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ORIGINAL ARTICLE

A prospective, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the efficacy and safety of SNZ TriBac, a three-strain *Bacillus* probiotic blend for undiagnosed gastrointestinal discomfort

Raunak Jay Soman[1] · Malisetty Venkat Swamy[1]

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Abstract

Purpose This prospective, randomized, double-blind, placebo-controlled, parallel-group study aimed to determine the efficacy and safety of a multistrain (*Bacillus coagulans* [SNZ 1969], *Bacillus clausii* [SNZ 1971], and *Bacillus subtilis* [SNZ 1972]) probiotic blend (SNZ TriBac) in managing symptoms of gastrointestinal (GI) discomfort in the absence of specific pathologies.

Methods Sixty adults with symptoms of GI discomfort were enrolled (mean age, 34.89 ± 9.95 years) and randomized to receive either SNZ TriBac or placebo. Changes from baseline in Severity of Dyspepsia Assessment (SODA), Gastrointestinal Symptom Rating Scale (GSRS), and Quality of Life (QoL) scales over the course of product use were determined at baseline and on days 30 and 37 as study outcomes.

Results On day 30, significant improvement with SNZ TriBac was noted in SODA burping/belching ($P = 0.025$), bloating ($P = 0.048$), sour taste ($P = 0.025$), and total ($P = 0.007$) scores as well as pain ($P = 0.003$), non-pain ($P = 0.04$), and satisfaction ($P = 0.03$) subscores. Significant improvement with SNZ TriBac was also observed in SODA burping/belching ($P = 0.011$), sour taste ($P = 0.011$), and total SODA scores ($P < 0.001$), and in SODA pain ($P = 0.005$), non-pain ($P = 0.06$), and satisfaction (P = 0.004) subscores on day 37. No adverse events were reported.

Conclusion Significant improvement in final SODA scores and subscores with SNZ TriBac versus placebo indicates improvement in several symptoms of gastrointestinal discomfort. This multistrain probiotic blend was well tolerated and could be an effective option for treatment of GI discomfort.

Trial registration Clinical Trials Registry of India (CTRI/2018/05/014071)

Keywords Gastrointestinal discomfort · Probiotic · *Bacillus coagulans* · *Bacillus clausii* · *Bacillus subtilis*

Introduction

There is growing evidence that digestive dysfunction is associated with lifestyle, including diet, lack of physical exercise, smoking, consumption of alcohol and tobacco, and other occupational and behavioral stresses [1]. The human gut microbiome comprises of microorganisms including bacteria, archaea, fungi, and viruses throughout the gastrointestinal (GI) tract [2]. The coordinated actions of these microbes play a key role in host metabolism and immunity by promotion of host cell differentiation, protection of the host from colonization of pathogens, and stimulation and modulation of the immune system [3]. The composition of the gut microbiome continues to alter in response to external factors, such as, environmental exposures, including diet, weaning, and breastfeeding status, successive introduction of different varieties of food, and antibiotic use, as well as, pH, oxygen levels, availability of nutrients, water activity, and temperature [4–7]. This state of dysbiosis has been seen to be associated with obesity and various GI disorders [8, 9].

Several studies have reported an association between lifestyle and GI discomfort [10, 11]. Approximately 63% of the GI symptoms were associated with meal type in a study by

✉ Raunak Jay Soman
Info@sanzymebiologics.com

Malisetty Venkat Swamy
mvswamy@sanzymebiologics.com

[1] Sanzyme Biologics Pvt. Ltd., Plot no. 13, Sagar Society, Road No. 2, Banjara Hills, Hyderabad 500034, India

Simren et al. 2001 [10]. In a large survey conducted in Japan, compared with control, few subjects with GI symptoms reported doing regular exercise and eating meals regularly, getting sufficient sleep, and vegetable consumption—not feeling stress in routine life [11]. The fast pace of modern life has particularly exacerbated these symptoms by causing a significant alteration of the composition of gut microbiota. Thus, healthy individuals with normal body mass index (BMI) have also been seen to experience symptoms of GI discomfort. While a diet rich in fat and spices are thought to contribute to discomfort [12]; supplementation with probiotics has been reported to alleviate GI discomfort by causing an alteration of the composition of the gut microbiota [13]. Hence, treatment with probiotics, when administered in adequate amounts, could help normalize the composition of gut microbiota, and in turn, alleviate the symptoms of GI discomfort.

The World Health Organization (WHO) defines probiotics as live microorganisms that are beneficial to the host when administered in adequate amounts [12]. The *Bacillus* species has been used as probiotics since several years [14]. These are spore-forming bacteria that are highly stable in extremely acidic environments and at high temperatures. Hence, they are preferred over non-spore-forming bacteria such as the *Lactobacillus* species in probiotic blend. Among the *Bacillus* species, *Bacillus coagulans* is a lactic acid–producing bacterium with characteristics of both *Lactobacillus* and *Bacillus* genera [14]. *Bacillus coagulans* SNZ 1969 spore preparation has been extensively studied, and it has been Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FDCA) [14]. *Bacillus subtilis* is considered an ideal probiotic because of its ability to produce B-vitamins and its highly enzymatic nature. It has been used to treat stomach discomfort, bloating, flatulence, indigestion, nausea, and irregular bowel movements [14]. *Bacillus clausii* helps replenish gut microbiota during antibiotic treatment due to its multi-antibiotic resistance, and is therefore, also used to treat chronic diarrhea [14]. Both *Bacillus subtilis* (SNZ 1972) and *Bacillus clausii* (SNZ 1971) also are GRAS certified.

Sanzyme Biologics has thus developed a multistrain probiotic blend, SNZ TriBac, which contains not less than 2 billion colony-forming units (CFUs) of *Bacillus coagulans* (SNZ 1969), *Bacillus clausii* (SNZ 1971), and *Bacillus subtilis* (SNZ 1972). Hence, this blend contains three strains of microorganisms that have been demonstrated to alleviate symptoms of GI discomfort and re-establish the balance of the composition of the gut microbiota, which has been shown to be useful for the treatment of GI dysfunction and indigestion-related symptoms [15]. The present study intended to evaluate the efficacy and safety of this multistrain probiotic blend, SNZ TriBac, in comparison with placebo in subjects reporting abdominal distress symptoms in the absence of specific pathologies.

Methods

Study design

This prospective, double-blind, placebo-controlled, parallel-group, comparative study was conducted at a single center. Before participation in the study, all subjects provided written informed consent. This study was performed in compliance with International Conference on Harmonisation (ICH E6[R2]) "Guidance on Good Clinical Practice," Declaration of Helsinki; Indian Good Clinical Practices Guideline; National Ethical Guidelines for Biomedical and Health Research involving human participants, Indian Council of Medical Research 2017; and, relevant standard operating protocols of Jehangir Clinical Development Centre, Pune, Maharashtra, India. This study was approved by the institutional review board of Jehangir Clinical Development Centre, Pune, Maharashtra, India, and was registered in the Clinical Trials Registry of India (CTRI/2018/05/014071).

Subjects ($N = 60$) were randomized 1:1 into intervention or placebo groups. Both study investigators and subjects were blinded to product assignment. Subjects were observed at five visits over the course of 5 weeks: a screening visit at − 7 days (1 week before) study initiation, a randomization visit on day 0, two treatment visits on days 15 and 30, and a follow-up visit on day 37 (Fig. 1). The study was conducted between July 2018 and November 2018. At screening, all subjects were evaluated for demographics, medical history, prior medication, physical examination, vital signs, height, body weight, clinical laboratory parameters, symptoms, severity, and pain intensity of GI discomfort, and adverse events. Compliance with treatment was assessed on the basis of number of units per container used by the subject and by review of the subject diary.

Subject population

All subjects were of either gender between 18 and 60 years of age (both inclusive) and had been attending the outpatient department with complaints of abdominal distress symptoms such as gas, pain, and abdominal distension (pain score and discomfort score ≥ 1 per Gastrointestinal Symptom Rating Scale (GSRS) and Severity of Dyspepsia Assessment (SODA) scale). All subjects were healthy, as determined by physical examination, medical history, and hematological and hepatic function tests and were willing to provide informed consent and comply with the protocol and scheduled visits. In addition, all subjects agreed to maintain dietary and exercise habits and avoided intake of probiotics, fiber supplements, and unpasteurized bacterial fermented food, including yogurt and cheese, for the entire duration of the study.

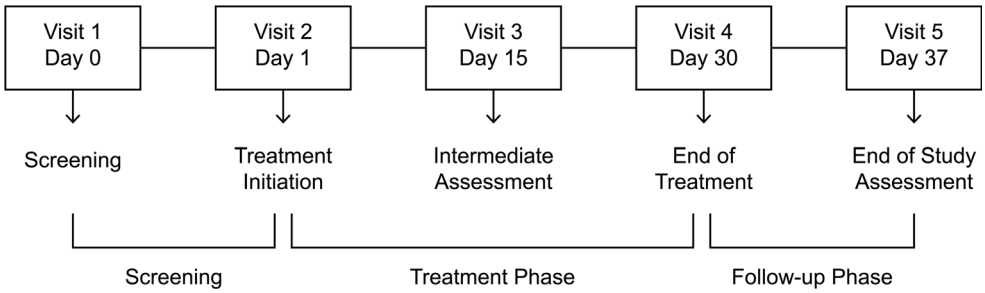

Fig. 1 Study design

Exclusion criteria for this study included history or diagnosis with specific pathologies such as short gut syndrome, food intolerance, inborn errors of metabolism, Crohn's disease, short bowel, ulcerative colitis, irritable bowel syndrome (IBS), constipation, or lactose intolerance, and use of GI medications for control of gut function such as antispasmodic, antimotility, and prokinetic agents or laxatives. Moreover, subjects suffering from any other disorder that was likely to be the cause of abdominal symptoms or likely to interfere with their participation in the study, including psychological disorders or use of drugs/alcohol that could interfere with compliance with study requirements, were excluded. Furthermore, subjects with known hypersensitivity to any of the components of the investigational study treatments, those on supplements/antibiotic treatment for GI symptoms in the past 4 weeks that could interfere with natural gut microbiota, concomitant heart disease, uncontrolled high blood pressure, renal or hepatic impairment, type I/II diabetes, immune disorders, unstable thyroid disease, Parkinson's disease, history of cancer, previous stomach/intestinal surgery, and any concomitant treatment were excluded. Subjects participating in another investigational study within 30 days of this study and pregnant and breastfeeding women were also excluded.

Intervention

The active product tested was the probiotic supplement SNZ TriBac. Each capsule of SNZ TriBac contained not less than 2 billion CFUs of *Bacillus coagulans* (SNZ 1969), *Bacillus clausii* (SNZ 1971), and *Bacillus subtilis* (SNZ 1972). The placebo was a capsule of calcium carbonate. Both SNZ TriBac and the placebo capsule were identical in color, shape, and size. One capsule of either SNZ TriBac or placebo was self-administered by the subject, once daily, after a major meal, at roughly the same time of the day, for a period of 30 days.

Assessments and endpoints

During the study, several questionnaires, each targeting a different symptom, were completed by the subjects. Assessments included SODA pain intensity, non-pain symptoms, and satisfaction with dyspepsia subscores, and total SODA score. Individual non-pain symptom scores of burping/belching, bloating, heartburn, sour taste, passing gas, nausea, and bad breath were also assessed using the SODA scale [16, 17]. GSRS assessment included GSRS dyspeptic syndrome, indigestion syndrome, and bowel dysfunction syndrome subscores, and total GSRS score [17, 18]. A visual analogue scale (VAS, 100 mm) for assessment of pain intensity and non-pain symptoms, and quality of life (QoL, QoLSF-36 v2) scores were also determined. Endpoints were defined as change in SODA and GSRS scores from baseline to day 30. For assessment of safety and adverse events, specific laboratory and clinical tests were performed as appropriate.

Statistical methods

Data were presented as mean ± standard deviation (SD) for continuous data and number (%) for categorical data. Student's unpaired *t* test was applied to compare continuous data. All statistical tests were two-tailed. Level of significance (α) was set at $P \leq 0.05$. Data were analyzed using SPSS v.15.0 (IBM Corp., NY, USA).

For determination of sample size, it was observed that 25 analyzable subjects per treatment arm would provide 80% power to achieve a significant result for a 0.8-sigma effect size [18–20]. A 15% drop-out rate was considered possible based on experience at the chosen site, and hence, a total of 30 subjects were recruited in each group.

Results

Of the total 60 subjects that were randomized to each group, two subjects from the placebo group were lost to follow-up (Fig. 2). Detailed baseline and demographic characteristics of the study subjects are described in Table 1. The mean height was 150.08 ± 9.74 cm and the mean weight was 63.38 ± 14.15 kg. The mean age of SNZ TriBac and placebo groups was 39.17 ± 9.11 and 34.89 ± 9.95 years, respectively. At baseline, no significant difference was noted for all variables between both groups.

Between visit 4 and visit 1 (the primary endpoint of this study), i.e., at the end of 30 days, analysis of SODA scale readings showed significant improvement in total SODA

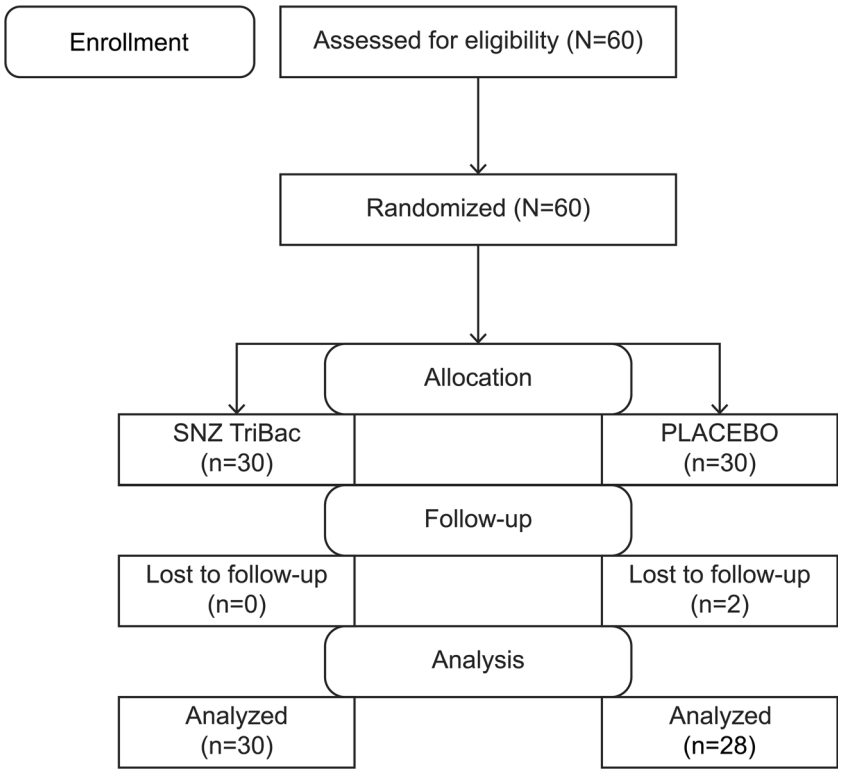

Fig. 2 Patient disposition

scores ($P = 0.007$), and SODA pain ($P = 0.003$) and non-pain ($P = 0.04$) subscores with SNZ TriBac. In addition, a significant increase in SODA satisfaction subscore ($P = 0.03$) was observed between visit 4 and visit 1 with SNZ TriBac. A significant improvement was also observed in SODA burping/belching ($P = 0.025$), bloating ($P = 0.048$), and sour taste scores ($P = 0.025$) with SNZ TriBac (Table 2).

Between visit 5 (7 days after end of the treatment) and visit 1, i.e., at the end of 37 days, significant improvement was observed in total SODA scores ($P < 0.001$), as well as, in SODA pain ($P = 0.005$) and non-pain ($P = 0.06$) subscores with SNZ TriBac. Additionally, significant increase in SODA satisfaction ($P = 0.004$) subscores was noted with SNZ TriBac versus placebo. Furthermore, significant improvement was also noted in parameters such as burping/belching ($P = 0.011$) and sour taste ($P = 0.011$) with SNZ TriBac as compared with placebo, per SODA scale (Table 3).

No significant differences were noted between SNZ TriBac and placebo groups for all tested parameters per GSRS on day 30 and day 37 which could be indicative of a strong placebo effect (Table 4). Furthermore, patients treated with SNZ TriBac experienced the symptoms as per SODA scale less frequently compared with placebo on day 30 (Fig. 3).

No significant differences were observed in VAS and QoL scores between SNZ TriBac and placebo groups at the end of day 30 and day 37. No adverse events were reported in any subject during the entire study period. Hence, SNZ TriBac is safe for human consumption.

Discussion

Several researchers across the world have investigated the clinical efficacy of probiotics for the treatment of GI discomfort [15, 17]. At the end of this study, significant improvement in SODA burping/belching, bloating, and sour taste scores, SODA pain and non-pain subscores, and total SODA scores indicates amelioration in these symptoms of GI discomfort following administration of the multistrain SNZ TriBac blend. In addition, significant improvement in SODA satisfaction subscores with SNZ TriBac versus placebo indicates an improvement in the subject's ability to deal with the symptoms of GI discomfort and a greater relief from these symptoms after consumption of this probiotic blend. Hence, multistrain SNZ TriBac can be useful in the treatment of GI discomfort.

Healthy gut microbiota is crucial for overall health and well-being of an individual as it plays an important role in nutrient metabolism, xenobiotic and drug metabolism, immune protection, immunomodulation, and maintenance of the integrity of the gut barrier and structure of the GI tract [21]. Any alteration in the gut microbiota is associated with several GI disorders including GI discomfort, gastroesophageal reflux disease (GERD), and irritable bowel syndrome (IBS) [22]. Probiotics are live microorganisms which when administered in adequate amounts provide health benefits by improving or restoring the composition of gut microbiota through various mechanisms, including inhibition of growth of pathogenic microorganisms and

Table 1 Baseline and demographic characteristics

Variable/parameter	SNZ TriBac (n = 30)	Placebo (n = 28)	P value
Age (years)	39.17 ± 9.11	34.89 ± 9.95	0.06
Gender			
Female	22 (73.3)	16 (57.1)	0.5
Male	8 (26.7)	12 (42.9)	
Ethnicity			
Non-Hispanic	30 (100.0)	28 (100.0)	NA
Race			
Asian	30 (100.0)	28 (100.0)	NA
Height (cm)	156.60 ± 9.83	150.08 ± 9.74	0.6
Weight (kg)	61.19 ± 15.51	63.38 ± 14.15	0.7
BMI (kg/m^2)	25.08 ± 6.26	24.97 ± 4.88	0.8
Smoking status			
Never smoked	30 (100.0)	28 (100.0)	NA
Alcohol status			
Never drank	30 (100.0)	28 (100.0)	NA
Diet			
Vegetarian	7 (23.3)	4 (14.3)	0.2
Non-vegetarian	23 (76.7)	24 (85.7)	
Pulse			
Normal	30 (100.0)	28 (100.0)	NA
Respiratory rate			
Normal	30 (100.0)	28 (100.0)	NA
SBP			
Normal	30 (100.0)	28 (100.0)	NA
DBP			
Normal	30 (100.0)	28 (100.0)	NA

Data: Mean ± SD or number (%)

BMI body mass index, *DBP* diastolic blood pressure, *NA* not applicable, *SBP* systolic blood pressure

restoration of intestinal permeability [23]. In a systematic review that evaluated the safety of probiotics containing *Lactobacillus* and *Bacillus* strains in reducing the risk, preventing, or treating GI disorders, no significant increase in the risk of adverse events was observed in children, adults, and older patients [24]. Hence, several clinical practice guidelines have endorsed the use of probiotics for inducing or maintaining remission in acute infectious diarrhea, antibiotic-associated diarrhea, *Clostridium difficile*–associated diarrhea, hepatic encephalopathy, ulcerative colitis, IBS, functional GI disorders, and necrotizing enterocolitis [25].

There is evidence that certain probiotics such as *Bacillus*-based probiotics may be capable of significantly reducing abdominal pain, abdominal distension, and flatulence in subjects with functional GI disorders [15, 17]. Based on the findings of our study, the multistrain *Bacillus*-based probiotic blend used was clearly more effective than placebo in

Table 2 Mean change from baseline per SODA scale on day 30

Characteristics	SNZ TriBac (n = 30)	Placebo (n = 28)	P value
Symptom scores			
Burping/belching	1.57 ± 0.82	1.04 ± 0.96	0.025
Heartburn	1.37 ± 0.89	0.96 ± 1.20	0.15
Bloating	1.30 ± 0.99	0.82 ± 0.86	0.048
Passing gas	0.93 ± 0.98	0.86 ± 0.80	0.7
Sour taste	1.37 ± 1.13	0.75 ± 0.89	0.025
Nausea	0.90 ± 0.92	0.92 ± 0.98	0.9
Bad breath	0.53 ± 0.86	0.36 ± 0.62	0.4
Subscores			
SODA pain score	13.47 ± 3.50	10.71 ± 3.68	0.003
SODA non-pain score	6.60 ± 3.16	4.89 ± 2.94	0.04
SODA satisfaction score	−4.43 ± 1.70	−3.00 ± 1.22	0.03
SODA total score	15.73 ± 3.71	12.60 ± 4.79	0.007

Data: Mean ± SD

SODA Severity of Dyspepsia Assessment

ameliorating symptoms of GI discomfort. Moreover, on comparing SNZ TriBac with placebo, reduction in burping/belching, bloating, and sour taste can be attributed to the use of multiple strains in SNZ TriBac, since each of the three strains of *Bacillus* used in this blend have demonstrated similar effects in other studies [17]. A significant reduction in

Table 3 Mean change from baseline per SODA scale on day 37

Characteristics	SNZ TriBac (n = 30)	Placebo (n = 28)	P value
Symptom scores			
Burping/belching	0.53 ± 0.68	−0.04 ± 0.96	0.011
Heartburn	1.37 ± 0.85	1.07 ± 1.12	0.3
Bloating	1.20 ± 1.06	0.82 ± 0.98	0.2
Passing gas	0.97 ± 1.03	0.86 ± 0.93	0.7
Sour taste	1.37 ± 1.03	0.71 ± 0.90	0.011
Nausea	0.90 ± 1.06	0.86 ± 0.97	0.9
Bad breath	0.57 ± 0.82	0.36 ± 0.62	0.3
Subscores			
SODA pain score	13.23 ± 2.91	10.43 ± 3.97	0.005
SODA non-pain score	6.53 ± 3.04	5.00 ± 3.01	0.06
SODA satisfaction score	−4.10 ± 1.56	−3.25 ± 2.37	0.004
SODA total score	19.31 ± 4.11	12.18 ± 4.61	< 0.001

Data: Mean ± SD

SODA Severity of Dyspepsia Assessment

Table 4 Mean change from baseline per GSRS subscore on day 30

Characteristics	SNZ TriBac (n = 30)	Placebo (n = 28)	P value
Subscores			
Dyspeptic syndrome subscore	3.33 ± 2.51	3.21 ± 2.47	0.9
Indigestion syndrome subscore	2.23 ± 1.89	2.32 ± 2.07	0.9
Bowel dysfunction syndrome subscore	1.60 ± 2.58	1.64 ± 2.39	0.9
GSRS total score	1.60 ± 2.58	7.18 ± 4.98	0.9

Data: Mean ± SD

GSRS Gastrointestinal Symptom Rating Scale

certain symptoms of GI discomfort with SNZ TriBac could be attributed to improved permeability.

When evaluating the effects of probiotics against indigestion or dyspepsia, patient-reported outcomes are important because of the absence of suitable biomarkers for functional GI disorders. Hence, the sensitivity of subjects to all gastric symptoms combined has been used as an endpoint to understand the effect of treatment with probiotics. Accordingly, in our study, the widely used, validated, and well established GSRS and SODA scales were used to assess symptoms of functional GI disorders [16, 26]. According to a double-blind randomized controlled trial to evaluate effects of a *Bacillus coagulans*–based product on functional intestinal gas symptoms, a lack of significance for certain efficacy endpoints could be attributed to either the placebo effect or a considerable amount of random variability in the efficacy endpoints [18]. Hence, in our study, non-significant differences between probiotic and placebo groups in GSRS and certain parameters of the SODA scale could also be due to the placebo effect.

An interesting observation in the present study was the persistent improvement in the SODA scores even seven days post treatment cessation, which suggests a beneficial effect even after discontinuation of treatment. These potentially long-lasting benefits of this multistrain probiotic blend make

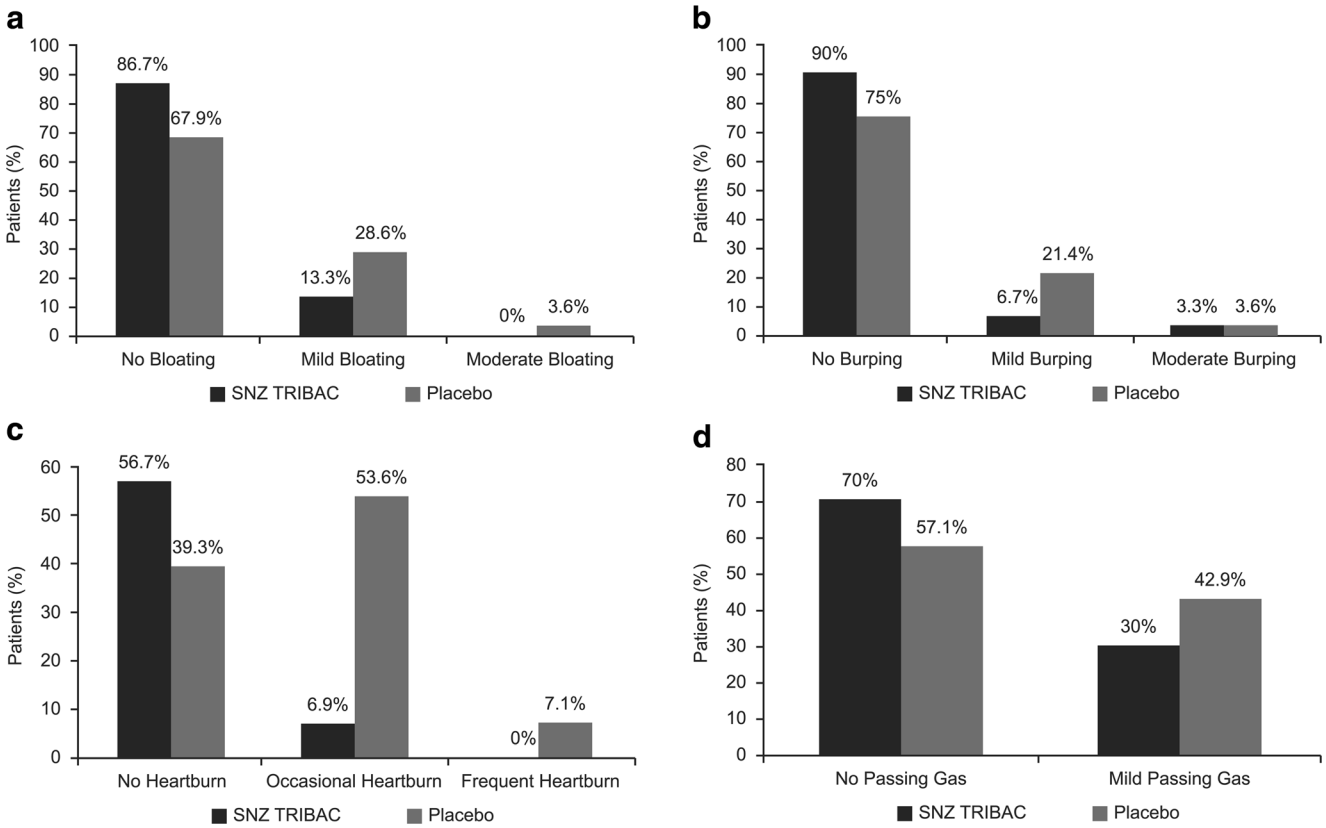

Fig. 3 Percentage of patients experiencing symptoms per SODA subscores on day 30. **a** SODA: bloating. **b** SODA: burping/belching. **c** SODA: heartburn. **d** SODA: passing gas

it clinically beneficial, particularly in patients with recurrent symptoms of GI discomfort. Furthermore, as no adverse events were observed in any subject after consumption of SNZ TriBac, this multistrain probiotic blend is very safe for human consumption.

A high placebo response has been commonly observed in the trials involving patients reported outcomes including GI symptoms [18]. Accordingly, additional research is warranted to determine the effects of this multistrain probiotic blend in a randomized placebo-controlled study involving a larger sample of subjects with functional GI disorders. Nevertheless, a potential benefit of this multistrain probiotic formulation could lie in its use for the treatment of several functional GI disorders, including GERD and IBS.

Conclusion

The multistrain probiotic blend SNZ TriBac containing *Bacillus coagulans* SNZ 1969, *Bacillus clausii* SNZ 1971, and *Bacillus subtilis* SNZ 1972 provided greater relief from certain symptoms associated with GI discomfort including burping/belching, bloating, and sour taste in comparison with placebo. Moreover, relief from burping/belching and sour taste lasted for one week after the last dose. Patients with indigestion/dyspepsia felt more satisfied with SNZ TriBac–induced reduction in associated symptoms. No adverse event was observed during the entire study period in any study group. This multistrain probiotic blend was well tolerated and could be an effective supplement for treatment of undiagnosed GI discomfort.

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Authors' contributions All authors made substantial contributions to study conception, acquisition of data, and preparation of this manuscript. All authors read and approved the final version of this manuscript.

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Compliance with ethical standards

Before participation in the study, all subjects provided written informed consent. This study was performed in compliance with International Conference on Harmonisation (ICH E6[R2]) "Guidance on Good Clinical Practice," Declaration of Helsinki; Indian Good Clinical Practices Guideline; National Ethical Guidelines for Biomedical and Health Research involving human participants, Indian Council of Medical Research 2017; and, relevant standard operating protocols of Jehangir Clinical Development Centre, Pune, Maharashtra, India. This study was approved by the institutional review board of Jehangir Clinical Development Centre, Pune, Maharashtra, India, and was registered in the Clinical Trials Registry of India (CTRI/2018/05/014071).

Conflict of interest R J Soman and M V Swamy are employees of Sanzyme Biologics Pvt Ltd. The authors have no other conflict of interest to declare with respect to this authored publication.

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