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Impact of probiotic supplements on behavioural and gastrointestinal symptoms in children with autism spectrum disorder: A randomised controlled trial

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ABSTRACT

Objective To investigate whether probiotic supplementation can improve behavioural and gastrointestinal (GI) symptoms in children with autism spectrum disorder (ASD) aged 2–9 years and further explore the correlation between these symptoms.

Design Single-blinded, randomised, placebo-controlled study.

Setting Five developmental paediatric outpatient clinics of 'Continua Kids'.

Patients Children aged 2–9 years diagnosed with ASD along with their caregivers.

Interventions Probiotic or placebo sachet reconstituted in 50 mL of lukewarm milk/water, taken two times per day for 3 months.

Main outcome measures Change in behavioural (measured by Social Responsiveness Scale-2 (SRS-2) and Aberrant Behaviour Checklist-2 (ABC-2) tools) and GI (measured by GI Severity Index (GSI) score) symptoms after receiving intervention for 3 months.

Results A total of 180 children with ASD were enrolled in the study (probiotic group: 90 and placebo group: 90). All children completed the study. The probiotic group showed a significant reduction in behavioural symptom severity as measured by the SRS-2 tool (47.77% vs 23.33%; p=0.000) compared with the placebo. Probiotic-treated children demonstrated significant reductions in severe symptoms, including social withdrawal/lethargy (40%), stereotypic behaviour (37.77%), hyperactivity (34.44%) and inappropriate speech (32.22%) post-intervention (p=0.000). They also had marked improvements in constipation (p=0.003) and diarrhoea (p=0.043) compared with the placebo group. Both groups exhibited a statistically significant correlation between behavioural and GI symptoms.

Conclusions Probiotic supplementation improved behavioural and GI symptoms in children with ASD with no adverse effects. Both symptoms were significantly correlated. However, these results need to be validated in a larger sample size.

Trial registration number CTRI/2021/11/038213.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Probiotics have shown improvements in gastrointestinal (GI) and behavioural symptoms of children with autism spectrum disorder (ASD) by improving gut health and microbiota balance.

WHAT THIS STUDY ADDS

⇒ In India, where the prevalence of ASD is high, clinical evidence of the effectiveness of probiotics in treating behavioural and GI symptoms is limited. The findings of this study indicate that probiotic supplementation can complement conventional ASD therapies.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ We anticipate the results of this study can potentially influence clinical guidelines and practice by incorporating probiotic supplementation in managing ASD in children and adults; however, further evidence is needed to confirm its effectiveness.

INTRODUCTION

Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder characterised by impairments in social communication and interaction, sensory abnormalities, repetitive behaviours and varying degrees of intellectual disability.[1] The aetiology of ASD is multifactorial, with both genetic and environmental factors contributing to its development.[2 3] The prevalence of ASD varies from 0.4% in Asia to 0.6% globally.[4–6] In India, the estimated prevalence of ASD is 1%–1.5%, with approximately 0.09% in urban and 0.11% in rural areas in children aged 1–10 years.[7]

Gut microbiota, a potential environmental factor, is crucial in modulating ASD symptoms by communicating with the central nervous system via the enteric nervous system and the vagus nerve, influencing cognitive, behavioural, neurological and psychological

functions.[8] This communication, known as the gut-brain axis, involves different pathways, namely anatomic, metabolic, humoral, endocrine and immune pathways.[9 10] In ASD, this axis is often disrupted due to dysbiosis, leading to increased pathogenic microbes, imbalanced microbial–host homeostasis and heightened intestinal permeability to exogenous dietary peptides and neurotoxic bacterial peptides.[11] Approximately 46%–84% of children with ASD experience gastrointestinal (GI) symptoms such as constipation, chronic diarrhoea, abdominal pain and gastro-oesophageal reflux disease, indicating a link between anomalous gut microbiota and ASD.[12–15] Recent studies have demonstrated dysbiosis in individuals with ASD and correlated GI symptom severity with ASD core symptoms.[12–16]

Over the years, probiotics have gained attention as a complementary therapeutic approach. They are administered orally and contain live microorganisms in adequate amounts, which can modulate neuroactivity, reduce gut inflammation and prevent the colonisation of harmful microbes in the gut.[16] Probiotics have shown improvements in both GI and behavioural symptoms of children with ASD,[17 18] primarily because they stabilise existing microbial communities, enhance gut-brain axis integrity and reduce inflammatory cytokines.[18] They have been effective in treating irritable bowel syndrome, maintaining gut health and improving GI symptoms of ASD with no adverse side effects.[19 20]

In India, although most studies are preclinical and clinical evidence is scarce, probiotics have shown effectiveness against GI and behavioural issues in ASD.[21–24] Given the high prevalence of ASD in India, probiotic supplementation can help resolve these symptoms in children with ASD—who, if left untreated, may develop severe difficulties in communication, social interactions and behaviour.[1–7] This study was conducted to investigate whether probiotic supplementation can improve the behavioural and GI symptoms in children with ASD aged 2–9 years and further explore the correlation between these symptoms.

METHODS

Study design

This single-blinded, randomised, placebo-controlled study was conducted at five developmental paediatric outpatient clinics of 'Continua Kids' (Centre of Neurotherapy in Uniquely Abled Kids) in Delhi, Faridabad, Ghaziabad, Gurgaon, and Noida, India, from December 2021 to June 2023 (18 months). This clinical trial is registered on www.ctri.nic.in (CTRI/2021/11/038213).

Study population and eligibility criteria

The study enrolled children aged 2–9 years who were medically diagnosed with ASD according to the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) criteria.[25] Diagnosis of ASD was re-confirmed using the INCLEN diagnostic tool for ASD[26] (a standardised questionnaire for detecting ASD), by the principal investigator (developmental paediatrician) and team at the centres included in the study. Children with confirmed brain abnormalities; neurological syndromes or focal neurological signs; a history of documented sequelae of birth asphyxia (preterm birth <28 weeks of gestational age or perinatal injuries); epilepsy (past or current); sensory impairments (deafness/blindness); coeliac disease; chronic medical conditions like diabetes (weight and height <3rd percentile for age); and organic GI disorders, such as gastro-oesophageal reflux, food allergies, inflammatory bowel disease or prior bowel surgery, were excluded from the study. Children treated with antibiotics (within 2 months or long-term use), psychotropic medications (within the preceding 3 months) or probiotic supplements (within the previous 6 months) were also excluded from the study. Furthermore, the study excluded children on special diets (gluten-free, casein-free, ketogenic, high-protein and lactose-free diets) in the preceding 3 months from the study start date.

Study treatment

All participating children were randomised (1:1) using a random allocation sequence generated by a statistician. The principal investigator enrolled the participants, and the clinic reception staff assigned intervention to participants as per the random sequence. Blocking was not implemented. Care providers of participants were blinded. Participants in the two groups (probiotic or placebo) received either a probiotic supplementation (5 g sachet containing 9 billion colony forming units (CFU) of 12 probiotic strains per sachet; patented, manufactured and packaged by Nutri Newron Pediatrix (patent number: 489143, HSN code: 21069099)) or a placebo, two times per day for 3 months (online supplemental file, table S1). Both interventions had to be reconstituted in 50 mL of lukewarm milk or water before feeding. The intervention was monitored using a communication diary, where a weekly record was maintained by the parents, who had an educational level above high school (most were graduates and few post-graduates; online supplemental file, table S2). At baseline and at the end of 3 months, assessments were conducted under the supervision of the principal investigator where the parents and caregivers of these children completed the questionnaire within 1.5 to 2 hours, over a single sitting. Children in both groups followed their regular diet along with the respective study treatments. However, regular rehabilitation and therapies, being the standard treatments for ASD, were not discontinued during the research. On an average, the children attended the centres for approximately 4–5 months to continue receiving them.

Study endpoints

The primary endpoint was to determine the change from baseline to end of 3 months in behavioural (assessed using the Social Responsiveness Scale-2 (SRS-2) and the Aberrant Behaviour Checklist-2 (ABC-2) tools) and GI

(assessed using the GI Severity Index (GSI) scale) symptoms.[25–27] The secondary endpoint was to determine the correlation between GI and behavioural symptoms experienced by these children.

Assessment tools

The SRS-2 tool is a 65-item standard questionnaire related to behaviour and rated on a Likert scale, where 1 refers to 'not true' and 4 refers to 'almost always true'. These items are further grouped into the social communication and interaction (SCI) domain (includes the subdomains of social awareness, social cognition, social communication and social motivation) and the restricted, repetitive behaviours and interests (RRBI) domain.[25]

The ABC-2 scale consists of a 58-item questionnaire further divided into five domains (irritability, agitation, crying (15 items); lethargy/social withdrawal (16 items); stereotypic behaviour (7 items); hyperactivity/non-compliance (16 items) and inappropriate speech (4 items)) and is rated on a 4-point Likert scale. It measures the psychiatric symptoms and behavioural disturbance in patients with intellectual developmental disabilities. Higher scores in both SRS-2 and ABC-2 scales reflect more severe problems.[25]

The GSI scale is a composite questionnaire of six symptoms—constipation, diarrhoea, stool consistency, stool smell, flatulence and abdominal pain. The symptoms are rated using a Likert scale (0–2), with a higher score indicating greater symptom severity.[27]

Statistical analysis

The sample size for this study was calculated considering a statistical power of 80% at a 90% confidence level using the formula $n = \frac{2*(z_{1-\alpha}-z_{1-\beta})^2 * \sigma_w^2}{(\mu_T-\mu_S-\delta)^2}$,where n is the required sample size, $z_{1-\alpha}$ is the critical z-score for a given α=1.96, $z_{1-\beta}$ is the critical z-score for a given β=0.84, μ_T is the mean of the treatment group, μ_S is the mean of the control group, δ is the superiority limit of the mean difference and σ^2_w is the variance within groups. Parametric data were analysed with Student's t-test/independent t-test/paired t-test and one-way ANOVA method for two or more groups. Bivariate regression analysis using analysis of covariance (ANCOVA) performed to determine the correlation between symptom scores. Continuous data were expressed as mean (SD), and categorical data were represented as absolute numbers (percentages). Nominal categorical data between groups were compared using the χ^2 test, and the correlation coefficient was determined to observe the linear relationship between variables. The statistical significance of $p<0.05$ was considered for all calculations.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting or dissemination of our research.

Table 1 Demographics of children with ASD included in the study

Characteristics	Probiotic (n=90)	Placebo (n=90)
Age (years, at baseline), mean (SD)	4.12 (1.27)	4.08 (1.43)
Boys, n (%)	70 (77.80)	69 (76.70)
BMI (kg/m^2), mean (SD)	19 (3.00)	17 (3.46)
Breastfed/formula-fed, n (%) (children aged 2–3 years)		
Breastfed	47 (52.22)	34 (52.31)
Formula-fed	12 (36.36)	13 (35.14)
Both	31 (54.39)	43 (55.13)
Diet, n (%) (children aged >3 to 9 years)		
Semisolid	14 (87.50)	3 (50.00)
Solid	4 (26.67)	9 (56.25)
Both	72 (48.32)	78 (49.37)
Behavioural therapy, n (%)		
None	44 (48.89)	56 (62.22)
Home	9 (10.00)	0 (0.00)
Special school	0 (0.00)	3 (3.33)
At clinic (hours/week)		
1–6	19 (21.11)	21 (23.33)
7–12	15 (16.67)	10 (11.11)
13–18	3 (3.33)	0 (0.00)

Data are expressed as mean (SD) and absolute numbers (percentages), as applicable.
ASD, autism spectrum disorder; BMI, Body Mass Index.;

RESULTS

Demographics

A total of 180 children with ASD were enrolled in the study—probiotic group: n=90; placebo group: n=90. All children completed the study (online supplemental file, figure S1). Both groups had comparable demographics (table 1). The mean (SD) age of children was 4.12 (1.27) years for the probiotic group and 4.08 (1.43) years for the placebo group. There were more boys in each group (probiotic: 77.80% and placebo: 76.70%) than girls (probiotic: 22.20% and placebo: 23.30%). A minor difference in dietary habits was observed in children (aged 2–3 years) in both groups. Most children (aged >3 to ≤9 years) in the probiotic group were on a semisolid diet (87.50%); whereas, those in the placebo group had a more solid diet (56.25%) (table 1). Children from both groups (probiotic: n=46/90 and placebo: n=34/90) received rehabilitation and standard therapies for ASD. Rehabilitation happened at the clinic for 1–6 hours/week (probiotic: 21.1% and placebo: 23.33%, table 1.)

Effect of probiotic supplement on behavioural symptoms

The mean SRS total T scores at baseline did not differ significantly between groups (table 2). There was a significant difference in mean SRS total T scores after

Table 2 Assessment of behavioural symptoms using the SRS-2 tool in children with ASD

Assessment tools	Level (score range)	Probiotic (n=90) Baseline n (%)	Probiotic (n=90) After intervention n (%)	Probiotic (n=90) P value	Placebo (n=90) Baseline n (%)	Placebo (n=90) After intervention n (%)	Placebo (n=90) P value
Mean (SD) SRS-2 total T score		76.59 (6.76)	70.49 (8.51)	0.340*	75.49 (8.57)	72.39 (7.03)	0.010†‡
Difference in mean score		6.10			3.10		
SRS-2 total T score	Normal (≤59)	0 (0.00)	7 (7.80)	0.000‡	7 (7.80)	1 (1.10)	0.000‡
	Mild (60–65)	8 (8.90)	19 (21.10)		11 (12.20)	6 (6.70)	
	Moderate (66–75)	24 (26.70)	49 (54.40)		24 (26.70)	56 (62.20)	
	Severe (≥76)	58 (64.40)	15 (16.70)		48 (53.30)	27 (30.00)	
SCI T score	Normal (≤59)	0 (0.00)	7 (7.80)	0.000‡	7 (7.80)	3 (3.30)	0.342
	Mild (60–65)	3 (3.30)	18 (20.00)		12 (13.3)	11 (12.20)	
	Moderate (66–75)	34 (37.80)	50 (55.60)		32 (35.60)	42 (46.70)	
	Severe (≥76)	53 (58.90)	15 (16.70)		39 (43.30)	34 (37.80)	
RRBI T score	Normal (≤59)	9 (10.00)	12 (13.30)	0.000‡	12 (13.30)	2 (2.20)	0.000‡
	Mild (60–65)	12 (13.30)	27 (30.00)		12 (13.30)	37 (41.10)	
	Moderate (66–75)	17 (18.90)	37 (41.10)		36 (40.00)	34 (37.80)	
	Severe (≥76)	52 (57.80)	14 (15.60)		30 (33.30)	17 (18.90)	

*Indicates *p* value determined between the two groups—probiotic and placebo at baseline.
†Indicates *p* value determined between the two groups—probiotic and placebo at the end of 3 months; data are expressed as mean (SD). All other *p* values are for differences from baseline within each group, and data are presented as absolute numbers (percentages).
‡Indicates statistically significant difference with p<0.05; χ^2 test was performed.
ASD, autism spectrum disorder; RRBI, restricted, repetitive behaviours and interests; SCI, social communication and interaction; SRS-2, Social Responsiveness Scale-2.

intervention (at the end of 3 months) across both groups (p=0.01), with a greater decline in the probiotic group than in placebo (6.10 units vs 3.10 units) (table 2). The probiotic group had a significant reduction in symptom severity after intervention than placebo (47.77% vs 23.33%; p=0.000; figure 1; table 2). More children in the probiotic group had mild and moderate symptoms after intervention than at baseline (an increase of 12.22% and 27.77%, respectively) (figure 1); only 16.7% had severe symptoms after intervention (table 2). In contrast, more placebo-treated children had moderate symptoms after intervention (62.2%, table 2). Similar trends were observed for the SCI T and RRBI T scores in both groups (figure 1; table 2). Consistently, the probiotic-treated children demonstrated a significant reduction in severe symptoms for social withdrawal or lethargy (40%), stereotypic behaviour (37.77%), hyperactivity (34.44%) and inappropriate speech symptoms (32.22%) after intervention (p=0.000, figure 2). However, symptoms of irritability did not show a prominent change in either group (table 3). There was a remarkable improvement in total behavioural scores, with a 26.66% reduction in severity and a subsequent 36.66% increase in mild symptoms in the probiotic group (figure 2).

Effect of probiotic supplement on GI symptoms

The probiotic-treated children showed significant improvements in constipation (p=0.003) and diarrhoea (p=0.043) than placebo-treated children (table 4). The average stool consistency in the probiotic-treated children improved (6.66% reduction in watery stools and an 18.88% increase in formed stools) considerably when compared with baseline. Among other GI symptoms, there was a 25.55% improvement in stool smell, a 22.22% reduction in daily flatulence and a 10% reduction in mild discomfort as well as moderate-to-severe abdominal pain after intervention in the probiotic group (figure 3). The GSI score improved by 27.77% in the probiotic group, with scores decreasing from ≥6 to <6.

Correlation between behavioural and GI symptoms in children with ASD

There was a statistically significant correlation between behavioural and GI symptoms in both groups (table 5). Both the SRS-2 total T score and the ABC-2 score showed a significant increase with a 1.00 unit rise in GSI score for the probiotic group (68.22 units and 53.17 units, respectively, p=0.000).

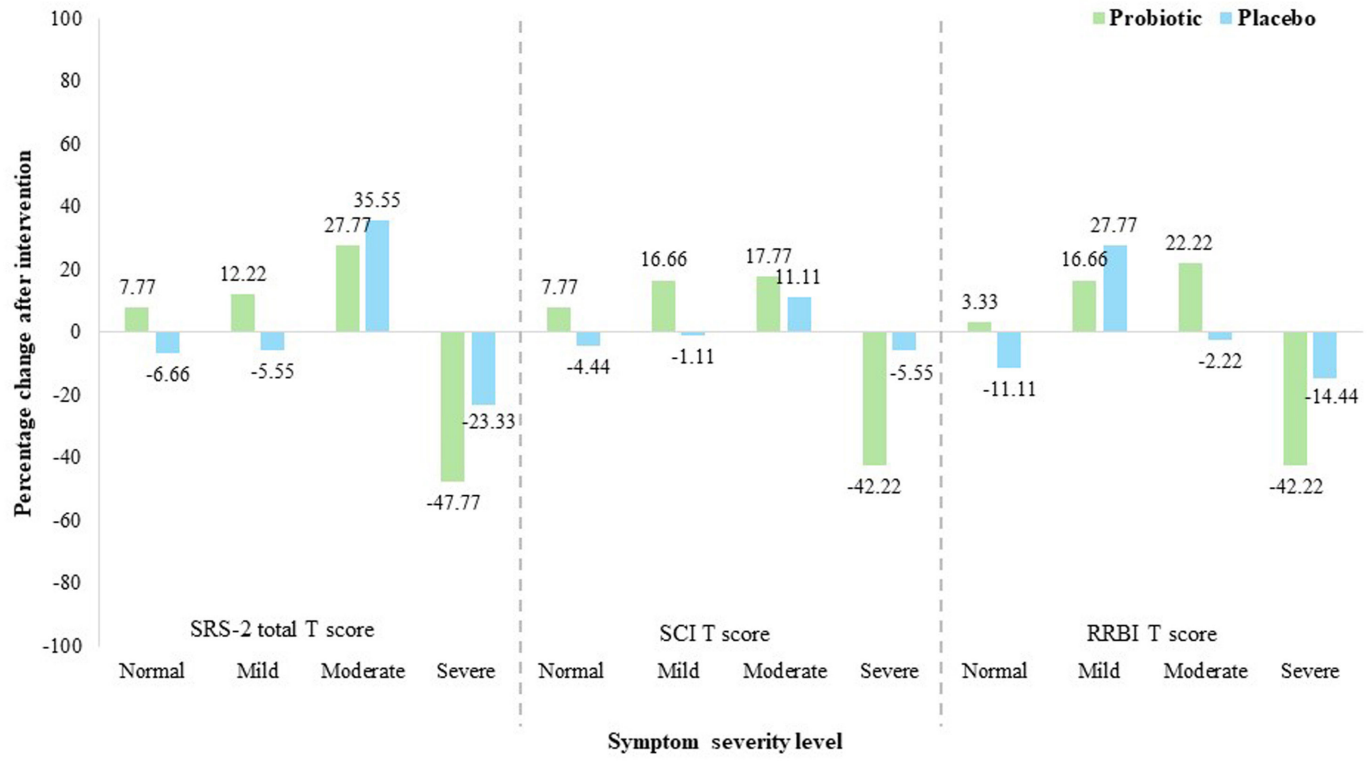

Figure 1 Change in behavioural symptoms assessed by SRS-2 T scores after intervention. RRBI, restricted repetitive behaviours and interests; SCI, social communication and interaction; SRS-2, Social Responsiveness Scale-2.

Safety outcomes

The intervention was well-tolerated among children with ASD without any adverse events. No visits to hospitals or interactions with other healthcare facilities due to minor illnesses during the study period were reported.

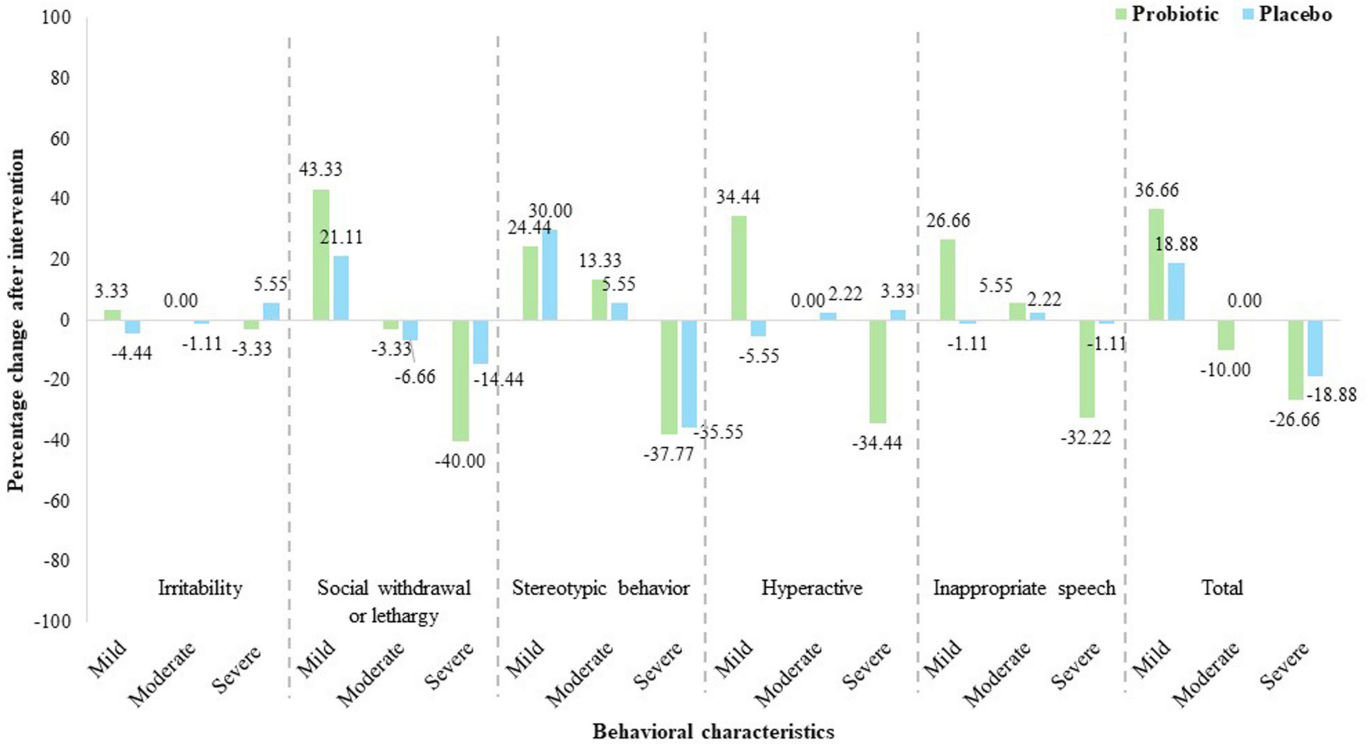

Figure 2 Change in behavioural characteristics assessed by ABC-2 scores after intervention. ABC-2, Aberrant Behaviour Checklist-2.

Table 3 Assessment of behavioural symptoms using the ABC-2 tool in children with ASD

Behavioural characteristics (scale range)	Level (score range)	Probiotic (n=90)			Placebo (n=90)		
		Baseline n (%)	After intervention n (%)	P value	Baseline n (%)	After intervention n (%)	P value
Irritability (0–60)	Mild (<15)	44 (48.89)	47 (52.23)	0.766	44 (48.89)	40 (44.44)	0.754
	Moderate (=15)	0 (0.00)	0 (0.00)		8 (8.89)	7 (7.78)	
	Severe (≥16)	46 (51.11)	43 (47.77)		38 (42.22)	43 (47.78)	
Social withdrawal or lethargy (0–64)	Mild (<18)	32 (35.56)	71 (78.89)	0.000*	29 (32.22)	48 (53.33)	0.006*
	Moderate (=18)	6 (6.66)	3 (3.33)		8 (8.89)	2 (2.22)	
	Severe (≥19)	52 (57.78)	16 (17.78)		53 (58.89)	40 (44.45)	
Stereotypic behaviour (0–28)	Mild (<4)	23 (25.55)	45 (50.00)	0.000*	17 (18.89)	44 (48.89)	0.000*
	Moderate (=4)	14 (15.56)	26 (28.89)		5 (5.55)	10 (11.11)	
	Severe (≥5)	53 (58.89)	19 (21.11)		68 (75.56)	36 (40.00)	
Hyperactive (0–64)	Mild (<24)	29 (32.22)	60 (66.67)	0.000*	42 (46.67)	37 (41.11)	0.687
	Moderate (=24)	2 (2.22)	2 (2.22)		5 (5.55)	7 (7.78)	
	Severe (≥25)	59 (65.56)	28 (31.11)		43 (47.78)	46 (51.11)	
Inappropriate speech (0–16)	Mild (<4)	36 (40.00)	60 (66.67)	0.000*	42 (46.67)	41 (45.55)	0.919
	Moderate (=4)	4 (4.44)	9 (10.00)		13 (14.44)	15 (16.67)	
	Severe (≥5)	50 (55.56)	21 (23.33)		35 (38.89)	34 (37.78)	
Total (0–232)	Mild (<66)	24 (26.67)	57 (63.33)	0.000*	37 (41.11)	54 (60.00)	0.028*
	Moderate (=66)	9 (10.00)	0 (0.00)		3 (3.33)	3 (3.33)	
	Severe (≥67)	57 (63.33)	33 (36.67)		50 (55.56)	33 (36.67)	

Data are expressed as absolute numbers (percentages).
A χ^2 test was performed.
*Indicates statistically significant difference with p<0.05.
ABC-2, Aberrant Behaviour Checklist-2; ASD, autism spectrum disorder.

DISCUSSION

This study evaluated the effectiveness of probiotics in treating behavioural and GI symptoms in children aged 2–9 years diagnosed with ASD. The probiotic-treated children showed significant improvement in ASD symptom severity scores from baseline, as evaluated by SRS-2 total T, SCI T and RRBI T scores. There was a notable reduction in symptom severity for social communication impairments and repetitive behaviour after probiotic supplementation, highlighting the efficacy of the probiotic supplementation. These findings align with studies reporting that probiotics, such as *Saccharomyces boulardii* and *Lactobacillus* strains, improved stereotypic and repetitive behaviour along with social interaction.[28 29] In our study, children treated with probiotics had marked improvements in hyperactivity, inappropriate speech, and social withdrawal or lethargy. These results were consistent with studies reporting improved speech/communication and sociability in children with ASD on probiotic supplementation, particularly with *Bifidobacterium* and *Lactobacillus* strains.[24–30]

Nearly half of the children with ASD frequently experience GI symptoms such as abdominal pain, bloating, diarrhoea and constipation, with more than 30% experiencing multiple concurrent symptoms.[31] Our study demonstrated a significant reduction in constipation and diarrhoea in children of the probiotic group, consistent with other studies.[17–32] Improved stool consistency, reduced flatulence and decreased abdominal pain were observed after probiotic supplementation, supporting the effectiveness of probiotics in managing GI symptoms in children with ASD.[17–20] Similarly, improved stool consistency and flatulence were reported in children diagnosed with ASD and treated with probiotics (*Bifidobacterium* and *Lactobacillus* strains) plus fructooligosaccharides or a combination of *Bifidobacterium infantis* and bovine colostrum.[18–30]

In children with ASD, GI symptoms are associated with challenging behaviour, self-injury, aggression, sensory issues, comorbid psychopathology and sleep problems, thereby reducing their quality of life.[31–33] Our study found a statistically significant correlation between behavioural and GI symptoms, supporting the gut–brain axis hypothesis. Although moderate, these findings are consistent with studies reporting a strong correlation between GI symptoms assessed by the GSI scores and the severity of ASD symptoms assessed by Autism Treatment Evaluation Checklist (ATEC) scores[17–34] and Childhood Autism Rating Scale (CARS) scores.[35] However, it must be noted that individuals with ASD often undergo simultaneous

Table 4 Assessment of GI symptoms using the GSI scale in children with ASD

GSI	Level definitions	Probiotic (n=90)			Placebo (n=90)		
		Baseline n (%)	After intervention n (%)	P value	Baseline n (%)	After intervention n (%)	P value
Constipation	0–2 stools/week	16 (17.78)	17 (18.89)	0.003*	17 (18.89)	2 (2.22)	0.001*
	3–4 stools/week	36 (40.00)	16 (17.78)		35 (38.89)	47 (52.22)	
	>5 stools/week	38 (42.22)	57 (63.33)		38 (42.22)	41 (45.56)	
Diarrhoea	0–1 stool/week	60 (66.67)	72 (80.00)	0.043*	75 (83.33)	48 (53.33)	0.000*
	2–3 stools/week	30 (33.33)	18 (20.00)		15 (16.67)	42 (46.67)	
Average stool consistency	Formed	55 (61.11)	72 (80.00)	0.009*	73 (81.11)	68 (75.56)	0.470
	Loose/unformed	28 (31.11)	17 (18.89)		17 (18.89)	22 (24.44)	
	Watery	7 (7.78)	1 (1.11)		0 (0.00)	0 (0.00)	
Stool smell	Normal	42 (46.67)	65 (72.22)	0.001*	53 (58.89)	61 (67.78)	0.321
	Abnormal	34 (37.78)	14 (15.56)		29 (32.22)	20 (2.22)	
	Unusually foul	14 (15.55)	11 (12.22)		8 (8.89)	9 (10.00)	
Flatulence	Normal	37 (41.11)	56 (62.22)	0.000*	61 (67.78)	66 (73.33)	0.811
	Frequent	28 (31.11)	29 (32.22)		27 (30.00)	22 (24.44)	
	Daily	25 (27.78)	5 (5.56)		2 (2.22)	2 (2.22)	
Abdominal pain	Normal	53 (58.89)	71 (78.89)	0.001*	69 (76.67)	69 (76.67)	1.000
	Mild discomfort	28 (31.11)	19 (21.11)		21 (23.33)	21 (23.33)	
	Moderate to severe	9 (10.00)	0 (0.00)		0 (0.00)	0 (0.00)	
GSI score	<6	56 (62.22)	81 (90.00)	0.000*	77 (85.56)	74 (82.22)	0.543
	≥6	34 (37.78)	9 (10.00)		13 (14.44)	16 (17.78)	

Data are expressed as absolute numbers (percentages).
*Indicates statistically significant difference with $p<0.05$; χ^2 test was performed.
ASD, autism spectrum disorder; GI, gastrointestinal; GSI, Gastrointestinal Severity Index.

therapies such as counselling and behavioural interventions, which might have contributed to significant improvements observed in the placebo group.

The observed improvements in both behavioural and GI symptoms on probiotic supplementation imply a correlation between these symptoms in children with ASD. This significant correlation emphasises the importance of addressing GI concerns in managing ASD-related behavioural issues to improve the quality of life in these children. It also highlights the potential of probiotics to restore gut microbiota balance, alleviate GI issues and positively impact neurobehavioural outcomes. This study further reinforces the idea that improving gut health through probiotics can positively affect behavioural outcomes, supporting the concept of the gut–brain axis.

Strengths and limitations of the study

The study follows a single-blinded, randomised, placebo-controlled design, conducted across multiple centres which minimises selection bias and enhances the reliability of the findings. Regular follow-ups ensured treatment compliance and accurate data collection. Although statistically calculated, the small sample size remains a major concern compromising the validity of the findings. The intervention duration was relatively short (3 months), and the long-term effects of probiotic supplementation were not assessed. Additionally, double-blinding was not considered, which might affect outcome assessment and reporting. The tools used to evaluate behavioural symptoms, although robust, were time-consuming. The GSI scale is subjective, lacks sensitivity and has limited validation. Moreover, individuals with ASD undergo additional psychotherapies, and such studies are susceptible to placebo effects as parents or caregivers are responsible for reporting outcomes, which might skew the dataset. Another limitation of this study is the higher proportion of children on semisolid diets in the probiotic group, which may have influenced stool quality independently of the intervention. Semisolid diets, especially those with fibre or prebiotics, can improve stool consistency, potentially confounding results. While participants were advised to maintain a balanced diet, future studies should standardise diets or stratify analyses to better isolate the effects of probiotics.

Clinical implications and future directions

Probiotic supplementation shows promise in reducing GI problems, such as diarrhoea, stool smell and flatulence,

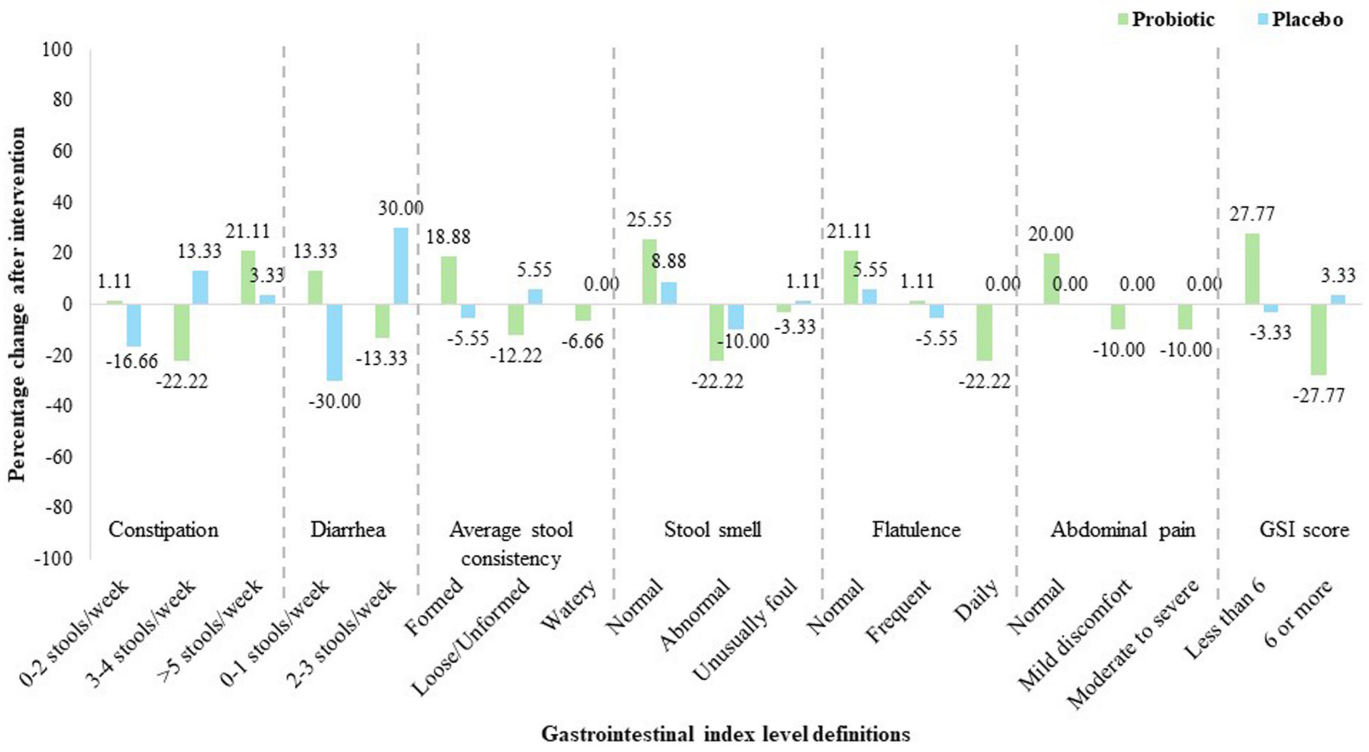

Figure 3 Change in GI symptoms assessed by GI scores after intervention. GI, gastrointestinal; GSI, GI Severity Index.

by improving gut health and microbiota balance. It may also alleviate the severity of behavioural symptoms in children with ASD, particularly improving hyperactivity, inappropriate speech, and social withdrawal or lethargy. This suggests that probiotic supplementation could complement conventional ASD therapies, aiding in managing behavioural symptoms associated with ASD, although subject to pending evidence for its efficacy.

Future research should focus on longitudinal studies to evaluate the long-term effect of probiotic supplementation on behavioural and GI symptoms in children with ASD. Investigating how probiotic strains influence neurobehavioural outcomes by modulating the gut–brain axis is crucial. Given the promising results of this study, expanding research to include a larger cohort of patients with diverse age groups and individuals with comorbidities having ASD will be vital for validating the efficacy of probiotic supplementation as a therapeutic strategy for ASD.

Table 5 Evaluating the correlation between behavioural and GI symptoms in children with ASD across the two groups

Groups	GI symptoms as assessed by GSI	Behavioural symptom scores as evaluated by SRS-2 total T scores					
		Unstandardised coefficient (B)	SE	Standardised coefficient (β)	t-value	P value	95% CI
Probiotic (n=90)	Constant*	68.22	1.21	—	56.41	0.000†	65.81—70.62
	GSI score	1.20	0.44	0.28	2.75	0.007	0.33—2.06
Placebo (n=90)	Constant*	71.19	1.08	—	65.91	0.000†	69.04—73.33
	GSI score	0.59	0.33	0.19	1.79	0.077	0.07— 1.25
		Behavioural symptom scores as evaluated by the ABC-2 tool					
Probiotic (n=90)	Constant*	53.17	2.87	—	18.50	0.000†	47.46— 58.88
	GSI score	2.56	1.05	0.25	2.45	0.016†	0.48 — 4.65
Placebo (n=90)	Constant*	59.96	3.18	—	18.86	0.000†	53.64—66.26
	GSI score	2.81	0.98	0.29	2.88	0.005†	0.87—4.75

Bivariate regression analysis was performed.
*The 'constant' value indicates the expected outcome value when the GI index score is assumed to be zero.
†Indicates statistically significant difference with p<0.05.
ABC-2, Aberrant Behaviour Checklist-2; ASD, autism spectrum disorder; GI, gastrointestinal; GSI, Gastrointestinal Severity Index; SRS-2, Social Responsiveness Scale-2.

CONCLUSION

Probiotic supplementation improved both behavioural and GI symptoms in children with ASD and was well-tolerated, with no adverse events reported. This study also found a statistically significant correlation between behavioural and GI symptoms. Further research with a larger sample size is necessary to substantiate these findings in clinical contexts.

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