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Impact of a synbiotic formulation on intestinal symptoms and quality of life in patients with irritable bowel syndrome

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Abstract

In a real-world setting, this study assessed the efficacy of a nutraceutical containing microencapsulated probiotics (*Lactobacillus plantarum* LP01, *Bifidobacterium breve* BR03), fructooligosaccharides, tryptophan, vitamins B3 and B6 in reducing symptoms and improving quality of life in patients suffering from any of the four irritable bowel syndrome subtypes. In this study, 121 patients received one stick pack of the nutraceutical twice daily for 60 consecutive days. Key outcome measures included the IBS-Severity Scoring System (IBS-SSS) and IBS-Quality of Life (IBS-QoL), administered at baseline (T0), 30 days (T1), and 60 days (T2). The IBS-SSS total score decreased significantly at T1 and T2 compared to T0 ($P<0.0001$), reflecting improved symptom management. By T2, 83.5% (101/121) of patients showed a reduction of at least 50 points, with a progressive shift towards disease remission and reduction in moderate-to-severe cases. Simultaneously, the IBS-QoL total score improved significantly at T1 and T2 ($P<0.0001$), with 67.8% (82/121) of patients showing clinically relevant improvement (an increase of 14 points or more). Improvements at T1 and T2 were consistent across IBS-SSS items and IBS-QoL subscales, regardless of the IBS subtype. During the treatment period, no patients reported adverse events related to the nutraceutical intake. These findings demonstrate that the tested nutraceutical, leveraging the synergistic action of its ingredients on the gut-liver-brain axis, is safe, well-tolerated, and effective for treating symptoms and enhancing the quality of life across all IBS subtypes.

Introduction

Irritable Bowel Syndrome (IBS) is a functional disorder characterized by abdominal pain or discomfort accompanied by changes in bowel habits in the absence of organic lesions. It is the most common chronic visceral pain syndrome.

IBS is defined using diagnostic criteria derived by consensus from an international panel of experts. These criteria are symptom-based and are known as the Rome IV criteria [1].

Based on the predominant alterations in bowel habits, four different IBS subtypes can be recognized: constipation predominant (IBS-C), diarrhea predominant (IBS-D), mixed (IBS-M), and undefined (IBS-U) [2]. IBS affects 3-5% of the population in Western countries [3]. A reliable estimate of the prevalence of IBS is difficult to obtain as there are no objective biomarkers for this condition. Its prevalence varies across different geographic regions due to variations in the interpretation and reporting of symptoms [4]. A recent cross-sectional survey promoted by the Rome Foundation reported that, using the Rome IV criteria, IBS prevalence rates range between 1.3% and 7.6%, with an overall prevalence of 4.1% [3].

Continuous research and the updating of the Rome criteria have changed the interpretation of the pathophysiological mechanisms of IBS over time, leading to the current approach based on gut-brain interaction [5]. IBS causes changes in intestinal function and abdominal pain and tends to overlap with other functional gastrointestinal diseases. It mainly affects young and female individuals, can seriously compromise the patient's quality of life and work activity, and has a considerable economic burden. According to a broad and comprehensive view, IBS can be defined as a chronic functional disorder of the gut-liver-brain interaction [6]. The gut and liver are recognized to mutually communicate through the biliary tract, portal vein, and systemic circulation. Intestinal products, such as microbial and host metabolites and microbe-associated molecular patterns, are transported to the liver via the portal vein and influence liver function. At the same time, the liver transports bile salts and antimicrobial molecules into the intestinal lumen via the bile ducts and thereby helps maintain intestinal eubiosis by modulating bacterial growth [7]. In general, increased intestinal permeability and consequent bacterial translocation can allow microbial metabolites to reach the liver, leading to inflammation, impaired bile acid metabolism, and intestinal dysmotility. These conditions promote intestinal dysbiosis, which subsequently exacerbates liver injury [8]. Alterations in fecal bacterial flora are described by changes in the composition of the dominant phyla producing Short-Chain Fatty Acids (SCFA), which represent an important energy source for intestinal epithelial cells and contribute to the regulation of secondary bile acid metabolism, immune processes and IgA production [8]. Therefore, intestinal dysbiosis can lead to metabolic disturbances in the liver, which in turn lead to liver damage. The impaired liver cannot effectively inhibit bacterial proliferation and remove harmful microbial byproducts, which results in accelerated disease progression. Furthermore, liver damage has been reported to be closely related to the severity of intestinal dysbiosis [9]. Ultimately, while the gut-brain axis plays an important role in the onset and development of IBS, the role of the liver cannot be ignored. In support of this, several studies demonstrate that IBS is associated with NAFLD [10,11].

With an overall prevalence of 37.6%, sleep disturbances are more common in IBS patients than in healthy subjects [12]. Poor sleep quality is related to IBS symptoms, and adult individuals with IBS commonly report difficulty falling asleep, shorter sleep times, frequent awakenings, or non-restorative sleep [13-17]. In a population-based study involving 2269 participants, the prevalence of IBS was found to be 33% among participants with sleep disturbances [18]. The risk of having IBS was 1.6 times greater in people with sleep disturbances compared to those without sleep disturbances, even after adjusting for age, sex, and somatization scores [18]. Several researchers have collected evidence of a positive association between poorer subjective sleep quality and increased frequency and severity of IBS symptoms [13,15,16]. Evidence has also been accumulating that sleep disturbances are associated with increased visceral sensitivity [19,20] and it has been proposed that sleep disturbances and gastrointestinal symptoms are part of a vicious cycle where poor sleep quality leads to hyperalgesia and hyperalgesia results in poor sleep quality [21]. Finally, one study found that IBS patients slept more hours per day but felt less rested than healthy controls [22]. The study found that IBS patients had sleep interrupted by more wakefulness episodes and this was associated with more abdominal pain and gastrointestinal disturbances that were negatively correlated with quality of life [22].

In conclusion, IBS causes significant suffering, has a significant impact on the quality of life of affected patients, represents a considerable burden on healthcare systems [23], and, to date, both the optimal diagnostic process and its most appropriate management remain uncertain.

The present study fits within this context. Sindrocol® (SND) is a nutraceutical, approved by the Italian Ministry of Health and marketed in Italy for several years, containing ingredients to which the European Food Safety Authority (EFSA) and the Italian Ministry of Health attribute health claims related to the maintenance of proper intestinal function. The physicians who participated in this study are accustomed to using SND in their daily clinical practice and often recommend SND to patients affected by IBS for a defined period. They also systematically employ questionnaires and scales to assess the efficacy of nutraceutical administration and better understand patient benefits. These favorable conditions facilitated the study.

Materials and methods

Tested nutraceutical

Sindrocol® (SND) is a nutraceutical listed in the Italian Ministry of Health's National Register of Food Supplements under code 194129. SND is manufactured by MèDISIN S.r.l. (Lissone, MB, Italy) and was released for sale in Italy on May 1, 2020. The qualitative and quantitative composition of SND is shown in Table 1. The probiotic bacteria are microencapsulated. Microencapsulation stabilizes the bacterial cells and increases their survival during passage through the gastroduodenal tract. In accordance with current Italian regulations, the following permitted health claims for nutraceutical ingredients are listed on the SND packaging: probiotic ferments promote a balanced intestinal flora, niacin contributes to the maintenance of normal mucous membranes, and vitamin B6 contributes to the normal function of the immune system.

Table 1: Qualitative and quantitative composition of Sindrocol®.

Component	Dose in two stick packs
Bifidobacterium breve BR03 (DSM16604)	$2 \cdot 10^9$ UFC
Lactobacillus plantarum LP01 (LMG P-21021)	$2 \cdot 10^9$ UFC
Fructooligosaccharides(FOS)	400 mg
L-tryptophan	200 mg
Vitamin B3 (Niacin)	16 mg
Vitamin B6 (Pyridoxine)	2,8 mg

Patients included in the study were given two stick packs per day.

Study population

To be enrolled, patients had to be men and women and between 18 and 65 years of age. Patients had to have been diagnosed with IBS using the Rome IV criteria: recurrent abdominal pain for at least one day per week in the past three months and two or more bowel-related symptoms associated with altered bowel frequency and altered stool appearance and consistency. The onset of symptoms had to be at least six months prior to diagnosis. Furthermore, patients had to have a score greater than 80 on the Irritable Bowel Syndrome - Severity Scoring System (IBS-SSS) questionnaire. Eligible participants included patients with IBS-D, IBS-C, IBS-M, or IBS-U who underwent prior specialist gastroenterological evaluation to confirm the diagnosis and rule out other gastrointestinal diseases. Finally, patients, after being informed, had to understand, accept, and sign the consent form for the trial and for the management of their personal data.

Patients were excluded if they had experienced a change in their IBS treatment regimen within the last four weeks, were taking products with a composition or action similar to SND, probiotics, antibiotics, anticholinergic drugs for painful intestinal spasms, antidepressants, and anxiolytic drugs. Furthermore, patients with a diagnosis of chronic inflammatory bowel disease, celiac disease, severe constipation not attributable to IBS, or other digestive conditions that could interfere with the study, as judged by the investigator, were excluded. Patients with signs and symptoms suggestive of depression or other psychiatric conditions, documented recent weight loss not attributable to dietary changes, bloody stools, anemia, or who had been treated with antibiotics within the four weeks prior to the study were also excluded. Finally, pregnant or breastfeeding women were excluded, as were patients with alcoholism, mental incapacity, language barriers hindering data collection, or a known allergy or hypersensitivity to any component of SND.

Study design

This is a prospective, open-label, single-arm study to assess the contribution of the nutraceutical SND to reducing symptoms and improving quality of life and sleep in patients with mild, moderate, and severe IBS-D, IBS-C, IBS-M, and IBS-U. The study was conducted within the context of the authors' routine clinical practice. Gastroenterology specialists and general practitioners located throughout Italy participated in the study. Outcome measures were collected via questionnaires, and adverse events were recorded on each patient's record.

Patients included in the study took one stick pack of SND twice daily for 60 consecutive days, fasting (at least one hour before or two hours after meals), with the second dose taken before bedtime.

Procedures

Each investigator identified IBS patients who met the inclusion and exclusion criteria and enrolled between 5 and 20 patients. Participants underwent a physical examination, and their demographic and medical history data were recorded. Data were entered into each patient's personal file, which was pseudonymized with an alphanumeric code to ensure anonymity to everyone except the attending investigator. The patient's personal file also included the questionnaires completed at T0, T1, and T2. After data collection, the anonymized forms were processed for statistical analysis and report writing.

To assess IBS symptoms, health-related quality of life, and sleep quality, enrolled patients were administered the Irritable Bowel Syndrome - Severity Scoring System (IBS-SSS), the Irritable Bowel Syndrome - Quality of Life (IBS-QoL), and the Sleep Quality Scale (SQS). These instruments were completed at baseline (T0), after 30 days of SND therapy (T1), and after 60 days of SND therapy (T2).

To ensure the safety of SND, the following procedure was adopted: patients were asked to report any adverse events to their physician via telephone during the treatment. Further verification of any adverse events occurred during the medical visits at T1 and T2. All safety-related information was recorded in the patient's record.

The study was conducted in compliance with the guidelines for Good Clinical Practice [24] and the Declaration of Helsinki [25]. In line with standard clinical practice and regulatory requirements, subjects were informed about the characteristics and purpose of the nutraceutical and signed a formal informed consent form, which included consent for personal data processing.

Outcomes

The primary outcome is the change in the IBS-SSS total score, which indicates a clinically significant improvement in IBS symptoms, from baseline (T0) to the end of the first 30 days of SND use (T1) and from baseline (T0) to the end of the overall 60 days of SND use (T2). According to the researchers who designed and implemented the IBS-SSS, a reduction in the IBS-SSS total score of at least 50 points identifies patients who have experienced a clinically significant improvement in symptoms following the therapy they underwent for IBS treatment [26]. Some researchers indicate that a 95-point reduction in the IBS-SSS total score is associated with a significant clinical improvement in IBS [27]. Therefore, we chose to evaluate the efficacy of SND treatment by considering both a 50-point reduction in the total score and a 95-point reduction in the total score.

Secondary outcomes are represented by changes in the total IBS-QoL and SQS scores. Regarding the interpretation of the results obtained with IBS-QoL, in addition to reaching statistical significance, a clinically significant response is determined by an increase in the total score of at least 14 points [28]. Therefore, we believe that treatment with SND is effective in improving the quality of life of patients affected by IBS if it increases the total score of the IBS-QoL questionnaire by at least 14 points. Regarding the effect of SND on sleep quality, this study has a purely exploratory value. In fact, the criteria for patient enrollment and the scale adopted to investigate the effect of SND on sleep quality allow for only an initial and summary assessment of the possible beneficial action of SND on sleep

quality. This initial approach to the action of SND on sleep quality serves as an exploration for a subsequent, more in-depth investigation, in which patients with sleep disorders who can be more confidently linked to IBS are selected and more refined assessment tools are adopted.

Irritable Bowel Syndrome - Severity Scoring System (IBS-SSS)

IBS symptoms and severity were assessed using the IBS-SSS questionnaire. This questionnaire includes 5 items regarding abdominal pain, number of days with abdominal pain, bloating/distention, satisfaction with bowel habits, and IBS-related quality of life [26]. Questions are answered using a visual analog scale from 0 to 100. The total questionnaire score is obtained by adding the scores of the individual items and ranges from 0 to 500. A total score between 75 and 175 identifies mild IBS, a score between 175 and 300 identifies moderate IBS, and a score above 300 identifies severe IBS. Patients with a score lower than 75 are in remission.

Irritable Bowel Syndrome - Quality of Life (IBS-QoL)

IBS-related quality of life was assessed using the IBS-QoL questionnaire, which consists of 34 items that assess the degree of interference between IBS and the patient's quality of life. Each item is rated on a 5-point Likert scale ranging from 1 to 5 depending on the response: 1 = not at all, 2 = slightly, 3 = moderately, 4 = somewhat, 5 = extremely. The total score ranges from 34 to 170 points. The outcome is assessed using both the total score and the scores for eight subscales. The questionnaire contains eight disease-relevant domains, corresponding to eight subscales: dysphoria, interference with activity, food avoidance, body image, health concerns, social reaction, sexual health, and relationships. To facilitate comparison, the total score and each of the eight subscale scores are transformed into scores ranging from 0 to 100, with a score of 0 indicating the worst quality of life and a score of 100 indicating the best quality of life in relation to IBS [29,30].

Sleep Quality Scale (SQS)

To assess sleep quality, a seven-point scale, previously validated in clinical studies [31,32], was adopted. Participants rated their overall sleep quality over the past two weeks using the following scores: very bad (1), very poor (2), poor (3), sufficient (4), good (5), very good (6), and excellent (7). The scale does not provide detailed information on patients' sleep characteristics. Nevertheless, we opted for a simple scale that provides a general indication of the potential of SND to improve sleep quality in IBS patients. Based on the results obtained in this study, we intend to further investigate this aspect by employing more appropriate tools that allow for a more precise and detailed investigation.

Statistical analysis

Descriptive statistics were used to summarize cohort characteristics, reporting median and Interquartile Range (IQR) or mean and Standard Deviation (SD) for continuous variables, and frequencies for categorical data, as appropriate. Treatment effect was estimated as the change in outcome scores from baseline (T0) to follow-up (T1 and T2) in treated patients. Differences were analyzed using the nonparametric Wilcoxon signed-rank test. Results were considered statistically significant at $P<0.05$. Statistical analysis was performed using GraphPad Prism version 8.0.2 for Windows (GraphPad Software, Boston, Massachusetts USA, www.graphpad.com).

Results

Between April 2023 and October 2024, 128 patients were enrolled. Five subjects were excluded due to incomplete data collection and two patients failed to meet the inclusion criteria. Therefore, 121 patients who met the inclusion and exclusion criteria and adhered to the study protocol requirements throughout the observation period were included in the study. The demographic and medical history data of the included patients are shown in Table 2.

The medications regularly taken by the patients included in the study are shown in Table 3.

Table 2: Demographic and medical history data of patients included in the study.

Age in years (n ± SD)		42.9 ± 16.5
Gender, % F (n)		68.6% (83)
Height in cm (n ± SD)		168.0 ± 9.71
Weight in kg (n ± SD)		67.4 ± 13.1
BMI in kg/m^2 (n ± SD)		23.8 ± 3.59
Smoking, % Yes (n)		20.7% (25)
Cups of coffee per day (n ± SD)		2.03 ± 1.13
Alcohol consumption	No, % (n)	38.0% (46)
	Occasional, % (n)	51.2% (62)
	Moderate, % (n)	10.8% (13)
Physical activity	No, % (n)	38.0% (46)
	Moderate, % (n)	52.1% (63)
	Intense, % (n)	8.3% (10)
	Competitive, % (n)	1.6% (2)
Degree di IBS	Mild, % (n)	10.7% (13)
	Moderate, % (n)	39.7% (48)
	Severe, % (n)	49.6% (60)
IBS subtype	IBS-D, % (n)	35.5% (43)
	IBS-C, % (n)	30.6% (37)
	IBS-M, % (n)	24.0% (29)
	IBS-U, % (n)	9.9% (12)
IBS symptoms for month (n ± SD)		45.7 ± 59,5
Familial history of digestive system diseases, %Sì (n)		11.6% (14)
Allergies, % yes (n)		12.4% (15)
Comorbidities, % (n)		43.8% (53)
Regular intake of drugs, % (n)		38.8% (47)

Table 3: Drugs regularly taken by patients included in the study.

Drug category	No. of patients who regularly take the drug
Proton pump inhibitors	15
Levothyroxine	9
Statins	7
Beta blockers	6
Oral hypoglycemics	4
Antiplatelet drugs	4
Sartans	4
ACE inhibitors	3
Ezetimibe	3
Cholecalciferol	3
Calcium antagonist drugs	2
Gabapentinoids	2
Alpha-adrenergic receptor antagonists	2
Corticosteroids	2
Oral contraceptive	1
Hormone replacement therapy	1
Alendronate	1
Ursodeoxycholic acid	1
Bronchodilator	1
Hydroxychloroquine	1
Sucralfate	1
5 alpha reductase inhibitor	1

The T1 follow-up was performed on average 32.8±5.97 days after T0; the T2 follow-up was performed on average 34.5±11.1 days after T1.

During the treatment period, no patients reported adverse events related to SND intake.

IBS-SSS

The IBS-SSS total score decreased significantly at T1 compared to T0 (P<0.0001) and at T2 compared to T1 and T0 (P<0.0001), demonstrating a reduction in IBS symptoms and the consequent reduction in the impact of the disease on patients' lifestyles (Table 4 & Figure 1).

Table 4: Total score and item scores of the Irritable Bowel Syndrome - Severity Scoring System.

Item	Parameter	T0	T1	T1 – T0	T2	T2 – T0
Total score	Median (25th; 75th)	290.0 (230.0; 360.0)	230.0 (180.0; 290.0)	-60.0 (-90.0; -30.0)	170.0 (115.0; 225.0)	-110.0 (-180.0; -65.0)
	Mean±SD	294.1±89.3	228.4±89.5	-65.7±55.2	168.0±89.4	-126.1±85.2
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Pain severity	Median (25th; 75th)	60.0 (40.0; 80.0)	50.0 (30.0; 60.0)	-10.0 (-20.0; 0.0)	30.0 (20.0; 40.0)	-20.0 (-40.0; -10.0)
	Mean±SD	58.9±21.6	45.2±20.4	-13.7±13.9	33.6±18.8	-25.3±20.0
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Duration of pain	Median (25th; 75th)	50.0 (30.0; 70.0)	40.0 (20.0; 50.0)	-10.0 (-20.0; 0.0)	30.0 (20.0; 40.0)	-20.0 (-45.0; -10.0)
	Mean±SD	53.9±25.4	39.2±22.4	-14.7±16.5	28.0±20.1	-25.9±22.5
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Abdominal distension	Median (25th; 75th)	60.0 (45.0; 70.0)	50.0 (30.0; 60.0)	-10.0 (-20.0; -10.0)	30.0 (20.0; 50.0)	-20.0 (-40.0; -10.0)
	Mean±SD	59.1±19.3	45.0±19.5	-14.0±13.7	33.7±19.1	-25.4±18.3
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Bowel satisfaction	Median (25th; 75th)	60.0 (50.0; 80.0)	50.0 (30.0; 60.0)	-10.0 (-20.0; 0.0)	40.0 (20.0; 50.0)	-20.0 (-40.0; -10.0)
	Mean±SD	60.7±23.8	48.7±21.8	-12.0±14.2	36.6±21.9	-24.0±22.0
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Interference with life	Median (25th; 75th)	60.0 (50.0; 80.0)	50.0 (40.0; 60.0)	-10.0 (-20.0; 0.0)	40.0 (20.0; 50.0)	-20.0 (-40.0; -10.0)
	Mean±SD	61.6±22.3	50.3±21.9	-11.2±15.5	36.0±22.9	-25.5±21.2
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	

Change in the median and mean of the total score and item scores of the IBS-SSS. Scores significantly decrease in all cases both from T0 to T1 and from T1 to T2 (P<0.0001). Due to rounding, the first decimal place of the difference between the values in column T1 and the values in column T0 and the first decimal place of the difference between the values in column T2 and the values in column T0 may not correspond to the first decimal place of the values reported in columns T1-T0 and T2-T0, respectively.

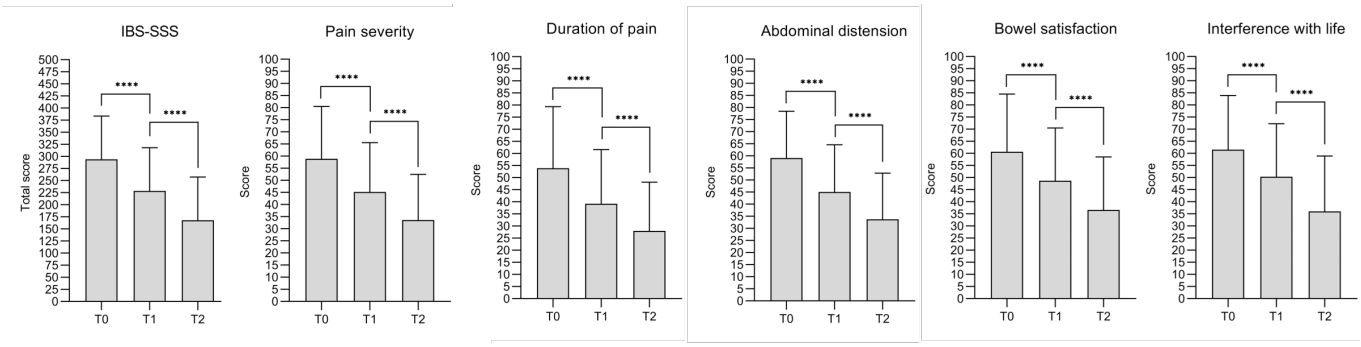

Figure 1: Change in the mean total score and item scores of the IBS-SSS. In all cases, scores decreased significantly from both T0 to T1 and from T1 to T2 (**** P<0.0001). The bar graphs represent the mean of the data at T0, T1, and T2, and the error bars represent the standard deviation.

Table 5: Change in disease severity as measured by change in the total IBS-SSS score of individual patients.

	T0	T1	T1 - T0	T2	T2 - T0	T2 - T1
IBS in remission	-	5,8% (7)	(+7)	16,5% (20)	(+20)	+185,7% (+13)
Mild IBS	10,7% (13)	17,3% (21)	+61,5% (+8)	37,2% (45)	+246,1% (+32)	+114,3% (+24)
Moderate IBS	42,2% (51)	56,2% (68)	+33,3% (+17)	40,5% (49)	-3,9% (-2)	-27,9% (-19)
Severe IBS	47,1% (57)	20,7% (25)	-56,1% (-32)	5,8% (7)	-87,7% (-50)	-72,0% (-18)

The values reported in the table represent the percentages and numbers (in parentheses) of patients with mild, moderate, or severe disease at T0 and who experience disease remission or have mild, moderate, or severe disease at T1 and T2.

The mean change in the IBS-SSS total score between T0 and T1 was -65.7±55.2, corresponding to a mean percentage change of -22.3%, while between T0 and T2 it was -126.1±85.2, corresponding to a mean percentage change of -42.9% (Table 4).

Between T0 and T1 and between T1 and T2 the trend of the total IBS-SSS score results in a progressive increase in the number of patients showing disease remission and a progressive decrease in the number of patients affected by moderate and severe disease (Table 5).

Regarding the transition of individual patients from one IBS severity category to another based on the IBS-SSS total score from T0 to T2, Table 6 further clarifies the individual benefit achieved.

Table 6: Patients transition between IBS severity categories from T0 to T2 based on the total IBS-SSS score.

From T0 to T2	Patients % (n)
Severe to moderate	49,1% (28)
Severe to mild	29,8% (17)
Severe to remission	8,8% (5)
Severe to severe	12,3% (7)
Moderate to mild	49,0% (25)
Moderate to remission	9,8% (5)
Moderate to moderate	41,2% (21)
Mild to remission	76,9% (10)
Mild to mild	23,1% (3)

The table shows the percentage of subjects who shifted categories or remained in the initial category from baseline to the end of the 60-day SND intake.

Compared to T0, at T1 a reduction of at least 50 points in the total IBS-SSS score was observed in 72 patients, representing 59.5% of the treated subjects, while between T0 and T2 a reduction of at least 50 points was observed in 101 patients, representing 83.5% of the treated subjects.

Compared to T0, at T1 a reduction of at least 95 points in the total IBS-SSS score was observed in 25 patients, representing 20.7% of the treated subjects, while between T0 and T2 a reduction of at least 95 points was observed in 71 patients, representing 58.7% of the treated subjects.

At T1, the percentage of subjects achieving a score below 75, indicating disease remission, was 5.8%, while at T2 it reached 16.5%.

The reduction in the score of all IBS-SSS items is significant ($P < 0.0001$) both at T1 and T2, demonstrating an improvement in bowel habits and a progressive reduction in the intensity and frequency of abdominal pain, the severity of abdominal distension and the impact of the disease on daily activities (Table 4, Figure 1).

At T0, the mean scores for individual items ranged from 53.9±25.4 (Duration of pain) to 61.6±22.3 (Interference with life) (Table 4). At T2, the mean scores for individual items decreased to values between 28.0±20.1 (Duration of pain) and 36.6±21.9 (Bowel satisfaction) (Table 4). The percentage reduction in the average score of the individual items is, in ascending order: -39.5% for Bowel satisfaction, -41.4% for Interference with life, -42.9% for Pain severity, -43.0% for Abdominal distension and -48.0% for Duration of pain.

IBS-QoL

The total IBS-QoL score significantly increases at T1 compared to T0 ($P<0.0001$) and at T2 compared to T1 ($P<0.0001$), demonstrating the improvement in patients' quality of life related to IBS (Table 7 & Figure 2).

Table 7: Total score and the subscales scores of Irritable Bowel Syndrome - Quality of Life.

Item	Parameter	T0	T1	T1 – T0	T2	T2 – T0
Total score	Median (25^{th}; 75^{th})	54.4 (34.9; 73.9)	66.9 (55.2; 80.2)	11.0 (4.77; 22.8)	81.6 (66.2; 92.3)	19.1 (11.0; 32.7)
	Mean±SD	53.3±24.1	67.2±19.7	13.9±12.3	77.9±18.3	24.5±19.6
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Dysphoria	Median (25^{th}; 75^{th})	53.1 (31.3; 75.0)	65.6 (51.6; 84.4)	12.5 (3.13; 25.0)	84.4 (68.8; 93.8)	21.9 (9.37; 40.6)
	Mean±SD	53.8±26.5	67.9±21.6	14.1±14.2	79.0±19.4	25.2±21.6
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Interference with activity	Median (25^{th}; 75^{th})	53.6 (32.1; 71.4)	64.3 (50.0; 82.1)	10.7 (3.57; 21.4)	78.6 (66.1; 92.9)	21.4 (7.14; 35.7)
	Mean±SD	52.3±25.4	65.6±20.9	13.4±13.9	76.9±18.7	24.7±21.4
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Food avoidance	Median (25^{th}; 75^{th})	33.3 (16.7; 54.2)	58.3 (41.7; 75.0)	16.7 (8.33; 33.3)	75.0 (58.3; 83.3)	33.3 (12.5; 50.0)
	Mean±SD	36.6±23.9	56.5±22.1	20.0±18.8	69.8±20.9	33.2±25.2
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Body image	Median (25^{th}; 75^{th})	56.3 (31.3; 78.1)	68.8 (50.0; 87.5)	12.5 (0.0; 21.9)	81.3 (68.8; 93.8)	18.8 (6.25; 31.3)
	Mean±SD	55.6±27.1	68.3±21.8	12.7±14.6	78.6±20.6	22.9±21.3
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	

Health worry	Median (25th; 75th)	50.0 (25.0; 75.0)	66.7 (50.0; 83.3)	16.7 (8.33; 25.0)	83.3 (66.7; 91.7)	25.0 (16.7; 41.7)
	Mean±SD	50.3±25.7	66.3±22.6	16.0±15.3	77.5±20.1	27.3±22.2
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Social reaction	Median (25th; 75th)	62.5 (37.5; 84.4)	68.8 (56.3; 87.5)	6.25 (0.0, 25.0)	81.3 (68.8; 93.8)	18.8 (6.25; 37.5)
	Mean±SD	58.3±28.3	70.0±20.8	11.8±14.2	79.8±18.6	21.5±22.1
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Sexual health	Median (25th; 75th)	75.0 (37.5; 100)	75.0 (62.5; 100)	0.0 (0.0; 25.0)	87.5 (62.5; 100)	12.5 (0.0; 25.0)
	Mean±SD	66.4±31.5	76.1±24.9	9.71±16.3	81.6±23.8	15.2±24.2
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	
Relationships	Median (25th; 75th)	58.3 (33.3; 75.0)	75.0 (50.0; 83.3)	8.34 (0.0; 25.0)	83.3 (66.7; 100)	16.7 (8.33; 33.3)
	Mean±SD	56.2±26.8	69.4±21.5	13.2±14.9	79.5±21.0	23.3±21.0
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	

Change in the median and mean of the total score and the scores of the IBS-QoL subscales. Scores increased significantly in all cases from both T0 to T1 and T1 to T2 (P<0.0001). Due to rounding, the first decimal place of the difference between the values in column T1 and the values in column T0 and the first decimal place of the difference between the values in column T2 and the values in column T0 may not correspond to the first decimal place of the values reported in columns T1-T0 and T2-T0, respectively.

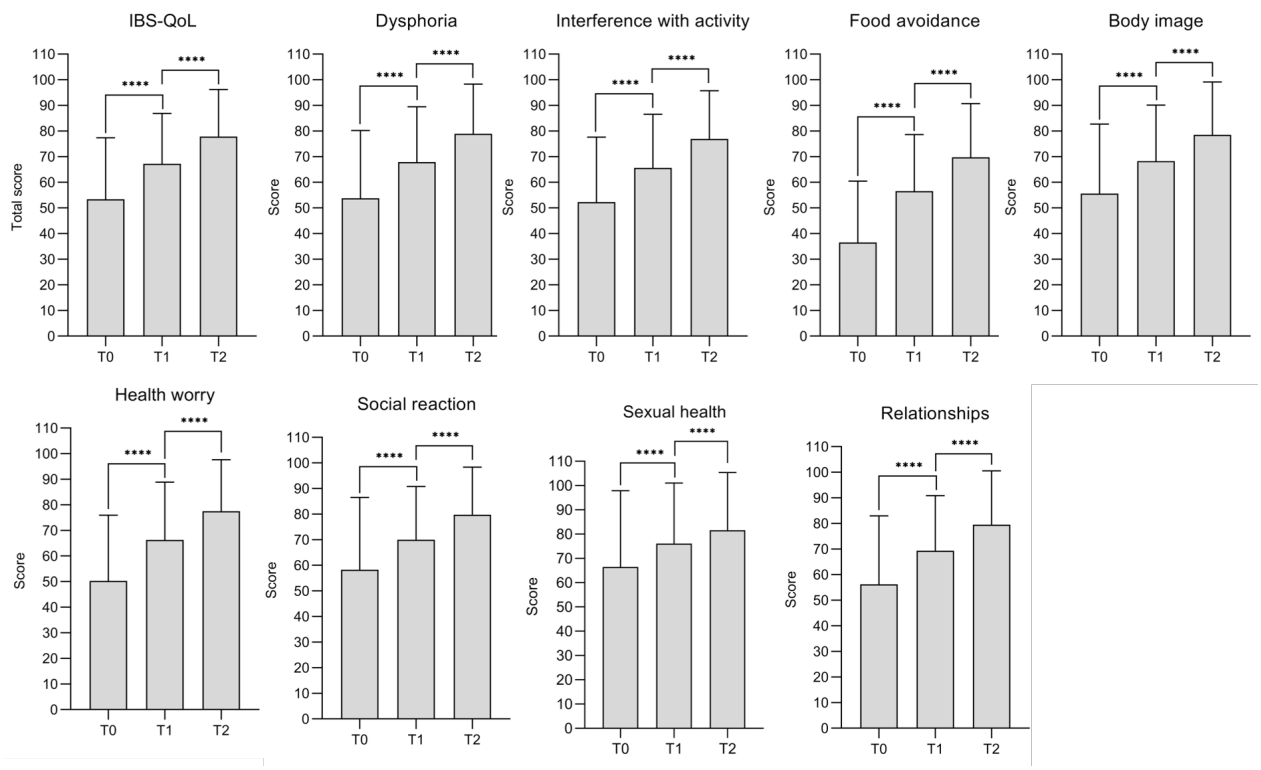

Figure 2: Change in the mean total IBS-QoL score and the scores of the eight IBS-QoL subscales. Scores increased significantly across all measures from T0 to T1 and from T1 to T2 (****P<0.0001). Bar graphs represent the mean data at T0, T1, and T2, with error bars representing the standard deviation.

The mean change in the total IBS-QoL score between T0 and T1 was +13.9±12.3, corresponding to a mean percentage change of +26.1%, while between T0 and T2 it was +24.5±19.6, corresponding to a mean percentage change of +46.0% (Table 7).

Between T0 and T1, a clinically significant response, equivalent to an increase of 14 points or more in the total IBS-QoL score, was observed in 51 subjects, representing 42.1% of patients, while between T0 and T2, an increase of 14 points or more was observed in 82 subjects, representing 67.8% of patients.

The increase in IBS-QoL subscale scores was significant between T0, T1, and T2 (Table 7, Figure 2). The Food Avoidance subscale is particularly significant: the mean score at T0 is the lowest among the IBS-QoL subscales, with a value of 36.6±23.9, while at T2 the score increases by 33.2±25.2 points, equivalent to an increase of +90.7% (Table 7). The second most significant increase is found for the Health Worry subscale, which increases by +54.3% between T0 and T2. Following, in decreasing order of percentage increase, are Interference with activity +47.2%, Dysphoria +46.8%, Body image +41.2%, Relationships +41.5%, Social reaction +36.9%, Sexual health +22.9%. This last subscale undergoes the smallest increase in score, but it is also the

subscale that at T0 records the highest average score, equal to 66.4±3 1.5 (Table 7).

SQS

At T0, median and mean SQS scores indicated sufficient sleep quality. SQS scores increased significantly at T1 compared to T0 (P<0.0001) and at T2 relative to T1 (P<0.0001), reflecting an improvement in sleep quality among IBS patients treated with SND. By the end of the 60-day treatment period, scores reached levels indicative of good sleep quality (Table 8 & Figure 3).

Table 8: Sleep quality scale score.

Item	Parameter	T0	T1	T1 – T0	T2	T2 – T0
Total score	Median (25^{th}; 75^{th})	4.0 (3.0; 5.0)	4.0 (4.0; 5.0)	0.0 (0.0; 1.0)	5.0 (4.0; 5.0)	1.0 (0.0; 2.0)
	Mean±SD	4.02±1.29	4.44±1.02	0.41±0.69	4.91±1.06	0.88±0.96
	P-value vs T0		<0.0001		<0.0001	
	P-value vs T1				<0.0001	

The SQS score increases significantly both from T0 to T1 and from T1 to T2 (P<0.0001). Due to rounding, the first decimal place of the difference between the value in column T1 and the value in column T0 and the first decimal place of the difference between the value in column T2 and the value in column T0 may not correspond to the first decimal place of the value reported in columns T1-T0 and T2-T0, respectively.

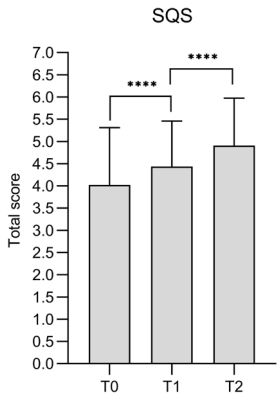

Figure 3: Change in SQS score. The score increased significantly from T0 to T1, and from T1 to T2 (**** P<0.0001). The bar graph represents the mean of the data at T0, T1, and T2, with error bars representing the standard deviation.

The mean increase in the total SQS score between T0 and T1 was 0.41±0.69, corresponding to a percentage increase of 10.2%, while between T0 and T2 it was 0.88±0.96, corresponding to a percentage increase of 21.9% (Table 8).

IBS subtypes

The total IBS-SSS and IBS-QOL scores show significant improvement between both T0 and T1 and between T1 and T2, in all four IBS subtypes (Table 9). It is noted that the scores achieved at T0, T1, and T2 by patients with IBS-D and IBS-M are higher in the IBS-SSS and lower in the IBS-QoL than those achieved by patients with IBS-C and IBS-U (Table 9). This demonstrates that in this study, patients with IBS-D and IBS-M on average experience worse symptoms and have a worse quality of life than patients with IBS-C and IBS-U. Looking at the data in Table 9, the IBS-SSS and IBS-QoL scores show the greatest improvements in patients with IBS-D and IBS-M.

Table 9: Total IBS-SSS, IBS-QoL, and SQS scores for patients by IBS subtype.

	IBS subtype	Parameter	T0	T1	T1 – T0	T2	T2 – T0
IBS-SSS	IBS-D (n=43)	Median (25^{th}; 75^{th})	350.0 (290.0; 400.0)	290.0 (230.0; 310.0)	-50.0 (-100.0; -20.0)	190.0 (140.0; 250.0)	-140.0 (-210.0; -70.0)
		Mean±SD	343.5±71.2	284.0±67.8	-59.5±43.9	206.3±90.4	-137.2±91.8
		P-value vs T0		<0.0001		<0.0001	
		P-value vs T1				<0.0001	
	IBS-C (n=37)	Median (25^{th}; 75^{th})	230.0 (155.0; 305.0)	170.0 (110.0; 230.0)	-50.0 (-80.0; -20.0)	130.0 (55.0; 180.0)	-100.0 (-130.0; -50.0)
		Mean±SD	238.4±86.1	174.3±83.1	-64.1±62.2	131.4±89.6	-107.0±76.7
		P-value vs T0		<0.0001		<0.0001	
		P-value vs T1				<0.0001	
	IBS-M (n=29)	Median (25^{th}; 75^{th})	290.0 (270.0; 355.0)	250.0 (205.0; 285.0)	-60.0 (-85.0; -40.0)	190.0 (145.0; 225.0)	-110.0 (-185.0; -70.0)
		Mean±SD	311.4±64.8	244.1±71.1	-67.2±53.5	179.7±63.1	-131.7±85.9
		P-value vs T0		<0.0001		<0.0001	
		P-value vs T1				<0.0001	
	IBS-U (n=12)	Median (25^{th}; 75^{th})	270.0 (142.5; 337.5)	185.0 (72.5; 242.5)	-75.0 (-105.0; -42.5)	120.0 (35.0; 180.0)	-105.0 (-187.5; -62.5)
		Mean±SD	247.5±97.7	158.3±80.2	-89.2±71.8	115.8±76.9	-131.7±84.0
		P-value vs T0		0.0005		0.0005	
		P-value vs T1				0.0010	
	IBS subtype	**Parameter**	**T0**	**T1**	**T1 – T0**	**T2**	**T2 – T0**
	IBS-D (n=43)	Median (25^{th}; 75^{th})	38.2 (23.5; 48.5)	55.9 (44.1; 66.9)	14.7 (3.67; 27.2)	75.0 (59.6; 86.0)	25.7 (14.0; 46.3)
		Mean±SD	38.8±22.2	54.5±17.8	15.6±13.1	69.4±20.6	30.5±23.1
		P-value vs T0		<0.0001		<0.0001	
		P-value vs T1				<0.0001	

IBS-QoL	IBS-C (n=37)	Median (25th; 75th)	70.6 (56.3; 82.0)	77.9 (66.9; 92.6)	7.36 (3.68; 12.9)	89.7 (77.2; 97.1)	15.4 (6.99; 19.5)
		Mean±SD	67.7±17.8	78.1±17.0	10.4±11.3	84.1±16.3	16.4±14.2
		P-value vs T0		<0.0001		<0.0001	
		P-value vs T1				<0.0001	
	IBS-M (n=29)	Median (25th; 75th)	48.5 (34.2; 61.8)	63.2 (55.2; 73.9)	14.0 (6.99; 20.6)	76.5 (71.0; 88.2)	27.2 (14.7; 35.7)
		Mean±SD	49.6±19.2	65.1±13.8	15.5±12.6	78.2±13.0	28.6±18.7
		P-value vs T0		<0.0001		<0.0001	
		P-value vs T1				<0.0001	
	IBS-U (n=12)	Median (25th; 75th)	82.4 (38.0; 88.6)	90.4 (65.6; 97.4)	9.93 (7.54; 25.4)	96.3 (75.7; 99.6)	12.5 (11.2; 28.1)
		Mean±SD	70.0±25.0	84.3±15.2	14.2±11.3	88.4±14.8	18.4±13.2
		P-value vs T0		0.0020		0.0010	
		P-value vs T1				0.0078	
	IBS subtype	**Parameter**	**T0**	**T1**	**T1 – T0**	**T2**	**T2 – T0**
SQS	IBS-D (n=43)	Median (25th; 75th)	4.0 (3.0; 5.0)	4.0 (4.0; 5.0)	0.0 (0.0; 1.0)	5.0 (4.0; 5.0)	1.0 (0.0; 2.0)
		Mean±SD	3.72±1.26	4.23±1.09	0.51±0.77	4.81±1.22	1.09±1.02
		P-value vs T0		<0.0001		<0.0001	
		P-value vs T1				<0.0001	
	IBS-C (n=37)	Median (25th; 75th)	4.0 (3.0; 5.0)	5.0 (4.0; 5.0)	0.0 (0.0; 1.0)	5.0 (4.0; 5.5)	0.0 (0.0; 1.0)
		Mean±SD	4.38±1.28	4.65±0.98	0.27±0.65	5.00±1.03	0.62±0.76
		P-value vs T0		0.0283		<0.0001	
		P-value vs T1				0.0010	
	IBS-M (n=29)	Median (25th; 75th)	4.0 (3.0; 5.0)	4.0 (4.0; 5.0)	0.0 (0.0; 1.0)	5.0 (4.0; 5.0)	1.0 (0.0; 2.0)
		Mean±SD	3.86±1.19	4.24±0.79	0.38±0.62	4.72±0.8	0.86±0.99
		P-value vs T0		0.0059		0.0001	
		P-value vs T1				0.0001	
	IBS-U (n=12)	Median (25th; 75th)	4.5 (3.0; 5.8)	5.0 (4.0; 5.8)	0.5 (0.0; 1.0)	5.0 (5.0; 6.0)	0.5 (0.0; 2.0)
		Mean±SD	4.42±1.44	5.0±1.21	0.58±0.67	5.42±1.08	1.00±1.13
		P-value vs T0		0.0313		0.0313	
		P-value vs T1				*0.1250*	

Change in the median and mean of the total IBS-SSS, IBS-QoL, and SQS scores for patients by IBS subtype. Scores improved significantly in all cases from T0 to T1 and from T1 to T2, except for the SQS score of IBS-U patients, which improved between T1 and T2 but did not reach significance. Non-significant P value is italicized. Due to rounding, the first decimal place of the difference between the values in column T1 and the values in column T0 and the first decimal place of the difference between the values in column T2 and the values in column T0 may not correspond to the first decimal place of the values reported in columns T1-T0 and T2-T0 respectively.

The SQS score improved significantly both between T0 and T1 and between T1 and T2 for patients affected by IBS-D, IBS-C, and IBS-M. For patients affected by IBS-U, there was a significant improvement between T0 and T1, while the improvement between T1 and T2 did not reach significance (P=0.1250; Table 9). It was observed that already at T1, patients affected by IBS-U reached a mean SQS score of 5.0±1.21, equivalent to the score achieved at T2 by patients affected by IBS-C (5.0±1.03) and higher than the score achieved at T2 by patients affected by IBS-D (4.81±1.22) and IBS-M (4.72±0.8).

Discussion

Treatment with SND for 60 consecutive days significantly improved IBS symptoms. Improvement was demonstrated by a significant reduction in the IBS-SSS total score and by reductions in the total score of at least 50 and 95 points, which were achieved and exceeded in a high percentage of treated patients. Treatment with SND produced a favorable response (IBS-SSS reduction ≥50 points) in 59.5% of patients (72/121) at 30 days (T1), a percentage that increased to 83.5% (101/121) after 60 days (T2). Analyzing the clinically significant benefit (IBS-SSS reduction ≥95 points), an increase from 20.7% (25/121) at T1 to 58.7% (71/121) at T2 was observed.

The reduction in IBS-SSS item scores was significant between T0, T1, and T2, demonstrating a progressive decrease in Pain severity, Duration of pain, Abdominal distension, and Interference with life and a progressive increase in Bowel satisfaction. After 60 days of treatment, the mean scores for the 5 items ranged from 28.0±20.1 (Duration of pain) to 36.6±21.9 (Bowel satisfaction), demonstrating a general and homogeneous improvement in IBS symptoms and a favorable impact on IBS-related quality of life.

Furthermore, 60-day SND treatment significantly improved the Quality of Life (QoL) of IBS patients. This is confirmed by the increase in the total IBS-QoL score. Specifically, an increase ≥14 points, indicative of a clinically relevant improvement, was observed in 42.1% of patients (51/121) after 30 days (T1) and in 67.8% (82/121) after 60 days (T2).

The increase in IBS-QoL subscale scores was significant between T0, T1, and T2, demonstrating a progressive reduction in emotional distress (Dysphoria), Interference with activity, Food avoidance, Health worry, and an improvement in Body image, Social reaction, Sexual health, and Relationships.

After 60 days of SND treatment, the mean scores across the 8 subscales ranged from 69.8±20.9 (Food avoidance) to 81.6±23.8 (Sexual health), demonstrating a general and consistent improvement in IBS-related quality of life. Particularly relevant is the case of the Food Avoidance subscale, whose mean score at T0 is the lowest among the IBS-QoL subscale scores and at T2 undergoes a percentage increase of 90.7%. From this data, it can be deduced that 60-day intake of SND allows for a broader

variety of foods that can be consumed by IBS patients, with an extremely favorable impact on their quality of life.

At T0, sleep quality was found to be "sufficient" (median SQS score: 4.0; mean 4.02±1.29), improving to a "good" level after 60 days of treatment. The mean change in SQS score was +0.41±0.69 (+10.2%) at 30 days (T1) and +0.88±0.96 (+21.9%) at 60 days (T2). Analyzing the clinical relevance, 34.7% of patients (42/121) recorded an increase ≥25% in the score at T1, a percentage that rose to 49.6% of patients (60/121) at T2. These data indicate that, after 60 days, almost half of the sample treated with SND achieved a significant improvement in sleep quality.

Responses to SND treatment were evaluated in IBS-D, IBS-C, IBS-M, and IBS-U subtypes. Due to the small number of patients with each IBS subtype, this assessment is indicative. Nevertheless, SND treatment induced a significant improvement in IBS-related symptoms and quality of life within the first 30 days and a further significant improvement in the subsequent 30 days in all IBS subtypes. Furthermore, a significant improvement in sleep quality was observed both in the first 30 days and in the subsequent 30 days of SND intake in patients with IBS-D, IBS-C, and IBS-M. However, in patients with IBS-U, the improvement was significant in the first 30 days, but in the subsequent 30 days of SND intake, further improvement did not reach statistical significance. We do not have the necessary elements to explain this result but we underline that already at T1, patients affected by IBS-U reached a mean score of the SQS scale equal to 5.0±1.21, equivalent to the score achieved at T2 by patients affected by IBS-C (5.0±1.03) and higher than the score achieved at T2 by patients affected by IBS-D (4.81±1.22) and IBS-M (4.72±0.8). It can be deduced that, with regards to sleep quality, patients with IBS-U responded more quickly to the intake of SND than patients with the other three IBS subtypes.

The scores obtained at T0, T1 and T2 by patients with IBS-D and IBS-M compared to those obtained by patients with IBS-C and IBS-U are higher in IBS-SSS and lower in IBS-QoL. This demonstrates that patients with IBS-D and IBS-M perceive worse symptoms and have a worse quality of life than patients with IBS-C and IBS-U. Regarding the quality of life related to IBS, this evidence emerged in our study is confirmed by the literature. In a study published in 2016 it was demonstrated that patients with IBS-D and IBS-M have a significantly worse quality of life measured with the IBS-QoL questionnaire than patients with IBS-C [33]. Patients with IBS-D show greater interference of the disease with their daily activities and have more problems with eating than patients with IBS-C. Similarly, patients with IBS-M showed greater interference in their activities, a greater impact on their relationships, and a lower social responsiveness score than patients with IBS-C [33]. In a further study, published in 2023, it was shown that patients with IBS-D had a worse IBS-related quality of life than patients with IBS-C [34]. The study found that IBS-D patients' bowel symptoms were also more likely to interfere with their daily activities, social interactions, food tolerance, and personal relationships [34].

In our study, despite worse scores, patients with IBS-D and IBS-M experienced the most marked improvements following SND intake compared to those with IBS-C and IBS-U.

What evidence is available in the literature to support the reliability of this study's results? As this is the first study to verify the effects of SND on IBS patients, direct comparison with previous research is not feasible. Consequently, justifying the mechanism of action of SND necessitates a review of the literature related to its individual ingredients.

SND contains two microencapsulated probiotic strains: *Bifidobacterium breve* BR03 (DSM 16604) and *Lactobacillus plantarum* LP01 (LMG P-21021). Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [35]. Numerous clinical studies have been published suggesting that probiotics may help in the treatment of IBS. This has led to several systematic reviews on the use of probiotics for the management of IBS. In a review published in 2022, the authors concluded that probiotic therapy could be effective in improving some IBS-D symptoms; however, the effects of probiotics on quality of life, stool frequency, and flatulence remained unclear [36].

Another review with meta-analysis published in 2022 compared conventional pharmacological treatments for IBS with probiotic interventions, finding that both approaches can effectively improve symptoms [37]. Although these results were promising, the substantial heterogeneity in the probiotics and medications studied, as well as the range of symptoms explored, suggested that these findings should be interpreted with caution. The availability of RCTs comparing similar products remained limited due to the vast array of therapeutic options [37]. Furthermore, a recent systematic review of 54 clinical trials confirmed that probiotics effectively alleviate IBS symptoms. Among these, the genera Lactobacillus and Bifidobacterium appear to be the most effective, with multi-strain formulations showing superior results compared to single-strain interventions [38].

The microencapsulated probiotic strains *B. breve* BR03 (DSM 16604) and *L. plantarum* LP01 (LMG P-21021), contained in SND, have been subjected to some experiments. In a randomized, double-blind, cross-over study, a comparison was carried out between the intestinal colonization induced by the two microencapsulated strains and that induced by the same two non-microencapsulated strains [39]. The study involved 44 healthy volunteers divided into 2 groups: 21 subjects received a mixture of *L. plantarum* LP01 (LMG P-21021) and *B. breve* BR03 (DSM 16604) in a non-microencapsulated form, and 23 other subjects received the same strains with gastro-resistant microencapsulation. The non-microencapsulated strains were administered at a rate of 5×10^9 colony-forming units/strain/day for 21 days, while the microencapsulated bacteria were administered at a rate of 1×10^9 colony-forming unit/strain/day for 21 days. At the end of the first treatment period, a three-week washout phase was performed, after which the groups were crossed over. The administered quantities of each strain were the same as in the first treatment. A statistically significant increase in Lactobacilli and Bifidobacteria was recorded in the feces of both groups at the end of each treatment, confirming the ability of the two strains to colonize the intestine, both in microencapsulated and non-microencapsulated form. The authors of the study conclude that microencapsulation significantly improves the gastro-resistance of the strains, thus enhancing their probiotic activity and allowing their use in quantities five times lower [39].

A double-blind, randomized, placebo-controlled study involved 300 healthy volunteers with evacuation disorders and hard stools, divided into three groups: 80 subjects received placebo, 110 subjects received a mixture of *L. plantarum*

LP01 and *B. breve* BR03 and 110 subjects received *B. animalis* subsp. *lactis* BS01 for 30 days [40]. Subjects treated with the probiotic strains reported a significant improvement in the number of weekly bowel movements and in the main bowel-related complaints, particularly stool consistency and ease of expulsion. Abdominal swelling, itching, burning, and anal pain also showed a significant improvement in the active groups receiving the probiotics. Thus, the intake of probiotic strains *L. plantarum* LP01 and *B. breve* BR03 can significantly alleviate evacuation disorders and hard stools [40].

In a randomized, placebo-controlled clinical trial [41], 60 patients with IBS were divided into three groups: 21 patients received *L. plantarum* LP01 and *B. breve* BR03 (5×10^9 CFU/ml each daily; Group A), 23 patients received *L. plantarum* LP0 1 and *L. acidophilus* LA0 2 (5×10^9 CFU/ml each daily; Group B), and 16 received a placebo for four weeks. The overall pain score in Group A decreased by 42% and 45% on day 14 and day 28, respectively; in Group B by 49% on both day 14 and day 28; in the placebo group by 25% and 29.5%. The overall symptom score in Group A decreased by 49.3% and 56% on day 14 and day 28, respectively; in Group B by 55.6%, both on day 14 and on day 28; in the placebo group by 8% and 14.4% [41].

Several in vitro studies have explored the mechanisms by which *L. plantarum* LP01 and *B. breve* BR03 can determine the effects observed in clinical studies. In one *in vitro* study, the two strains show inhibitory action against the enteropathogenic *E. coli* biotypes ATCC 8739, ATCC 10536, ATCC 35218 and ATCC 25922 and the diarrheal serotype O157:H7 [42]. In another *in vitro* study, it was demonstrated that the two strains positively modulate the balance between inflammatory and anti-inflammatory cytokines [43]. In the same study, the integrity of the epithelial barrier, simulated by a monolayer of CaCo-2 cells, was altered by two proinflammatory cytokines (TNF-α and IL-1β). To measure the ability of the two probiotic strains to prevent damage and restore epithelial integrity, Transepithelial electrical resistance was evaluated. *B. breve* BR03 and *L. plantarum* LP01 exerted a protective and restoring action on the epithelial barrier [43].

SND contains Fructooligosaccharides (FOS) with prebiotic function. A prebiotic is defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit [44]. Host microorganisms include producers of SCFA, such as butyric acid, particularly the genera Bifidobacterium and Lactobacillus [45]. Thus, FOS from SND nourish the probiotics contained in SND and the bacteria already present in the intestinal microbial flora that transform FOS into SCFA. The latter have anti-inflammatory and immunoregulatory activity [46], act on myenteric neurons and facilitate colonic peristalsis [47], nourish colonocytes, as butyrate is the preferential source of energy for these cells [48], and strengthen the intestinal barrier [49]. Finally, SCFAs participate in the modulation of hepatic lipid metabolism and attenuate inflammation and hepatic steatosis through the gut-liver axis, thereby contributing to liver health and maintenance of proper intestinal functions [50].

SND also contains a dose of tryptophan which, like FOS, has been added for the purpose of nourishing SND's probiotics and the resident gut microbiota. The metabolization of tryptophan by intestinal microbes results in the production of several molecules, including indoles and their derivatives. Several indole derivatives have been shown to act as endocrine molecules capable of activating the aryl hydrocarbon receptor [51]. Among these molecules, Indole-3-Propionic Acid (IPA), indole-3-acetaldehyde, indole-3-acrylic acid, indole-3-aldehyde and indole-3-acetate have been described. IPA has been shown to improve metabolism and strengthen the intestinal barrier function, modulating the immune response and exerting anti-inflammatory effects in several animal models [52,53]. In two human studies, IPA was associated with a lower risk of developing type 2 diabetes and the abundance of this metabolite was shown to be inversely related to low-grade systemic inflammation [54,55]. Furthermore, indole and indole-3-acetate modulate host metabolism by reducing hepatic inflammation or stimulating L cells to secrete GLP-1 [56-58]. The mentioned effects show that tryptophan metabolites produced by the intestinal microbiota may contribute to the preservation of the intestine, liver and their functions, with favorable effects on the gut-liver axis.

A portion of the tryptophan introduced with SND escapes metabolization by intestinal bacteria and is exploited by enterochromaffin cells to synthesize serotonin, thanks to the enzyme Trp Hydroxylase 1 (TpH1) [59]. Serotonin is a gastrointestinal signaling molecule for intrinsic or extrinsic neurons and influences intestinal peristalsis, motility, secretion, vasodilation, and nutrient absorption [60]. The gut microbiota is involved in intestinal serotonin production [61] and its role has been demonstrated in germ-free mice presenting altered serotonin production in the colon and low serotonin concentrations in the blood. The mechanisms through which the gut microbiota modulates serotonin production are not fully understood, but the role of SCFAs in stimulating TpH1 expression has been suggested [62]. Furthermore, some secondary bile acids, such as deoxycholate produced by the microbial biotransformation of cholate, can also stimulate serotonin biosynthesis [61]. These latest findings confirm the crucial role of FOS supplementation for the gut microbiota and the importance of liver health in modulating intestinal function.

SND contains vitamin B6. In an exploratory cross-sectional study, a significant inverse association emerged between dietary vitamin B6 intake and the severity of IBS symptoms [63]. A subsequent study in 105 IBS patients confirmed that low vitamin B6 intakes are found in these patients. Furthermore, an inverse association emerged between vitamin B6 intake and extraintestinal symptoms and fatigue associated with IBS [64]. There are several explanations for the association between low vitamin B6 levels and IBS symptoms. First, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid is a vitamin B6 derivative and a purinergic P2X receptor antagonist. This antagonistic action is thought to influence intestinal propulsion and attenuate abdominal pain and visceral hypersensitivity in IBS subjects [65]. Secondly, low vitamin B6 levels correlate with increased inflammation [66] and, consequently, heightened symptom severity, given that inflammation is a proposed mechanism underlying IBS pathophysiology [67].

SND also contains vitamin B3, which works with vitamin B6 to facilitate liver regeneration, ensure proper hepatic function [68,69], and combat non-alcoholic fatty liver disease [70]. The function of these vitamins within the composition of SND is primarily aimed at improving the function of the gut-liver axis.

Overall, we can state that the literature regarding the individual ingredients of SND, which we have briefly presented thus far, confirms the reliability of the results of this study.

An original aspect of this study is the aim of achieving a reduction in the total IBS-SSS score of at least 50 and 95

points and an increase in the total IBS-QoL score of at least 14 points. A comparison between patients' conditions before and after treatment is often considered sufficient. Probiotics have already been shown to be effective in treating IBS symptoms [38] and in improving the quality of life of IBS patients [71]. Therefore, a statistically significant improvement in the parameters chosen for clinical evaluation is easily achievable. In our opinion, the statistical significance of the improvement should not be considered sufficient: in fact, a patient who has experienced a statistically significant change in the total score of a questionnaire assessing symptoms or quality of life is not necessarily also satisfied with the result obtained. Therefore, we established a minimum threshold for total questionnaire score changes to enhance the realism of SND efficacy verification and improve the reliability of optimal treatment duration determination.

A further element of originality of the study lies in the analysis of the effect of SND on sleep quality. Many studies have been published on the association between IBS and sleep quality, but few have proposed a possible treatment for sleep disturbances associated with IBS. We hope that our contribution will be useful in directing IBS treatment toward improving sleep quality.

The lack of a placebo-treated control arm can be considered a limitation of the present study. We would like to point out that we do not consider a comparison with a placebo-treated group necessary, primarily because probiotics have already been extensively tested in placebo-controlled studies and there are placebo-controlled clinical trials involving the two strains contained in SND [40,41]. In these studies, probiotics have proven more effective than placebo, which suggests that SND could easily produce effects superior to those of placebo in treated subjects. Furthermore, our ambition is not just to prescribe nutraceuticals that outperform placebos in treating patients with functional intestinal disorders, a goal that is very easily achievable, but rather to try to restore patients to optimal health. Finally, this study was conducted as part of our regular clinical practice, and we do not believe it is ethically correct to treat patients who trust us to alleviate or resolve their ailments with a placebo.

Conclusion

In conclusion, over 60 days of treatment with SND, the primary outcome of this study was achieved, and SND proved effective in reducing the severity of IBS symptoms as measured by the IBS-SSS. The secondary outcomes of quality of life and sleep quality were also achieved. Treated patients reported no adverse effects attributable to SND intake. Overall, these results demonstrate that SND, thanks to the synergistic action of the microencapsulated probiotics *L. plantarum* LP01 and *B. breve* BR03, FOS, tryptophan, vitamin B3 and vitamin B6 is safe, well tolerated and effective for treating the symptoms of patients with the four IBS subtypes. They also demonstrate that treatment continued for 60 consecutive days is more effective than treatment continued for 30 days.

The authors believe that SND is a good candidate for further research in IBS and are aware that confirmation of the results obtained in this study requires further clinical studies with adequate statistical power and also aimed at exploring potential dietary confounders and mechanisms of action.

Declarations

Conflict of interest: Emma Baiano, Filippo Bove, Davide Cantone, Nadia Cautela, Alessandro Cavalotti, Daniela De Benedictis, Nicola Francesco Le Rose, Claudio Licci, Clorinda Lungo, Vito Emanuele Maccora, Mauro Donato Pappagallo, Irene Sorrentino, Davide Wild declare that they do not have any business relationships that could pose a conflict of interest in relation to the submitted article. Stefano Agostini, who wrote the article, is the scientific director of MèDISIN S.r.l.

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