinical Study Design and Selection of Study Subjects

This prospective, interventional trial was a randomized, double-blind, parallel, multi-arm, placebo controlled clinical study which had total 6 visits to clinical site by all the registered and selected participants, and a seventh visit as telephonic consultation follow-up to assure the safety of individual participant. The selection of subjects was merely on the basis of pre-defined inclusion and exclusion criteria.

Inclusion Criteria
(i) Male and female participants (age: 18-65 years) diagnosed with chronic functional constipation induced by selected classes of drugs followed by Rome IV criteria [following the International Classification of Diseases, 10th Ed. (ICD-10-CM); Medical Diagnosis Code K59.03] and under treatment with constipation causing drugs for at least 3 months prior to screening; (ii) Willing and able to provide written informed consent prior to any study-related activities being performed.

Exclusion Criteria. The participants with following symptoms were excluded from the said clinical study: (i) Known hypersensitivity to contents of drug product or related class of drugs or to any of the excipients of the formulation; (ii) Addicted alcoholics and/or drug abusers; (iii) History or presence of coronary, renal, pulmonary and thyroid disease, and/or active or a history of inflammatory bowel disease; (iv) Any abdominal surgery, except for hernia repair or appendectomy; (v) Active treatment with prescribed medication for any of the FGID symptoms within 4 weeks prior to screening; (vi) Diagnosed with infectious gastroenteritis within 6 weeks prior to screening; (vii) Antibiotics within 14 days prior to

screening, used antipsychotic medications within 3 months prior to screening, and/or used systemic steroids within 1 month prior to screening; (viii) Suffered from a major psychiatric disorder within the past 2 years from screening; (ix) In addition, participants having adverse or severe adverse ailments, in pregnancy or lactation phase, known HBsAg positive, Anti HCV and HIV positive; (x) Participation in any other clinical study within 30 days before the first dose of Investigational Product.

Samples, Randomization and Treatment Procedures

A total of 168 participants were enrolled for the said clinical study on the basis of pre-defined inclusion criteria. The participants were allocated as 28 randomized subjects in each group, namely placebo arm, and atorvastatin, atenolol, metformin, amitriptyline, and calcium in test arm. The test arm contained a total of 140 subjects (28 per drug group), and the subjects were asked to orally consume the IP containing *Bacillus coagulans* LBSC (2×10^9 spores) powder (1 g sachet) with water, 3 times a day. Whereas, the subjects in the placebo arm were only on maltodextrin and the dosing schedule was same. The total treatment period with IP was up to 35 days and total duration of the study was nearly 74 days. The random distribution of subjects (followed by block randomization), allocation of treatment and procedures to be followed are presented schematically in Figure 1. The subjects, investigators, physicians and officers in the study remained blind until the accomplishment of the clinical trial, while un-blinding was done strictly after the completion of post-clinical phase of the trial. All the supportive treatments, if required, were recommended and administered to the subjects if thought essential by the investigator/physician, but use of antibiotics in combination with IP was not recommended. The designed protocol was austerely followed, and no further amendments or changes were incorporated after commencement of the trial. Further, during the entire study period, no interim analysis of data was performed.

Endpoint Analysis: Efficacy and Safety Variables

The evaluation of primary and secondary endpoints measured different efficacy and safety indicators of *Bacillus coagulans* LBSC for treating the patients with constipation associated with FGID symptoms. The primary endpoints were set to determine the efficacy outcomes measuring the changes occurred in constipation associated with FGID symptoms such as occurrence of constipation using Bristol Stool Form Scale, and assessment of degree of constipation on 4-point likert, occurrence of hard stool, scale and degree of stool expulsion on 3-point scale, and defecation frequency. The changes in severity of FGID symptoms, visual analogue scale and tolerance to IP at SOT to EOT were also evaluated. The severity of FGID symptoms was also evaluated by physicians investigation for feeling of a lump, fullness or something stuck in throat, pain or discomfort in the middle of chest, heartburn, epigastric pain, bloating, early satiety, nausea, vomiting, diarrhoea, urgency, food or drinks get stuck after swallowing or go down slowly through chest, regurgitation, epigastric burning, belching, postprandial fullness, abdominal pain, abdominal discomfort, abdominal distension, feeling of incomplete evacuation, and straining during defecation. The endpoint results of test arm were compared with respective findings of placebo arm. The evaluation of clinical safety of *Bacillus coagulans* LBSC was assessed by examination of general physical health, adverse events reports, and vital biomarkers, i.e. the secondary endpoint. The haematological and biochemical parameters were also determined to confer the clinical safety, and performed using standard medical protocols. The haematological parameters/indicators quantified were complete blood count (hematocrit, hemoglobin, RBC, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, WBC and its differential counts, platelet count), while the biochemical parameters/ indicators measured were aspartate transferase, alanine transaminase, serum creatinine, blood urea nitrogen, random blood sugar, total cholesterol, total albumin and globulin, and C-Reactive Protein. The adverse events is defined as any medically untoward event detected in clinical study subject after use of the study agents, whether or not caused by the use of the agents. On the other hand, severe adverse events are any untoward medical incidence which is life-threatening and results into death or hospitalization, disability or incapacity, and congenital anomaly.

Sample Size and Power, and Statistical Analysis

A total of 168 patients with drug-induced constipation associated with FGID symptoms (ICAWFGID) were enrolled and randomized into 2 arms, i.e. placebo or treatment arms. Test arm had randomized 140 patients and placebo arm had 28 patients so that at the end of study, at least 85.715% evaluable patients are available for the assessments of safety and efficacy endpoints in both the arms.

Following available trial outcomes and empirical assumptions, 168 patients are required to have 80% chance of detecting, as significant at the 5% level, an increase in the primary outcome measure from 91.4% in the control group to 100% in the experimental group.

Calculation based on the formula:

$$n = \frac{f(\alpha, \beta) \times [p1 \times (100 - p1) + p2 \times (100 - p2)]}{(p2 - p1)^2}$$

Where, p1 and p2 are the percent 'success' in the placebo and test group, respectively, and $f(\alpha, \beta) = [\Phi^{-1}(\alpha) + \Phi^{-1}(\beta)]^2$

Φ^{-1} is the cumulative distribution function of a standardized normal deviate. The mean outcome is compared between the experimental and standard treatment groups.
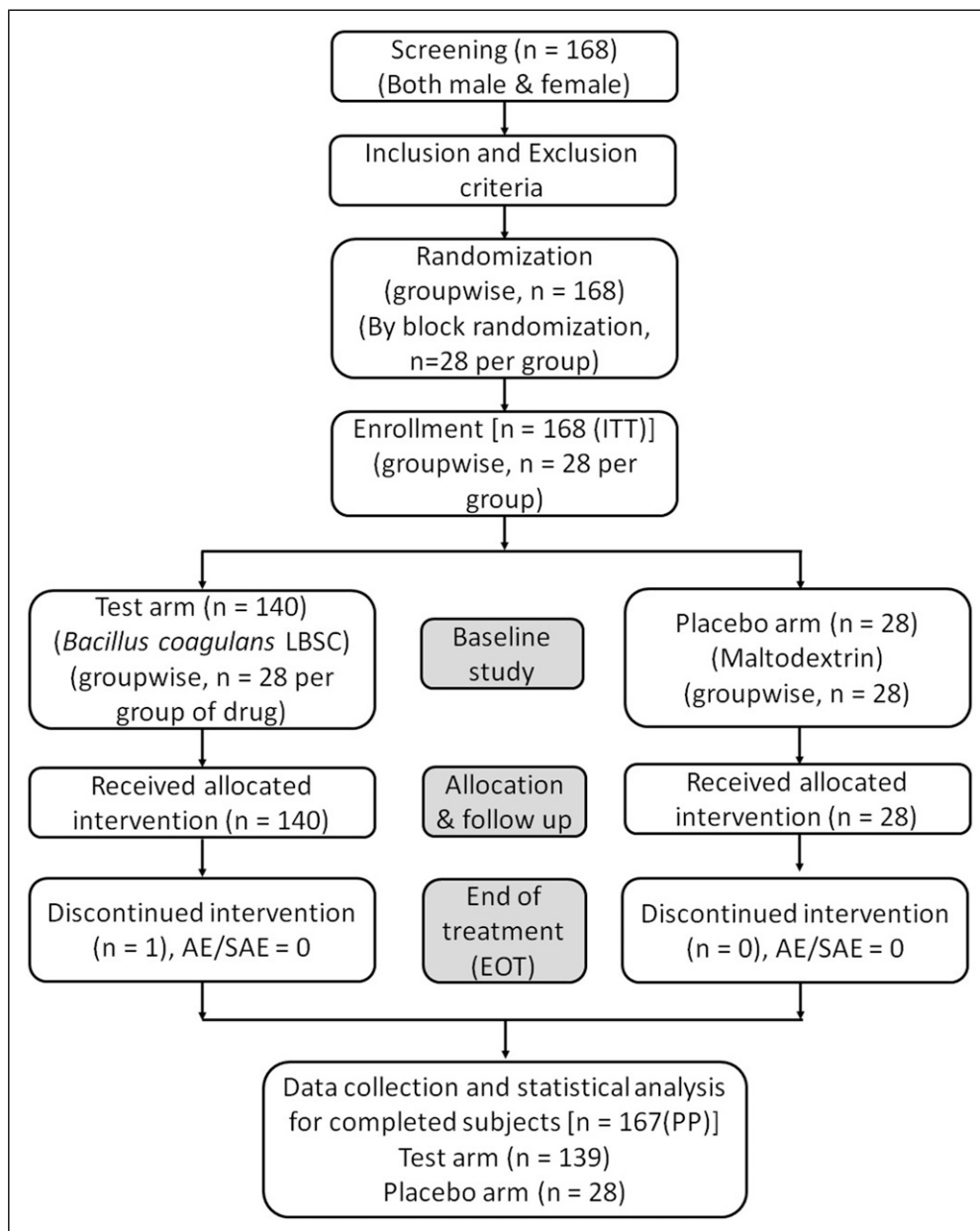
Figure 1. Schematic representation of current clinical study on investigating the clinical efficacy and safety of *Bacillus coagulans* LBSC in the treatment of drug induced constipation associated with functional gastrointestinal disorder (FGID) symptoms.

All the data was analysed using GraphPad Prism (version 9.5.1) software. Multiple unpaired t-tests were performed for all the parameters to compare difference between control and test. The results of all the experiments are expressed as mean ± standard error; $P \leq 0.05$ was considered statistically significant unless specified.

Results

The clinical trial to assess the efficacy and safety of *Bacillus coagulans* LBSC for the treatment of drug-induced constipation associated with functional gastrointestinal disorder

(FGID) symptoms began in November 2021 and ended in July 2022. A total 168 patients with an "intention to treat" participated in the study, which included 125 male (M) and 43 female (F) subjects (Table 1). Of the 2 treatment groups, the test arm was assigned with 140 subjects (107 M and 33 F) while placebo arm with 28 (18 M and 10 F) subjects. The test arm contained 28 subjects each for atorvastatin (22 M and 6 F), atenolol (19 M and 9 F), metformin (22 M and 6 F), amitriptyline (21 M and 7 F), and calcium (25 M and 5 F) induced constipation associated with FGIDs. The assessment of study regulations along with safety protocols and efficacy assays of the subjects during each visit was performed as per

Table 1. Demographic Details of Subjects in Both Treatment Groups, Test and Placebo, Participated in the Mentioned Clinical Study and Their Descriptive Statistics (ICAWFGID - Induced Constipation Associated With FGIDs).

| | Treatment Groups | | | | | |
| | Test arm Supplemented with *Bacillus coagulans* LBSC | | | | | |
Parameters	Atorvastatin ICAWFGID	Atenolol ICAWFGID	Metformin ICAWFGID	Amitriptyline ICAWFGID	Calcium ICAWFGID	Placebo arm
Number of subjects	28	28	28	28	28	28
Age (Years) [Min/ Max]	43.07 ± 9.93 [25/59]	42.93 ± 11.17 [20/62]	39.5 ± 8.77 [25/59]	42.93 ± 11.22 [20/59]	37.75 ± 8.95 [19/55]	39.21 ± 10.62 [25/65]
Gender [n (%)]						
Male	22 (78.57%)	19 (67.86%)	22 (78.57%)	21 (75%)	23 (82.14%)	18 (64.29%)
Female	6 (21.43%)	9 (32.14%)	6 (21.43%)	7 (25%)	5 (17.86%)	10 (35.71%)
Race [n (%)]						
Asian	28 (100%)	28 (100%)	28 (100%)	28 (100%)	28 (100%)	28 (100%)
Ethnicity [n (%)]						
Hispanic or Latino	0 (0%)	0 (0%)	01 (3.57%)	0 (0%)	0 (0%)	0 (0%)
Not-Hispanic or Latino	28 (100%)	28 (100%)	27 (96.43%)	28 (100%)	28 (100%)	28 (100%)

the schedule of events by the clinical trial team and principal investigator (Table 2). The conclusion of clinical study was made after a final follow-up i.e. the end of treatment on visit 06, and lastly followed by a telephonic safety call on visit 07 of the last enrolled subject and accomplishment of target population size as per study protocols.

Primary Endpoint: Efficacy Evaluation

Occurrence of Constipation (%)

The treatment of drug induced constipation associated with FGIDs with the probiotic strain *Bacillus coagulans* LBSC has caused a gradual reduction in the occurrence of constipation (Figure 2). At the baseline (SOT) ie, zero day, all the subjects (i.e. 100%) in placebo arm and test arm showed occurrence of constipation. After 14 days of supplementation of IP, 22 out of 28 subjects (ie, 79.6%) showed occurrence of constipation in amitriptyline ICAWFGID arm, while it was 82.1% (23/28), 88.89% (24/27), and 89.29% (25/28) in the constipation induced with atenolol, atorvastatin and metformin ICAWFGID arms, respectively after 14 days. The occurrence in constipation in calcium ICAWFGID arm was 92.9% and was same as that of placebo arm. With the progression of treatment, a gradual relief from occurrence of constipation was seen in the subjects. After the completion of the intervention period (35 days of treatment with IP), there were only 1.4% (2/139) subjects in the combined test arm that displayed the occurrence of constipation, and this was significantly superior over the placebo arm (25%, 7/28). After comparing the individual drug test group with placebo, zero percent subjects each in the atorvastatin ICAWFGID (*P*-value

0.0054; CI: −70.28, −42.22), atenolol ICAWFGID (*P*-value 0.0047; CI: −71, −43.29), and calcium ICAWFGID (*P*-value 0.0047; CI: −71, −43.29) arms, and 3.6% subjects each in the metformin ICAWFGID (*P*-value 0.0219; CI: −70.62, −16.88) and amitriptyline ICAWFGID (*P*-value 0.0219; CI: −70.62, −16.88) arms displayed the occurrence of constipation, and this was statistically significant over the placebo arm (25%). This statistically significant difference in the scores highlighted the efficacy of IP in relieving the occurrence of constipation in the subjects with constipation induced by prolonged consumption of atorvastatin, atenolol, metformin, amitriptyline and calcium. The intra group analysis of scores at start of treatment (SOT) and end of treatment (EOT) revealed a significant reduction in occurrence of constipation (*P*-value <0.001) after supplementation with *Bacillus coagulans* LBSC.

Co-primary Efficacy Endpoints

Degree of Constipation

The degree of constipation was assessed on a 4 point likert scale. There was a gradual reduction in the score for degree of constipation with a progression in the treatment duration with IP in both the arms, placebo and test, and this decrease was higher in test arm over the placebo arm. The scores of placebo arm and combined test arm declined from 2.59 ± 0.12 to 1.05 ± 0.06, and 2.57 ± 0.055 to 0.86 ± 0.047, and these were 59.55% and 66.54% low from respective EOT to SOT (Figure 3). This decrease in score in test arm over the placebo arm indicated the change in degree of constipation towards "only bearing down and discomfortable sensation" to

Table 2. Schematic Schedule of the Clinical Trial Conducted for the Functional Gastrointestinal Disorder Symptoms.

Visits	Visit 01 (Day 0)[a]	Visit 02 (Day 01)[b]	Visit 03 (Day 14 ± 1)[c]	Visit 04 (Day 22 ± 2)[d]	Visit 05 (Day 36 ± 2)[e]	Visit 06 (Day 50 ± 2)[f]	Visit 07 (Day 60 ± 2)[g]
Informed consent	✓						
Inclusion and exclusion criteria check	✓	✓	✓				
Demographics*	✓						
Medical history and medication history	✓						
Physical examination	✓	✓	✓	✓	✓	✓	
Temperature, pulse rate, blood pressure and respiratory rate	✓	✓	✓	✓	✓	✓	
Body weight, height and BMI	✓						
Urine pregnancy test	✓	✓	✓	✓	✓	✓	
Continuation of drugs causing constipation	✓	✓	✓	✓	✓	✓	✓
Patient diary issuance		✓	✓	✓	✓		
Randomization			✓				
IP Dispensing			✓#				
Evaluation of constipation symptoms	✓	✓	✓	✓	✓	✓	
Evaluation of constipation scoring system		✓	✓	✓	✓	✓	
Evaluation of FGID symptoms	✓	✓	✓	✓	✓	✓	
Estimation of serum concentrations of drug causing constipation			✓			✓	
HRQoL questionnaires			✓			✓	
Laboratory tests**		✓				✓	
Retrieval of IP				✓	✓	✓	
Retrieval of patient diary and its check			✓	✓	✓	✓	
IP compliance check				✓	✓	✓	
AE/SAE Recording	✓	✓	✓	✓	✓	✓	✓
Concomitant medications	✓	✓	✓	✓	✓	✓	✓

[a]Visit 01: Screening phase.
[b]Visit 02: Baseline, enrolment and run-in period initiation.
[c]Visit 03: Run-in period completion, basement assessment and randomization.
[d]Visit 04: Interim follow-up 1.
[e]Visit 05: Interim follow-up 2.
[f]Visit 06: End of treatment/early discontinuation.
[g]Visit 07: End of study (Telephonic safety call); *Demographics include age, gender, race and ethnicity; ** Laboratory tests includes complete blood count [Hematocrit, hemoglobin, erythrocytes count (RBC), mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, leukocytes (WBC) and its differential counts, platelet count] and biochemical test [AST, ALT, serum creatinine, BUN, RBS, total cholesterol, total albumin and globulin, C-Reactive Protein]; #IP treatment will start on day 15.

"normal defecation" from "often abdominal pain or anal burning sensation to influence defecation". In the individual drug arms, the decrease in score for degree of constipation in atorvastatin test arm was highest (69.55%), followed by calcium (67.67%), atenolol (66.71%), amitriptyline (65.42%), and metformin (63.17%) ICAWFGID arms. This reduction in score of degree of constipation was considerably higher in the test arm supplemented with *Bacillus coagulans* LBSC. Further, there was a statistically significant difference in mean change (EOT-SOT) scores of atorvastatin ICAWFGID arm (*P*-value 0.0334; CI: −0.580, −0.080) revealing the therapeutic potential of IP to relieve the degree of constipation in subjects with constipation induced by long term intake of atorvastatin. The intra group analysis of scores at start of treatment (SOT) and end of treatment (EOT) revealed a significant reduction in the degree of constipation (*P*-value <0.001) after supplementation with *Bacillus*

coagulans LBSC. Further, the inter group differences in atenolol, metformin, amitriptyline and calcium test arms were considerably higher but statistically insignificant (*P*-value ≥0.05) over the placebo arm.

Degree of Stool Consistency

The degree of stool consistency was determined on a 7 Bristol Stool Form Scale. Both the arms, placebo and test, displayed a steady improvement in the scores for degree of stool consistency with the treatment period (Figure 4). The scores for degree of stool consistency (SOT to EOT) increased from 1.57 ± 0.15 to 4.17 ± 0.24 in the placebo arm, and 1.47 ± 0.058 to 4.74 ± 0.076 in combined test arm, and this increase was significantly higher (*P*-value 0.0232; CI: 0.1870, 1.1629). This revealed the effectiveness of IP in enhancing the stool consistency. The individual drug test arms also
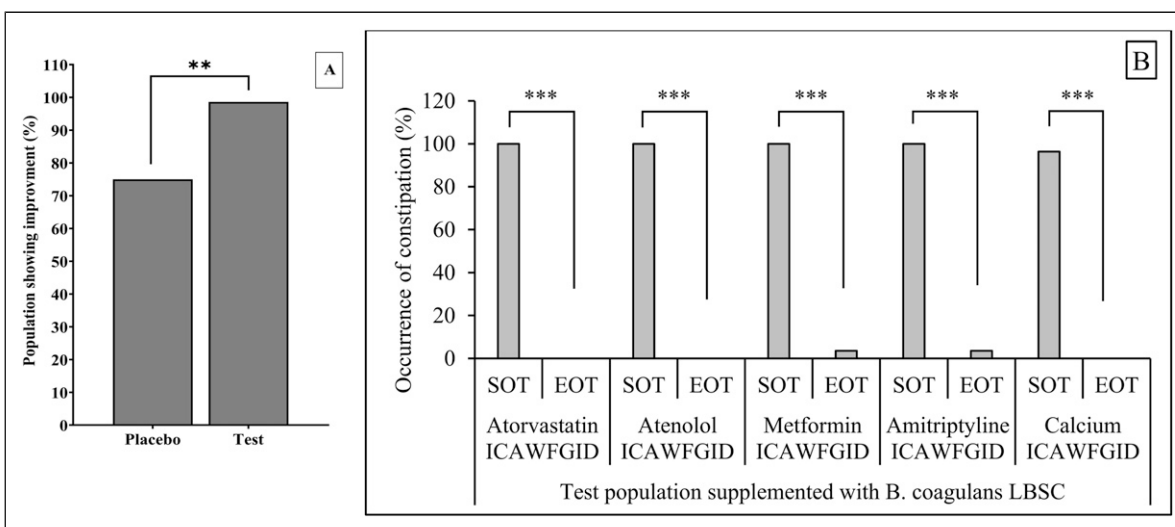
Figure 2. Study population showing (A) change in occurrence of constipation (EOT-SOT) of placebo v/s test after treatment with *Bacillus coagulans* LBSC for 35 intervention days; (B) Occurrence of constipation at SOT and EOT after treatment with *Bacillus coagulans* LBSC for 35 intervention days in patients with constipation associated with FGID induced by individual drug (**P-value ≤ 0.01, intergroup analysis; ***P-value ≤ 0.001, intragroup analysis at SOT and EOT using the unpaired, one-tailed t test).
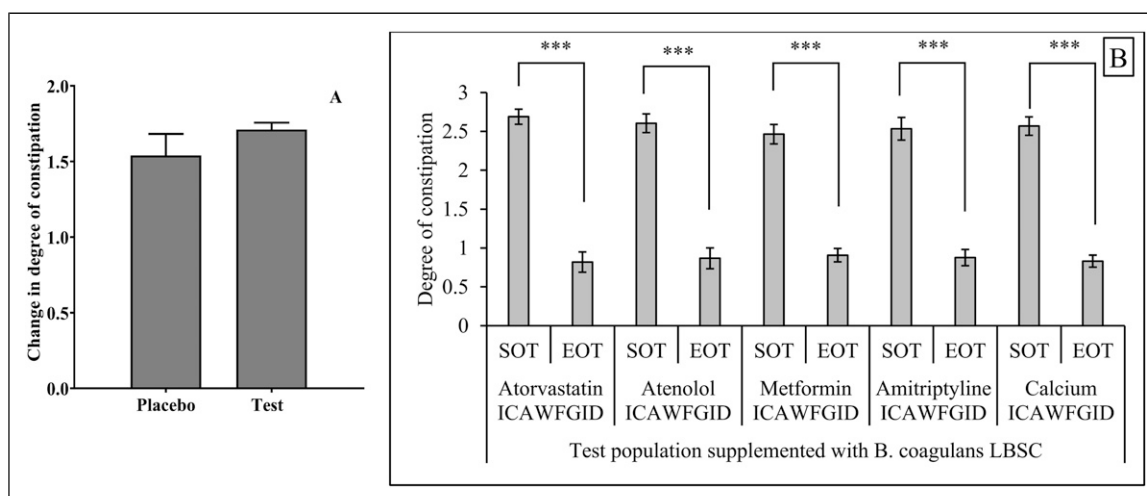

Figure 3. (A) Change in score of degree constipation (EOT-SOT) of placebo v/s test after treatment with *Bacillus coagulans* LBSC for 35 intervention days. (B) Degree of constipation at SOT and EOT after treatment with *Bacillus coagulans* LBSC for 35 intervention days in patients with constipation associated with FGID induced by individual drug (**P-value ≤ 0.01, intergroup analysis; ***P-value ≤ 0.001, intragroup analysis at SOT and EOT using the unpaired, one-tailed t test).

showed an increase in the scores for degree of stool consistency from 1.37 ± 0.11 to 4.96 ± 0.16, 1.42 ± 0.11 to 4.60 ± 0.13, 1.74 ± 0.17 to 4.45 ± 0.19, 1.34 ± 0.11 to 4.92 ± 0.21, and 1.48 ± 0.14 to 4.79 ± 0.13 in atorvastatin, atenolol, metformin, amitriptyline, and calcium ICAWFGID arms, respectively. Further, there was a statistically significant difference in mean change (EOT-SOT) scores of atorvastatin ICAWFGID arm (P-value: 0.0102; CI: 0.3604, −1.6311), amitriptyline ICAWFGID arm (P-value: 0.0104; CI: 0.3540, −1.6131) and calcium

ICAWFGID arm (P-value: 0.0451; CI: 0.0829, −1.3420) over the placebo arm indicating the therapeutic potential of *Bacillus coagulans* LBSC to significantly enhance the degree of stool consistency in subjects with constipation induced by atorvastatin, amitriptyline and calcium. The intra group analysis revealed that treatment with *Bacillus coagulans* LBS has caused significant improvement in the scores of degree of stool consistency (P-value <0.001) in all the patients with drug induced constipation associated with functional gastrointestinal disorder.
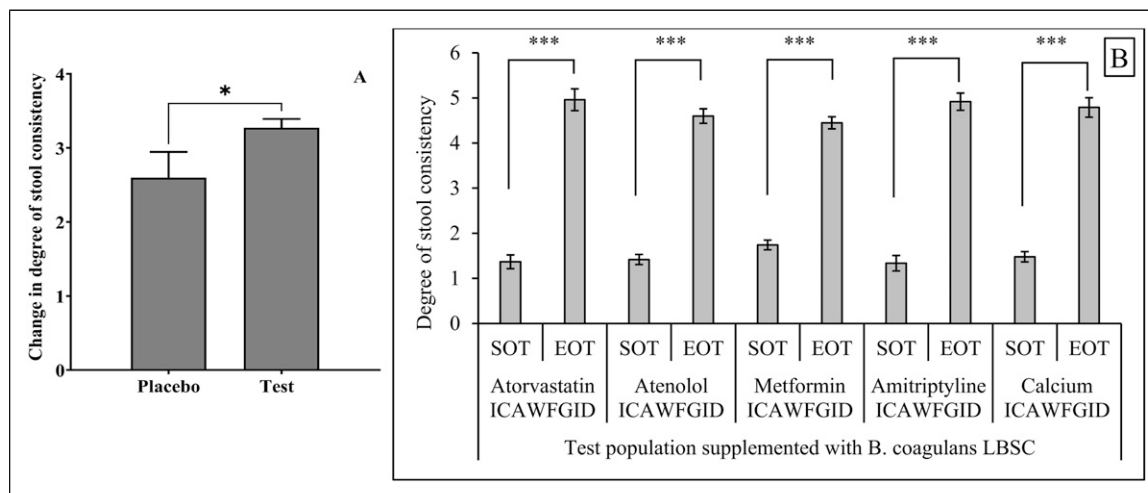
Figure 4. (A) Change in score of degree stool consistency (EOT-SOT) of placebo v/s test after treatment with *Bacillus coagulans* LBSC for 35 intervention days. (B) Degree of stool consistency at SOT and EOT after treatment with *Bacillus coagulans* LBSC for 35 intervention days in patients with constipation associated with FGID induced by individual drug (**P-value ≤ 0.01, intergroup analysis; ***P-value ≤ 0.001, intragroup analysis at SOT and EOT using the unpaired, one-tailed *t* test).

Degree of Stool Expulsion

The assessment of degree of stool expulsion was performed on a 3 point scale [Grade I (1 point): difficult; Grade II (2 point): satisfactory; Grade III (3 point): complete]. With the progress of the treatment period, there was a continuous enhancement in the degree of stool expulsion in both the arms, placebo and test (Figure 5). The scores for degree of stool expulsion (SOT to EOT) increased from 1.13 ± 0.05 to 2.53 ± 0.14 in the placebo arm, and 1.13 ± 0.026 to 2.80 ± 0.039 in combined test arm, and this increase was borderline significantly superior (P-value 0.0553; CI: 0.0378, −0.4939). This increase in scores indicated the efficacy of IP in changing the stool expulsion from "difficult" to "satisfactory". Further, the increase in EOT-SOT scores of the atorvastatin, atenolol, metformin, amitriptyline, and calcium ICAWFGID arms after the intervention with *Bacillus coagulans* LBSC for 35 days was 1.21, 1.24, 0.98, 1.30 and 1.24 times over the placebo arm, respectively. A statistically significant difference in mean change (EOT-SOT) scores of amitriptyline ICAWFGID arm (P-value 0.0431; CI: 0.1151, −0.7085) and calcium ICAWFGID arm (P-value 0.0449; CI: 0.0450, −0.6331) was seen over the placebo. In addition, the intra group analysis at SOT and EOT revealed that treatment with *Bacillus coagulans* LBS has caused significant improvement in the scores of degree of stool expulsion (P-value <0.001) in all the patients with drug induced constipation associated with functional gastrointestinal disorder. These increased scores indicated the therapeutic potential of *Bacillus coagulans* LBSC to prominently improve the degree of stool expulsion in subjects with constipation induced by amitriptyline and calcium. The inter group analysis displayed variations in atorvastatin, atenolol and metformin test arm were considerably higher but

statistically insignificant (P-value ≥0.05) as compared to placebo arm.

In addition, the inter group analysis of occurrence of hard stools (Figure S1, Supplementary file), constipation scoring system (Figure S2, Supplementary file), and defecation frequency were marginally changed but statistically insignificant (P-value ≥0.05) over the placebo arm. The findings are described in results and discussion section of supplementary file.

Secondary Endpoint: Safety Evaluation

Assessment of IP Tolerance, Adverse Events, Serious Adverse Events and Systemic Biomarkers

The investigational product (IP) *Bacillus coagulans* LBSC was well tolerated by all the participants in the study. During the entire clinical investigation, there were no reports related to adverse event, serious adverse events and/or systemic biomarkers, indicating the safety of IP for oral consumption.

The changes in medical outcomes which includes physical functioning, role functioning, social functioning, mental health, health perceptions, and bodily pain have shown no statistical significant variations in the differences of EOT and SOT (P-value ≥0.05) (Table S1, Supplementary file). The investigation of vital indicators such as body temperature, pulse rate, respiratory rate, and systolic and diastolic blood pressure of each subject was performed during every visit by the principal investigator (Table S2, Supplementary file). There was no statistical significance difference (P-value ≥0.05) in the vital indicators in both, placebo arm and test arm during all the visits and the obtained results were in normal working regime. Further, the haematological and
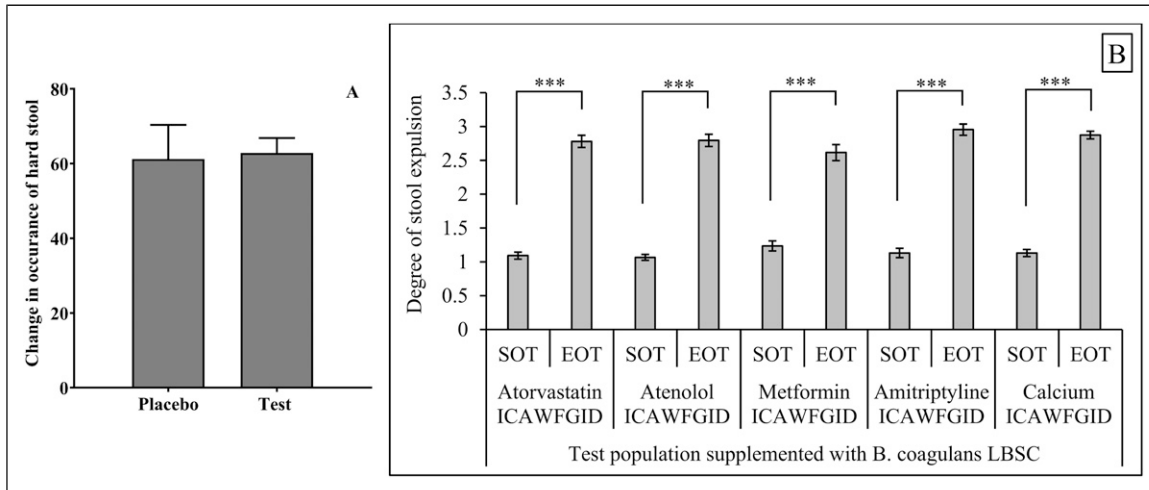
Figure 5. (A) Change in score of degree stool expulsion (EOT-SOT) of placebo v/s test after treatment with *Bacillus coagulans* LBSC for 35 intervention days. (B) Degree of stool expulsion at SOT and EOT after treatment with *Bacillus coagulans* LBSC for 35 intervention days in patients with constipation associated with FGID induced by individual drug (**P-value ≤ 0.01, intergroup analysis; ***P-value ≤ 0.001, intragroup analysis at SOT and EOT using the unpaired, one-tailed t test).

biochemical indicators were also analysed at the SOT and EOT in both, placebo arm and test arm. The haematological indicators included the measurement of complete blood count (hemoglobin, red blood cell, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, white blood cell and its differential counts, platelet count) (Table S3, Supplementary file) and biochemical indicators comprised of aspartate transferase, alanine transaminase, serum creatinine, blood urea nitrogen, random blood sugar, total cholesterol, total albumin and globulin, C-Reactive Protein, respectively (Table S4, Supplementary file). There was no statistically significant difference (P-value ≥0.05) in both, haematological and biochemical indicators, of placebo arm and test arm at end of treatment and SOT. Further, the obtained values of indicators in haematological and biochemical tests were within the standard domain of the reference values.

Assessment of Quality by Visual Analogue Scale Changes in Symptoms of FGID

Out of twenty FGID symptoms, the abdominal pain, abdominal discomfort, abdominal distension, feeling of incomplete evacuation, and straining during defecation are directly associated with drug induced constipation. Herein, the treatment of subjects with the investigational product (IP) *Bacillus coagulans* LBSC (6×10^9 spores/day) for 35 days showed a reduction in symptoms of FGIDs based visual analogue scale. Overall, the processed responses clearly signified a considerable positive improvement (non-significant, P-value ≥0.05) in other symptoms of constipation associated with FGIDs as determined by the changes in the EOT-SOT score (Table 3).

Discussion

In the current CONSORT compliant, prospective, interventional, randomized, double-blind, parallel, multi-arm, placebo controlled clinical study, *Bacillus coagulans* LBSC was investigated for its therapeutic efficacy and potential in management of drug induced constipation associated with functional gastrointestinal disorder symptoms. Herein, the patients with complaints of constipation due to prolonged consumption of regularly prescribed drugs used for the treatment of some chronic disease were selected to assess the therapeutic effect of *Bacillus coagulans* LBSC. The patients undergoing treatment with atorvastatin, atenolol, metformin, amitriptyline and calcium belonging to different class of drugs (statins, β-blockers, antidiabetic drugs, tricyclic antidepressant and mineral supplement) were considered to cover a wide acumen of drugs and broad category of diseases.

Regular consumption of prescribed drugs without failure is a pre-requisite for the patients with different chronic diseases for maintaining proper function of body, healthy state of mind and meet the needs of health care. The medications for treatment/management of chronic diseases have to be taken for a prolonged duration or life time depending upon the severity of disease and psycho-physiological state of patients. Previously published reports claims the development of constipation and its associated symptoms in patients after regular or prolonged intake of medicines for chronic diseases. The possible causes include antimicrobial properties,[6] water secretion and pharmacological impact on intestinal motility by statins,[1] blockade of β-receptors with β-blocker medications,[2] changes in the bile acid pool inside the intestine,[3] inhibition of transient receptor potential channel canonical

Table 3. Changes in Symptoms of Individual Drug Induced Constipation Associated With FGIDs (ICAWFGID) at SOT and EOT After Treatment With *Bacillus coagulans* LBSC for 35 Intervention Days (mean ± SE).

Parameters	Arms Supplemented with *B coagulans* LBSC	SOT	EOT	Mean change	Intergroup Mean Difference	*P*-Value	95% CI
Feeling of a lump, fullness or something stuck in throat	Placebo	0.46 ± 0.19	0.04 ± 0.04	−0.43 ± 0.19			
	Atorvastatin ICAWFGID	0.26 ± 0.16	0.00 ± 0.00	−0.26 ± 0.15	0.17	0.2875	−0.092, −0.4315
	Atenolol ICAWFGID	0.11 ± 0.11	0.00 ± 0.00	−0.11 ± 0.11	0.32	0.0421	0.0616, −0.5812
	Metformin ICAWFGID	0.32 ± 0.14	0.04 ± 0.04	−0.29 ± 0.14	0.14	0.3650	−0.116, −0.4026
	Amitriptyline ICAWFGID	0.04 ± 0.04	0.00 ± 0.00	−0.04 ± 0.04	0.39	0.0131	0.1330, −0.6526
	Calcium ICAWFGID	0.25 ± 0.11	0.00 ± 0.00	−0.25 ± 0.11	0.18	0.2577	−0.081, −0.4383
Pain or discomfort in the middle of chest	Placebo	0.04 ± 0.04	0.00 ± 0.00	−0.04 ± 0.03			
	Atorvastatin ICAWFGID	0.41 ± 0.15	0.00 ± 0.00	−0.41 ± 0.15	−0.37	0.1683	−0.815, −0.0723
	Atenolol ICAWFGID	0.46 ± 0.30	0.00 ± 0.00	−0.46 ± 0.30	−0.42	0.1091	−0.868, −0.0114
	Metformin ICAWFGID	0.39 ± 0.28	0.04 ± 0.04	−0.36 ± 0.27	−0.32	0.2290	−0.761, −0.1185
	Amitriptyline ICAWFGID	0.11 ± 0.08	0.00 ± 0.00	−0.11 ± 0.08	−0.07	0.7890	−0.511, −0.3685
	Calcium ICAWFGID	0.39 ± 0.17	0.00 ± 0.00	−0.39 ± 0.18	−0.35	0.1815	−0.797, −0.0828
Heartburn	Placebo	0.21 ± 0.13	0.07 ± 0.07	−0.14 ± 0.08			
	Atorvastatin ICAWFGID	0.48 ± 0.17	0.00 ± 0.00	−0.48 ± 0.17	−0.32	0.4224	−1.034, −0.3568
	Atenolol ICAWFGID	0.75 ± 0.58	0.00 ± 0.00	−0.75 ± 0.58	−0.61	0.1471	−1.296, −0.0819
	Metformin ICAWFGID	0.25 ± 0.20	0.00 ± 0.00	−0.25 ± 0.20	−0.11	0.7977	−0.796, −0.5819
	Amitriptyline ICAWFGID	0.39 ± 0.17	0.00 ± 0.00	−0.39 ± 0.17	−0.24	0.5499	−0.939, −0.4390
	Calcium ICAWFGID	0.36 ± 0.28	0.00 ± 0.00	−0.36 ± 0.28	−0.21	0.6083	−0.903, −0.4747
Epigastric pain	Placebo	1.14 ± 0.21	0.10 ± 0.06	−1.04 ± 0.22			
	Atorvastatin ICAWFGID	1.25 ± 0.24	0.10 ± 0.06	−1.15 ± 0.23	−0.11	0.8446	−1.092, −0.8606
	Atenolol ICAWFGID	1.56 ± 0.91	0.10 ± 0.05	−1.47 ± 0.88	−0.43	0.4619	−1.400, −0.5357
	Metformin ICAWFGID	1.02 ± 0.22	0.11 ± 0.05	−0.90 ± 0.22	0.14	0.8219	−0.835, −1.100
	Amitriptyline ICAWFGID	0.91 ± 0.20	0.03 ± 0.02	−0.88 ± 0.20	0.16	0.7937	−0.814, −1.1214
	Calcium ICAWFGID	1.09 ± 0.22	0.06 ± 0.04	−1.03 ± 0.22	0.01	0.9903	−0.960, −0.9750

Table 3. (continued)

Parameters	Arms Supplemented with *B coagulans* LBSC	SOT	EOT	Mean change	Intergroup Mean Difference	P-Value	95% CI
Bloating	Placebo	0.52 ± 0.18	0.27 ± 0.09	−0.25 ± 0.16			
	Atorvastatin ICAWFGID	0.76 ± 0.22	0.02 ± 0.01	−0.74 ± 0.21	−0.48	0.3293	−1.322, −0.3387
	Atenolol ICAWFGID	1.07 ± 0.72	0.08 ± 0.03	−0.99 ± 0.72	−0.74	0.1386	−1.563, −0.0823
	Metformin ICAWFGID	0.84 ± 0.20	0.19 ± 0.06	−0.65 ± 0.19	−0.40	0.4228	−1.223, −0.4226
	Amitriptyline ICAWFGID	0.22 ± 0.10	0.05 ± 0.02	−0.17 ± 0.09	0.08	0.8716	−0.742, −0.9037
	Calcium ICAWFGID	0.77 ± 0.28	0.06 ± 0.03	−0.71 ± 0.26	−0.46	0.3557	−1.284, 0.3616
Early satiety	Placebo	0.38 ± 0.16	0.06 ± 0.04	−0.32 ± 0.16			
	Atorvastatin ICAWFGID	0.44 ± 0.17	0.08 ± 0.05	−0.37 ± 0.17	−0.05	0.9398	−1.032, −0.9415
	Atenolol ICAWFGID	1.09 ± 0.77	0.06 ± 0.03	−1.04 ± 0.76	−0.71	0.2290	−1.692, −0.2634
	Metformin ICAWFGID	0.73 ± 0.38	0.13 ± 0.06	−0.60 ± 0.37	−0.28	0.6343	−1.259, −0.6956
	Amitriptyline ICAWFGID	0.45 ± 0.20	0.03 ± 0.02	−0.43 ± 0.20	−0.11	0.8566	−1.084, −0.8706
	Calcium ICAWFGID	0.94 ± 0.50	0.06 ± 0.04	−0.89 ± 0.50	−0.57	0.3417	−1.542, −0.4134
Nausea	Placebo	0.84 ± 0.22	0.12 ± 0.07	−0.71 ± 0.21			
	Atorvastatin ICAWFGID	0.71 ± 0.23	0.01 ± 0.01	−0.70 ± 0.22	−0.01	0.9780	−0.707, −0.7318
	Atenolol ICAWFGID	0.64 ± 0.43	0.06 ± 0.04	−0.57 ± 0.42	0.06	0.7425	−0.571, −0.8553
	Metformin ICAWFGID	0.65 ± 0.22	0.03 ± 0.02	−0.62 ± 0.21	0.09	0.8326	−0.621, −0.8046
	Amitriptyline ICAWFGID	1.12 ± 0.23	0.08 ± 0.07	−1.04 ± 0.23	−0.33	0.4587	−1.033, −0.3925
	Calcium ICAWFGID	0.90 ± 0.42	0.01 ± 0.01	−0.89 ± 0.42	−0.18	0.6847	−0.888, −0.5375
Vomiting	Placebo	0.07 ± 0.08	0.02 ± 0.02	−0.07 ± 0.07			
	Atorvastatin ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07	0.8445	−0.528, −0.6718
	Atenolol ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07	0.8431	−0.523, −0.6663
	Metformin ICAWFGID	0.32 ± 0.34	0.00 ± 0.00	−0.32 ± 0.34	−0.25	0.4886	−0.844, −0.3448
	Amitriptyline ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	−0.00 ± 0.00	0.07	0.8431	−0.523, −0.6663
	Calcium ICAWFGID	0.50 ± 0.50	0.00 ± 0.00	−0.50 ± 0.50	−0.43	0.2355	−1.023, −0.1663

(continued)

Table 3. (continued)

Parameters	Arms Supplemented with *B coagulans* LBSC	SOT	EOT	Mean change	Intergroup Mean Difference	P-Value	95% CI
Diarrhea	Placebo	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
	Atorvastatin ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−0.00	1.000	−0.397, −0.3975
	Atenolol ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−0.00	1.000	−0.393, −0.3939
	Metformin ICAWFGID	0.43 ± 0.41	0.04 ± 0.04	−0.39 ± 0.41	−0.39	0.1009	−0.786, −0.0010
	Amitriptyline ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	−0.00 ± 0.00	−0.00	1	−0.393, −0.3939
	Calcium ICAWFGID	0.04 ± 0.04	0.00 ± 0.00	−0.04 ± 0.04	−0.04	0.8812	−0.429, 0.3582
Urgency	Placebo	0.07 ± 0.08	0.07 ± 0.06	0.00 ± 0.00			
	Atorvastatin ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	1.000	−0.992, −0.9921
	Atenolol ICAWFGID	0.57 ± 0.57	0.00 ± 0.03	−0.57 ± 0.57	−0.57	0.3383	−1.554, −0.4116
	Metformin ICAWFGID	1.21 ± 0.81	0.00 ± 0.04	−1.21 ± 0.81	−1.21	0.0424	−2.197, −0.231
	Amitriptyline ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	−0.00 ± 0.00	−0.00	1.000	−0.983, −0.9830
	Calcium ICAWFGID	0.18 ± 0.16	0.04 ± 0.04	−0.14 ± 0.13	−0.14	0.8107	−1.125, −0.8402
Food or drinks get stuck after swallowing or go down slowly through chest	Placebo	0.18 ± 0.12	0.04 ± 0.00	−0.14 ± 0.12			
	Atorvastatin ICAWFGID	0.26 ± 0.16	0.00 ± 0.00	−0.26 ± 0.16	−0.12	0.8181	−0.950, −0.7178
	Atenolol ICAWFGID	0.82 ± 0.54	0.00 ± 0.00	−0.82 ± 0.54	−0.68	0.1766	−1.505, −0.1480
	Metformin ICAWFGID	0.39 ± 0.28	0.00 ± 0.00	−0.39 ± 0.28	−0.25	0.6692	−1.040, −0.6123
	Amitriptyline ICAWFGID	0.18 ± 0.10	0.00 ± 0.00	−0.18 ± 0.01	−0.04	0.9432	−0.862, −0.7909
	Calcium ICAWFGID	0.89 ± 0.58	0.00 ± 0.00	−0.89 ± 0.58	−0.75	0.1354	−1.576, −0.0766
Regurgitation	Placebo	0.08 ± 0.05	0.06 ± 0.04	−0.02 ± 0.02			
	Atorvastatin ICAWFGID	0.30 ± 0.15	0.00 ± 0.00	−0.30 ± 0.15	−0.28	0.5094	−0.991, −0.4247
	Atenolol ICAWFGID	0.77 ± 0.51	0.02 ± 0.02	−0.75 ± 0.50	−0.73	0.0878	−1.430, −0.026
	Metformin ICAWFGID	0.58 ± 0.28	0.07 ± 0.03	−0.52 ± 0.27	−0.50	0.2408	−1.201, −0.2018
	Amitriptyline ICAWFGID	0.14 ± 0.11	0.01 ± 0.01	−0.13 ± 0.11	−0.11	0.7929	−0.813, −0.5900
	Calcium ICAWFGID	0.79 ± 0.39	0.03 ± 0.03	−0.77 ± 0.37	−0.75	0.0789	−1.451, −0.048

Table 3. (continued)

Parameters	Arms Supplemented with *B coagulans* LBSC	SOT	EOT	Mean change	Intergroup Mean Difference	*P*-Value	95% CI
Epigastric burning	Placebo	0.61 ± 0.19	0.10 ± 0.07	−0.51 ± 0.18			
	Atorvastatin ICAWFGID	1.08 ± 0.22	0.06 ± 0.04	−1.01 ± 0.21	−0.53	0.1988	−1.149, −0.1417
	Atenolol ICAWFGID	0.94 ± 0.51	0.06 ± 0.04	−0.88 ± 0.50	−0.40	0.3436	−1.007, −0.2720
	Metformin ICAWFGID	0.41 ± 0.17	0.17 ± 0.08	−0.24 ± 0.17	0.27	0.4846	−0.368, −0.9113
	Amitriptyline ICAWFGID	0.86 ± 0.22	0.03 ± 0.02	−0.84 ± 0.21	−0.32	0.4027	−0.964, −0.3149
	Calcium ICAWFGID	0.74 ± 0.24	0.03 ± 0.02	−0.71 ± 0.23	−0.20	0.6065	−0.839, −0.4399
Belching	Placebo	0.07 ± 0.06	0.04 ± 0.02	−0.04 ± 0.04			
	Atorvastatin ICAWFGID	0.04 ± 0.05	0.00 ± 0.00	−0.04 ± 0.04	−0.00	0.9983	−1.044, −1.0414
	Atenolol ICAWFGID	1.11 ± 1.03	0.00 ± 0.00	−1.11 ± 1.02	−1.07	0.0881	−2.104, −0.038
	Metformin ICAWFGID	0.21 ± 0.14	0.11 ± 0.09	−0.11 ± 0.12	−0.07	0.9093	−1.104, −0.9618
	Amitriptyline ICAWFGID	0.29 ± 0.16	0.00 ± 0.00	−0.29 ± 0.16	−0.25	0.6900	−1.283, −0.7832
	Calcium ICAWFGID	0.32 ± 0.20	0.00 ± 0.00	−0.32 ± 0.17	−0.28	0.6486	−1.318, −0.7475
Postprandial fullness	Placebo	0.67 ± 0.21	0.19 ± 0.08	−0.48 ± 0.20			
	Atorvastatin ICAWFGID	0.59 ± 0.20	0.02 ± 0.02	−0.56 ± 0.20	−0.08	0.8960	−1.105, −0.9427
	Atenolol ICAWFGID	1.05 ± 0.76	0.04 ± 0.02	−1.02 ± 0.76	−0.54	0.3862	−1.548, −0.4809
	Metformin ICAWFGID	0.74 ± 0.28	0.17 ± 0.06	−0.56 ± 0.26	−0.08	0.8957	−1.095, −0.9338
	Amitriptyline ICAWFGID	0.25 ± 0.13	0.02 ± 0.02	−0.23 ± 0.13	0.25	0.6855	−0.765, −1.2638
	Calcium ICAWFGID	1.18 ± 0.58	0.01 ± 0.01	−1.16 ± 0.56	−0.68	0.2682	−1.696, −0.3324
Abdominal pain	Placebo	1.73 ± 0.17	0.31 ± 0.10	−1.42 ± 0.18			
	Atorvastatin ICAWFGID	1.43 ± 0.20	0.10 ± 0.05	−1.33 ± 0.21	0.09	0.8625	−0.779, −0.9622
	Atenolol ICAWFGID	2.15 ± 0.74	0.17 ± 0.05	−1.98 ± 0.73	−0.56	0.2826	−1.425, −0.2999
	Metformin ICAWFGID	1.96 ± 0.28	0.17 ± 0.05	−1.80 ± 0.28	−0.38	0.4734	−1.238, −0.4874
	Amitriptyline ICAWFGID	1.42 ± 0.17	0.14 ± 0.08	−1.28 ± 0.18	0.14	0.7807	−0.717, −1.0085
	Calcium ICAWFGID	1.67 ± 0.24	0.15 ± 0.06	−1.52 ± 0.22	−0.10	0.8528	−0.959, −0.7656

Table 3. (continued)

Parameters	Arms Supplemented with B coagulans LBSC	SOT	EOT	Mean change	Intergroup Mean Difference	P-Value	95% CI
Abdominal discomfort	Placebo	0.29 ± 0.13	0.08 ± 0.06	−0.21 ± 0.11			
	Atorvastatin ICAWFGID	0.57 ± 0.20	0.07 ± 0.04	−0.50 ± 0.19	−0.29	0.6498	−1.373, −0.7799
	Atenolol ICAWFGID	1.34 ± 0.88	0.08 ± 0.04	−1.26 ± 0.86	−1.05	0.1054	−2.116, −0.0166
	Metformin ICAWFGID	1.10 ± 0.57	0.15 ± 0.06	−0.95 ± 0.55	−0.74	0.2514	−1.809, −0.3237
	Amitriptyline ICAWFGID	0.28 ± 0.13	0.04 ± 0.03	−0.24 ± 0.11	−0.03	0.9604	−1.098, −1.0345
	Calcium ICAWFGID	0.83 ± 0.33	0.04 ± 0.03	−0.79 ± 0.30	−0.58	0.3686	−1.648, −0.4845
Abdominal distension	Placebo	0.14 ± 0.10	0.00 ± 0.00	−0.14 ± 0.09			
	Atorvastatin ICAWFGID	0.15 ± 0.11	0.04 ± 0.04	−0.11 ± 0.11	0.03	0.9474	−0.761, −0.8253
	Atenolol ICAWFGID	0.61 ± 0.62	0.00 ± 0.00	−0.61 ± 0.60	−0.47	0.3308	−1.250, −0.3221
	Metformin ICAWFGID	0.54 ± 0.46	0.04 ± 0.04	−0.50 ± 0.45	−0.36	0.4542	−1.143, −0.4292
	Amitriptyline ICAWFGID	0.00 ± 0.00	0.00 ± 0.00	−0.00 ± 0.02	0.14	0.7646	−0.643, −0.9292
	Calcium ICAWFGID	0.39 ± 0.19	0.00 ± 0.00	−0.39 ± 0.17	−0.25	0.6003	−1.036, −0.5363
Feeling of incomplete evacuation	Placebo	1.89 ± 0.15	0.38 ± 0.10	−1.49 ± 0.21			
	Atorvastatin ICAWFGID	1.95 ± 0.16	0.04 ± 0.04	−1.92 ± 0.16	−0.42	0.5090	−1.408, −0.6027
	Atenolol ICAWFGID	2.69 ± 0.82	0.06 ± 0.03	−2.63 ± 0.82	−1.12	0.0654	−2.113, −0.120
	Metformin ICAWFGID	2.22 ± 0.39	0.17 ± 0.06	−2.05 ± 0.38	−0.54	0.3736	−1.534, −0.4583
	Amitriptyline ICAWFGID	1.97 ± 0.14	0.11 ± 0.08	−1.86 ± 0.16	−0.35	0.5627	−1.346, −0.6465
	Calcium ICAWFGID	2.30 ± 0.36	0.09 ± 0.05	−2.22 ± 0.34	−0.71	0.2452	−1.699, −0.2933
Straining during defecation	Placebo	1.85 ± 0.14	0.27 ± 0.10	−1.58 ± 0.19			
	Atorvastatin ICAWFGID	1.93 ± 0.16	0.11 ± 0.04	−1.82 ± 0.17	−0.40	0.7595	−1.551, −1.0657
	Atenolol ICAWFGID	2.94 ± 1.01	0.11 ± 0.05	−2.82 ± 0.99	−1.12	0.1153	−2.538, −0.0553
	Metformin ICAWFGID	2.39 ± 0.64	0.14 ± 0.05	−2.25 ± 0.65	−0.54	0.3972	−1.963, −0.6303
	Amitriptyline ICAWFGID	1.97 ± 0.15	0.07 ± 0.07	−1.90 ± 0.16	−0.35	0.6835	−1.617, −0.9760
	Calcium ICAWFGID	2.43 ± 0.44	0.28 ± 0.09	−2.14 ± 0.48	−0.70	0.4742	−1.860, −0.7335

type 4 responsible for Ca^{+2} permeability and GI excitability,[4] and alteration in neuromuscular excitability by high calcium levels,[7] along with alterations in gut microbiome.[5]

Constipation associated with FGIDs could potentially misbalance the gut-brain axis through the autonomic, central, and enteric nervous systems.[31] Probiotics are known to offer promising health benefits against numerous disorders including gastrointestinal ailments like IBS, IBD, constipation, intestinal gas and bloating, diarrhea, indigestion, ulcerative colitis, pouchitis, Crohn's disease, nutrient malabsorption, small intestine bacterial overgrowth, etc.[17,26,27,32,33] The

therapy with supplementation of probiotics aids in restoring the non-pathogenic microbiome balance of gut and re-establish the healthy and complex microbiome-host interactions.[34] Numerous short chain fatty acids produced by probiotics confer potential health benefits by modulation of gut microbiota, improving the gut brain axis and symptoms of constipation.[35,36] Probiotics are capable of producing a wide array of neurotransmitters, neuroactive compounds, and metabolites such as γ-amino butyric acid, serotonin, tryptamine, norepinephrine, dopamine, acetylcholine, histamines, etc. along with the short chain fatty acids such as acetate, butyrate, lactate, and propionate.[35,37] These neurochemical compounds could reduce the visceral hyper-sensitivity by expression of mu-opioid and cannabinoid receptors in the intestinal epithelial cells,[38] activating the antinociceptic activity by inhibition of transient receptor vanilloid 1 channels for reliving the symptoms of abdominal pain,[39] and/or modulating the integrity of proteins in tight junction such as ZO-1, JAM-A, occuludin, and claudin-1.[40] It is worth noticing that the clinical efficacy of most of the probiotics, including *Bacillus coagulans*, is strain specific and the therapeutic potential is predominantly associated with clinical conditions of individuals such as digestive and non-digestive related conditions.[41] Further, the variations in design of clinical study may also demonstrate alterations in the efficacies of probiotics under investigation in the gastro-intestinal disorders.[42]

The current study demonstrates the therapeutic potential of *Bacillus coagulans* LBSC as evident from the statistically significant reduction in the occurrence of constipation in test arm over the placebo arm (≥98.6% and P-value <0.05). The assessment of co-primary endpoints showed significant improvements in degree of stool consistency (P-value <0.05), borderline significant superiority in degree of stool expulsion, while the other co-primary efficacy endpoints displayed considerably higher values over the placebo arm (non-significant, $P \leq 0.05$). The intra group analysis of scores at SOT and EOT indicated a significant reduction in symptoms for occurrence of constipation and degree of constipation, while significant enhancement in scores for degree of stool consistency, degree of stool expulsion (P-value <0.001) after the intervention period. The administration of IP might have helped to reverse the symptoms of drug induced constipation and restore the normal functioning of gut by any of the mechanisms mentioned in the above sections. These changes in symptoms of drug induced constipation has further caused improvement in relief of FGIDs. The specificity of probiotic strain and psychological factors might have also played vital roles in the improvement of indications of drug induced constipation associated with FGIDs.

Previously, similar findings revealed the release of functional constipation by improvement in the number of bowel movements and achievement of normal stool consistency after administration of *Bacillus coagulans* Unique IS2 (2 × 10⁹ CFU/day) for 4 weeks.[22] Minamida et al[33] documented

considerable enhancement in the changes in the average scores of self-reported fecal size, sensation of incomplete evacuation, and defecation frequency in patients with constipation after administration of *Bacillus coagulans* ilac-01 (1 × 10⁸ CFU/day) for 2 weeks. Venkataraman et al[16] also reported a significant reduction in the symptoms of functional constipation such as abdominal-pain, defecation and sensation of incomplete evacuation after administration of *B coagulans* Unique IS2 (2 × 10⁹ spores/day) with lactulose for 4 weeks. Similarly, the other randomized, double-blind, placebo controlled, parallel-group trial with two arms has shown a significant improvement in colonic transit time, complete spontaneous bowel movement score, and bowel discomfort symptom after the administration of *B coagulans* SNZ 1969 (2 × 10⁹ CFU/day) in adults with mild intermittent constipation for 8 weeks.[43] Other 4 weeks investigation has shown a significant improvement in symptoms such as abdominal pain, abdominal distention, and global assessment score in patients with IBS after a daily supplementation of 2 × 10⁹ CFU *Bacillus coagulans* PROBACI.[44] Tandon et al[45] reported significant improvement in scores for Quality of Life in Reflux and Dyspepsia Questionnaire (QOLRAD) score and Global Overall Symptom (GOS) scale in paediatric patients with functional abdominal pain after daily supplementation of *Bacillus coagulans* GBI-30, 6086 along with digestive enzymes for 6 weeks. A 4 weeks randomized, double-blind, placebo-controlled study with supplementation of *Bacillus coagulans* MTCC 5856 (2 × 10⁹ CFU/day) has showed a significant reduction in abdominal distension, borborygmi, burping, and flatulence. Further, there were also significant changes in cumulative scores for abdominal pain, reflux syndrome, diarrhea, and constipation, and no adverse events were reported proving the safety upon consumption.[17] The data represented in the current study, along with the information in the previously published articles, establishes the effectiveness of *Bacillus coagulans* in curing and/or managing the symptoms drug induced constipation associated with the FGID symptoms.

Bacillus sp. are not considered as indigenous inhabitants of gut microbiota, but are transient microbes making their presence in gut after the ingestion of fermented vegetables and food.[25] Hence, it is necessary to primarily establish the stability and safety of the probiotic strain before advising it for human administration. The phenotypic and genomic safety, and stability of *Bacillus coagulans* LBSC [DSM17654; GenBank: CP022701.1 & GenBank: ATW84696.1 (gyrB)] has already been reported by sequence analysis of whole genome,[46] and following the FAO/WHO guidelines (FAO and WHO Expert Consultation, 2001).[47,48] Further, this Gram-positive spore-forming *Bacillus coagulans* can also be seen in the Qualified Presumption of Safety (QPS) list given by European Food Safety Authority (EFSA, 2018)[49] for intentional addition into the food and feed chain. *Bacillus coagulans*, being a spore former, has stability against osmolarity, temperature, and desiccation, and the ability of

resistance against gastric and intestinal juices.[50,51] Further, the endospores of *Bacillus coagulans* possess the ability of germinating in the host intestine and can alleviate the symptoms of constipation associated with FGIDs upon stimulation and establishment.[23,52] In addition, the clinical efficacy of *Bacillus coagulans* LBSC has also been proven for the treatment of irritable bowel syndrome,[27] acute diarrhea and abdominal discomfort,[17] and in modulation of human gut microbiome.[53]

Herein, the subjects were randomly distributed in 2 arms viz. Placebo group, and the atorvastatin, atenolol, metformin, amitriptyline, calcium ICAWFGID in test arm, for the current clinical investigation. The values of vital indicators along with levels of both, haematological and biochemical indicators, were within the normal defined domain and no significant differences were seen between baseline and final visit of placebo and test arm. This clinically significant findings potentially illustrates the safety of *Bacillus coagulans* LBSC upon oral consumption. In addition, at the defined dose there were no reports on adverse events (AEs) or serious adverse events (SAEs) during the entire study period, and thus signifies the safety of investigational product for therapeutic use. The safety and efficacy of another *Bacillus coagulans* Unique IS-2[16] and *B coagulans* SNZ 1969[43] were previously investigated in functional constipation by administering a dose of 2×10^9 spores/day and 2×10^9 CFU/day. In addition to the wide acumen of application of *Bacillus coagulans* in treatment of numerous disorders, the promising effects in modulation of gut microbiome, metabolism and immune response has been covered in a review article by Mu and Cong.[24] Further, the findings of various clinical studies have signified the therapeutic dose of *Bacillus coagulans* to be in the range of 0.1×10^9 CFU/day to 20×10^9 CFU/day.[54]

To the best of our knowledge, this is a first scientific report illustrating the therapeutic safety and efficacy of *Bacillus coagulans* LBSC on the drug induced constipation associated with FGIDs. Overall, we found that the probiotic supplement could positively reduce the symptoms of drug induced constipation associated with FGIDs. Current data suggests the oral administration of *Bacillus coagulans* LBSC could benefit the patients with constipation induced due to prolonged intake of drugs used to treat/manage chronic disease/s. Hence, regular intake of *Bacillus coagulans* LBSC could confer health benefits to patients and help manage the drug induced constipation, as the medications for the chronic diseases are generally prescribed for a long duration or life-long.

Conclusion

In conclusion, the investigational product containing *Bacillus coagulans* LBSC with a total dose of 6×10^9 spores/day was well tolerated by the population under study and found to be safe. This IP showed excellent compliance and considerable enhancement in the treatment and management of drug induced constipation associated with FGIDs. There was a significant improvement in symptoms of primary end point (occurrence of constipation) (*P*-value <0.05), and some co-primary endpoints of constipation induced by individual drug. On the other hand, the symptoms of occurrence of hard stool, constipation scoring system, patient health questionnaire on a 15 item somatic symptom severity scale, along with the FGIDs displayed positive variations but the changes were statistically insignificant, over the placebo arm. Zero reports of adverse events and serious adverse events, no use of rescue and/or emergency medicine and/or treatment further established the safety and effectiveness of *Bacillus coagulans* LBSC. Current data suggests the oral administration of *Bacillus coagulans* LBSC, could benefit the patients with constipation induced due to prolonged intake of some drugs used to treat/manage chronic disease/s. The use of probiotics could be a novel, safe and alternate strategy for relieving the symptoms of drug induced constipation associated with FGIDs. Even the findings of the clinical investigation signified the *Bacillus coagulans* LBSC to be a novel and alternate strategy for management of drug induced constipation associated with FGIDs, there is need of further prospective, double blind, larger scale interventional trials with prolonged follow-up periods to establish the therapeutic potential. In addition, the studies with placebo arm of individual drugs must be performed to warrant the detailed therapeutic efficacy, and also establish the underlying mechanism.

Author's Contributions

Conceptualization, methodology: AR, and RP; Data curation, formal analysis: AR; Visualization: RP; Writing – original draft: AR; Writing – review & editing: AR and RP.

Consent for Publication

Informed written consent was obtained from all participants. No vulnerable subject participated in the study.

Clinical Trial Registration

The clinical trial registry of India CTRI/2021/08/035889 [Registered on: 24.08.2021].

ORCID iD

Ankit Rathi ⓘ https://orcid.org/0000-0002-7173-5587

Data Availability Statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Supplemental Material

Supplemental material for this article is available online.

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