Supplementary Materials

Efficacy of EB-F01, Heat-Killed *Akkermansia muciniphila* Strain EB-AMDK19, in Patients with Respiratory Symptoms: A Multicenter Clinical Trial

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Table S1. The complete list of inclusion and exclusion criteria.

Inclusion Criteria
1) Adults aged 19 to 70 years
2) Presence of two or more of the following symptoms (cough, sputum, or shortness of breath/chest tightness) for one month to less than three months, with a BCSS score of 3 or above but less than 9
3) FEV1/FVC ratio of 70% or higher
4) Written informed consent for participation provided before the commencement of the trial
Exclusion Criteria
1) Currently undergoing treatment for severe cardiovascular, immune, gastrointestinal/hepatic/biliary, renal/urinary, neurological, musculoskeletal, psychiatric, infectious diseases, or malignant tumors (individuals with cancer in remission for at least 5 years are eligible)
2) Clinically significant respiratory findings in chest X-ray results
3) Diagnosis of and current medication treatment for chronic obstructive pulmonary disease or asthma
4) Chronic bronchitis (consistent BCSS score of 9 or above for at least 2 years with persistent cough and sputum symptoms lasting at least 3 months annually)
5) Respiratory infection diagnosed by viruses or bacteria within the last four weeks
6) Use of systemic corticosteroids or immunosuppressants within four weeks prior to visit 1
7) Medication adjustment for bronchodilators within one week prior to visit 1
8) Current smokers or individuals who quit smoking or vaping less than six months ago
9) Alcohol consumption exceeding 30 g/day for males or 20 g/day for females within the last four weeks from visit 1
10) Creatinine levels exceeding twice the upper limit of normal
11) AST(GOT) or ALT(GPT) levels exceeding three times the upper limit of normal
12) Uncontrolled hypertension (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg, measured after 10 minutes of stability for trial participants)
13) Uncontrolled diabetes (fasting blood glucose ≥180 mg/dL)
14) Use of antibiotics or antidiarrheals within two weeks prior to visit 1
15) Use of probiotics, prebiotics, or regular intake (≥4 times per week) of probiotic products within two weeks prior to visit 1
16) Pregnancy, lactation, or planning to become pregnant during the trial
17) Sensitivity or allergy to ingredients in the trial-specific food
18) Participation in another interventional clinical trial (including human trials) within three months before visit 1 or planning to participate in another interventional clinical trial (including human trials) after the commencement of this trial
19) Other reasons deemed by the investigator as unsuitable for participation

Figure S1. Mean change from baseline in BCSS total score in the per-protocol set.

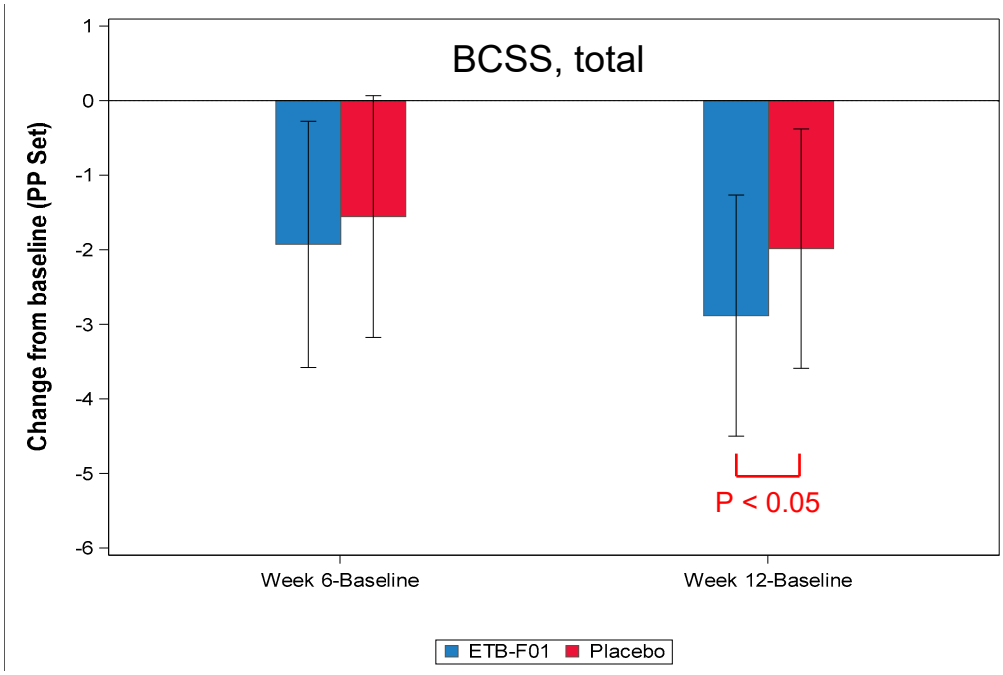


Figure S2. Mean change from baseline in BCSS total score in the full analysis set.

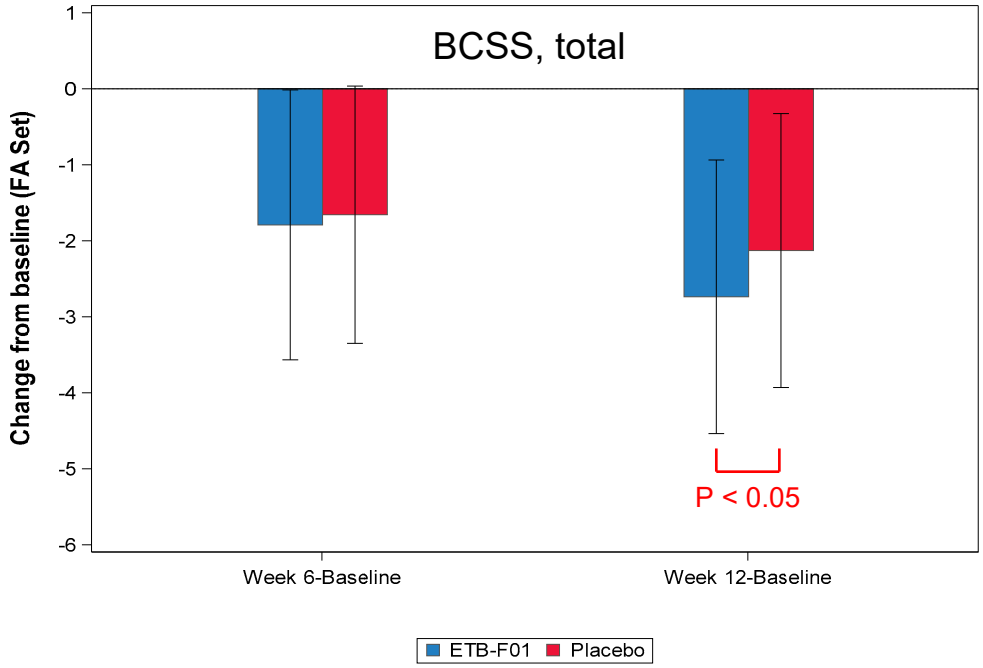


Figure S3. Mean change from baseline in BCSS breathlessness score in the per-protocol set.

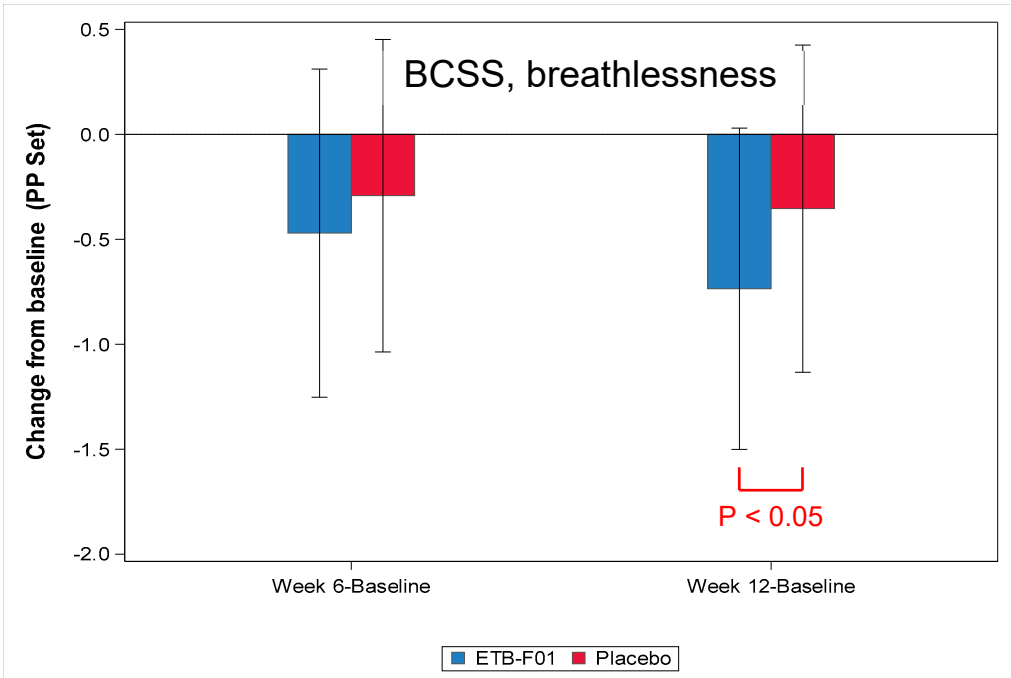


Figure S4. Mean change from baseline in BCSS breathlessness score in the full analysis set.

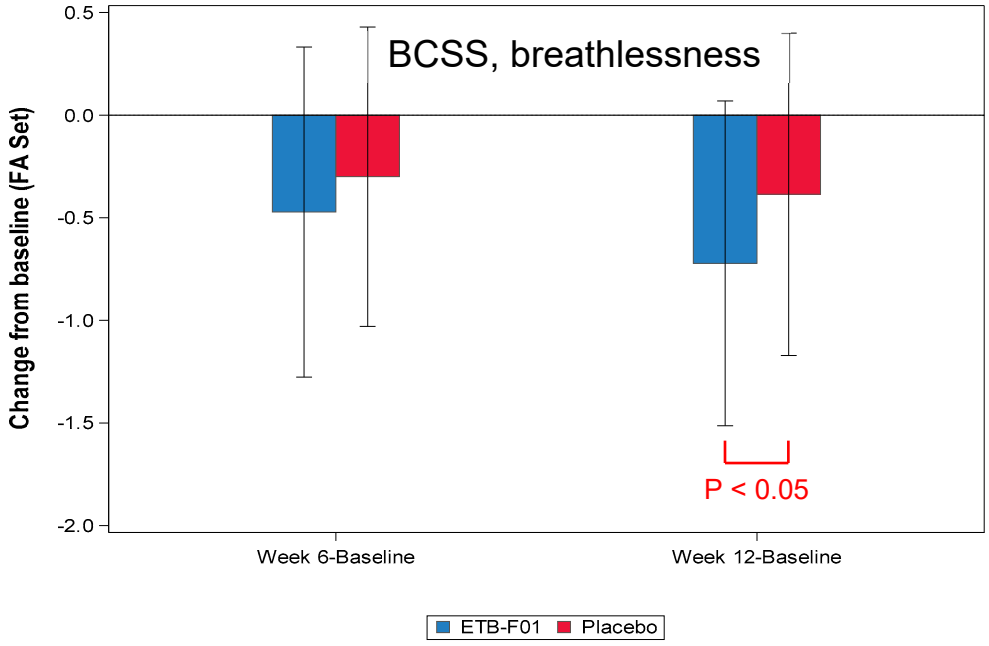


Figure S5. Mean change from baseline in BCSS cough score in the per-protocol set.

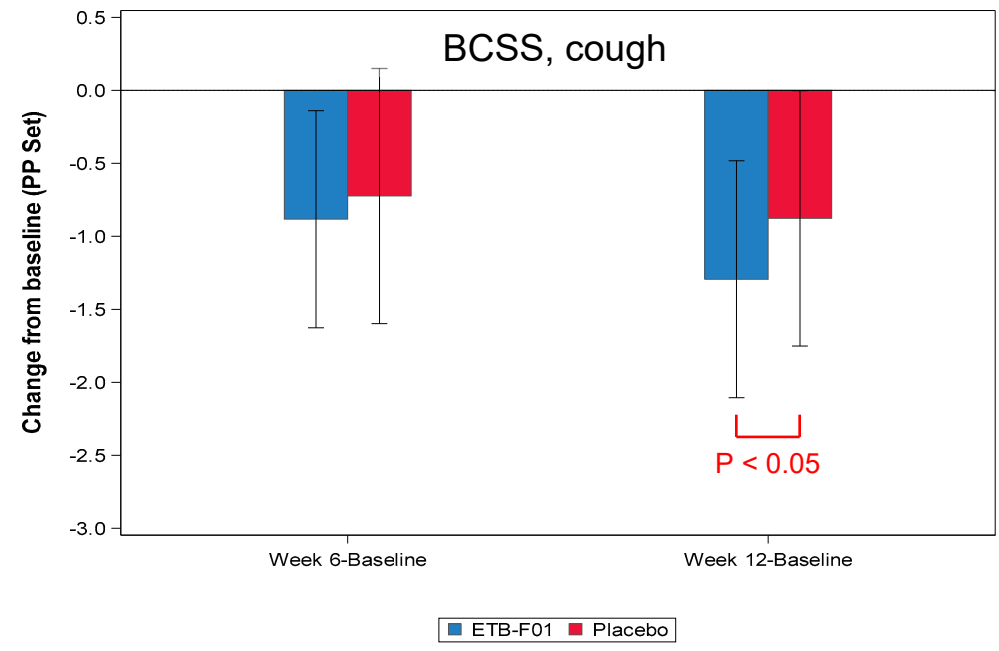


Figure S6. Mean change from baseline in BCSS cough score in the full analysis set.

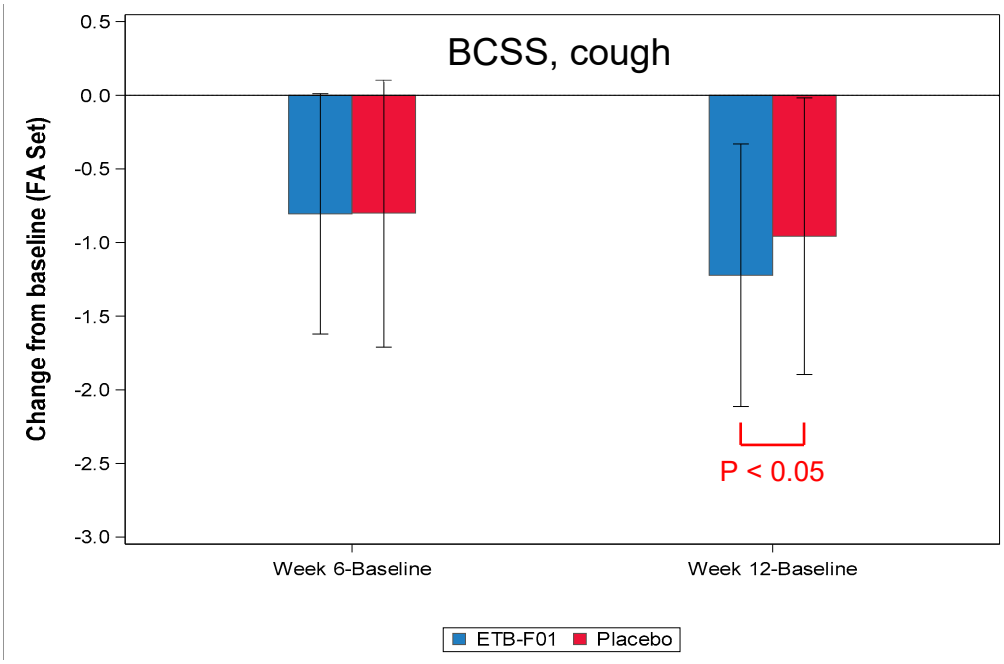


Figure S7. Mean change from baseline in BCSS sputum score in the per-protocol set.

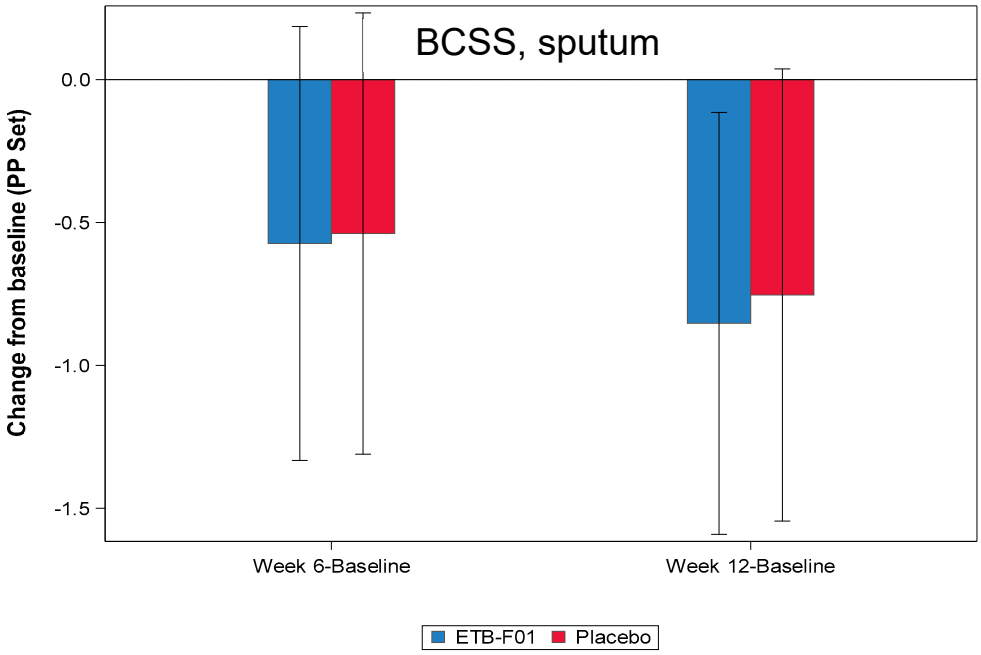


Figure S8. Mean change from baseline in BCSS sputum score in the full analysis set.

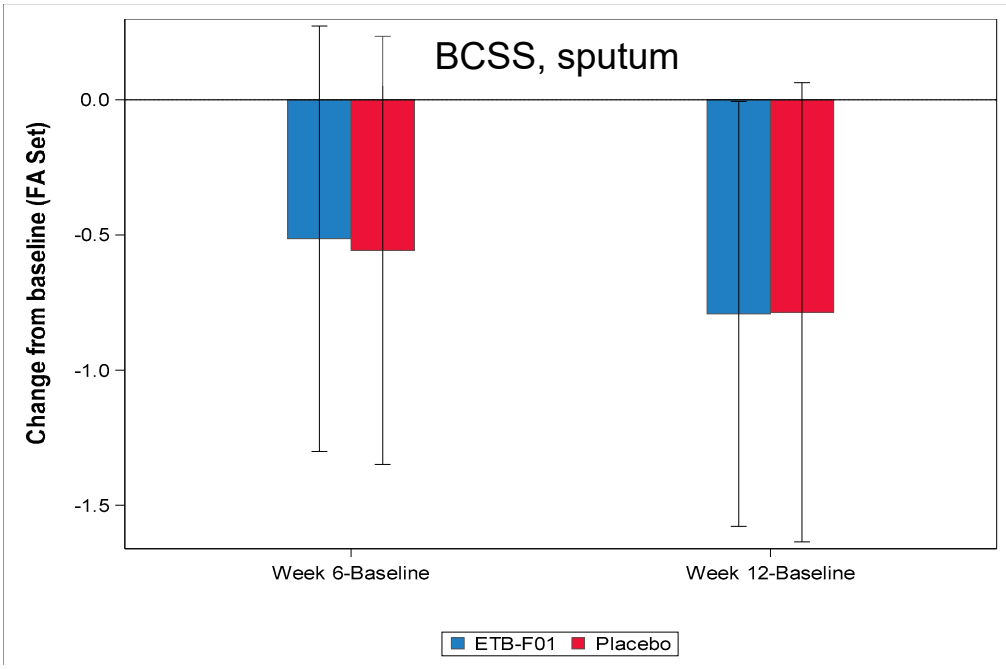


Table S2. mMRC and SGRQ.

Outcome	ETB-F01 (n=68)	Placebo (n=65)
mMRC		
At week 12, mean ± SD	0.6±0.6	0.6±0.5
Change from baseline to week 12, mean ± SD	-0.6±0.8	-0.6±0.6
P-value within groups	<0.001	<0.001
P-value between groups	0.961	
Change from baseline to week 12, LS mean ± SE	-0.5±0.2	-0.5±0.2
Difference in change from baseline to week 12, LS mean, (95% CI)	0.01 (-0.2, 0.3)	
P-value[a]	0.937	
SGRQ		
At week 12, mean ± SD	7.2±11.3	12.3±13.9
Change from baseline to week 12, mean ± SD	-15.4±17.5	-13.7±15.9
P-value within groups	<0.001	<0.001
P-value between groups	0.944	
Change from baseline to week 12, LS mean ± SE	-10.6±4.4	-9.6±3.9
Difference in change from baseline to week 12, LS mean, (95% CI)	-1.0 (-6.8, 4.9)	
P-value[a]	0.745	

CI, confidence interval; LS, least squares; mMRC, Modified Medical Research Council Dyspnea Scale; SD, standard deviation; SE, standard error; SGRQ, St. George's Respiratory Questionnaire. [a] p-value for ANCOVA-adjusted smoking history.

Table S3. VAS.

Outcome	ETB-F01 (n=68)	Placebo (n=65)
VAS breathlessness, mm		
At week 12, mean ± SD	6.2±11.0	9.3±12.4
Change from baseline to week 12, mean ± SD	-18.5±20.3	-15.4±18.5
P-value within groups	<0.001	<0.001
P-value between groups	0.392	
Change from baseline to week 12, LS mean ± SE	-23.5±5.1	-19.6±4.5
Difference in change from baseline to week 12, LS mean, (95% CI)	-3.9 (-10.7, 2.9)	
P-value[a]	0.258	
VAS cough, mm		
At week 12, mean ± SD	8.0±10.3	15.2±15.5
Change from baseline to week 12, mean ± SD	-24.1±20.0	-18.0±18.9
P-value within groups	<0.001	<0.001
P-value between groups	0.074	
Change from baseline to week 12, LS mean ± SE	-28.6±5.1	-21.9±4.5
Difference in change from baseline to week 12, LS mean, (95% CI)	-6.8 (-13.6, 0.0)	
P-value[a]	0.051	
VAS sputum, mm		
At week 12, mean ± SD	11.1±14.3	14.9±16.1
Change from baseline to week 12, mean ± SD	-23.5±20.9	-23.3±21.5
P-value within groups	<0.001	<0.001
P-value between groups	0.973	
Change from baseline to week 12, LS mean ± SE	-28.1±5.6	-27.1±4.9
Difference in change from baseline to week 12, LS mean, (95% CI)	-1.0 (-8.4, 6.5)	
P-value[a]	0.798	

CI, confidence interval; LS, least squares; SD, standard deviation; SE, standard error; VAS, Visual Analog Scale. [a] p-value for ANCOVA-adjusted smoking history.

Table S4. Blood test results.

Variables	ETB-F01 (*n* = 77)			P-value[1]	Placebo (*n* = 73)			P-value[1]	ETB-F01 vs. Placebo P-value[2]
	Baseline	12 Weeks	Mean Change		Baseline	12 Weeks	Mean Change		
Hematological parameters									
WBC (10^3/μL)	6.21 ± 1.58	6.08 ± 1.43	-0.08 ± 1.20	0.58 (P)	6.13 ± 1.72	6.08 ± 1.43	-0.01 ± 1.56	0.60 (S)	0.99 (W)
RBC (10^6/μL)	4.52 ± 0.42	4.49 ± 0.40	0.00 ± 0.20	0.85 (S)	4.56 ± 0.41	4.54 ± 0.46	-0.03 ± 0.25	0.38 (P)	0.57 (W)
Hb (g/dL)	13.50 ± 1.30	13.43 ± 1.24	0.01 ± 0.62	0.92 (P)	13.50 ± 1.40	13.43 ± 1.47	-0.07 ± 0.66	0.36 (P)	0.46 (T)
Hct (%)	40.54 ± 3.55	40.27 ± 3.29	-0.06 ± 2.03	0.74 (S)	40.67 ± 3.39	40.34 ± 3.87	-0.38 ± 1.98	0.12 (P)	0.38 (W)
Platelet (10^3/μL)	259.06 ± 47.07	254.66 ± 56.33	-3.77 ± 30.00	0.31 (S)	270.12 ± 52.27	263.74 ± 54.89	-6.71 ± 28.13	0.05 (P)	0.35 (W)
Neutrophil (%)	55.28 ± 8.62	55.03 ± 8.85	0.16 ± 8.18	0.59 (S)	56.61 ± 8.99	56.57 ± 9.63	-0.26 ± 8.88	0.81 (P)	0.95 (W)
Lymphocyte (%)	35.08 ± 7.89	35.22 ± 8.49	-0.15 ± 6.83	0.73 (S)	33.90 ± 7.88	33.34 ± 8.68	-0.37 ± 7.92	0.70 (P)	0.66 (W)
Monocyte (%)	6.59 ± 1.97	6.77 ± 1.95	0.14 ± 1.73	0.51 (P)	6.20 ± 1.68	6.50 ± 1.82	0.34 ± 1.35	0.01 (S)	0.35 (W)
Eosinophil (%)	2.12 ± 1.70	2.04 ± 1.07	-0.17 ± 1.62	0.53 (S)	2.39 ± 2.18	2.73 ± 2.09	0.35 ± 1.69	0.06 (S)	0.47 (W)
Basophil (%)	0.56 ± 0.32	0.60 ± 0.29	0.03 ± 0.26	0.23 (S)	0.60 ± 0.32	0.62 ± 0.32	0.02 ± 0.24	0.58 (P)	0.60 (W)
Biochemical parameters									
AST (GOT)(IU/L)	20.66 ± 5.49	21.86 ± 7.42	1.52 ± 6.82	0.10 (S)	20.23 ± 5.05	20.00 ± 6.48	0.04 ± 6.40	0.34 (S)	0.07 (W)
ALT (GPT)(IU/L)	17.81 ± 10.48	19.20 ± 14.99	2.54 ± 13.12	0.15 (S)	17.25 ± 9.23	16.65 ± 8.23	-0.59 ± 6.09	0.51 (S)	0.14 (W)
Total Cholesterol (mg/dL)	195.60 ± 28.79	194.01 ± 31.02	-0.07 ± 20.85	0.83 (S)	196.13 ± 36.72	200.16 ± 39.46	2.24 ± 19.56	0.36 (P)	0.79 (W)
Glucose (mg/dL)	95.39 ± 13.81	94.96 ± 12.83	-0.59 ± 16.29	0.95 (S)	96.82 ± 11.75	93.68 ± 11.06	-2.85 ± 14.11	0.16 (S)	0.34 (W)
Total Protein (g/dL)	7.44 ± 0.35	7.36 ± 0.36	-0.07 ± 0.32	0.07 (P)	7.47 ± 0.39	7.41 ± 0.44	-0.06 ± 0.39	0.07 (S)	0.63 (W)
BUN (mg/dL)	12.26 ± 3.08	12.58 ± 3.50	0.13 ± 2.92	0.71 (P)	12.84 ± 3.68	13.24 ± 2.99	0.38 ± 3.55	0.38 (P)	0.65 (T)
Creatinine (mg/dL)	0.71 ± 0.14	0.73 ± 0.14	0.02 ± 0.11	0.20 (S)	0.71 ± 0.14	0.74 ± 0 .14	0.02 ± 0.09	0.14 (S)	0.91 (W)
Uric acid (mg/dL)	4.51 ± 1.03	4.59 ± 1.04	0.12 ± 0.65	0.14 (P)	4.69 ± 1.19	4.95 ± 1.45	0.23 ± 0.76	0.02 (P)	0.35 (T)
Ca (mg/dL)	9.53 ± 0.41	9.54 ± 0.35	0.01 ± 0.33	0.99 (S)	9.58 ± 0.38	9.51 ± 0.39	-0.06 ± 0.36	0.20 (P)	0.32 (W)

The values are presented as the mean ± SD.
[1] Compared within groups; P-value for paired t-test (P) or Wilcoxon signed rank test (S).
[2] Compared between groups; P-value for two-sample t-test (T) or Wilcoxon rank sum test (W).

Table S5. BCSS score according to sex groups.

Variables	12 Weeks	Mean Change	P-value[1]	12 Weeks	Mean Change	P-value[1]	ETB-F01 vs. Placebo P-value[2]
Females	**ETB-F01 (*n* = 55)**			**Placebo (*n* = 46)**			
BCSS total score	1.38±1.24	-2.93±1.61	< 0.0001	2.37±1.61	-1.89±1.55	< 0.0001	0.0017
BCSS breathlessness score	0.35±0.55	-0.75±0.75	< 0.0001	0.70±0.63	-0.28±0.81	0.0219	0.0067
BCSS cough score	0.49±0.60	-1.31±0.84	< 0.0001	0.91±0.72	-0.87±0.83	< 0.0001	0.0156
BCSS sputum score	0.55±0.57	-0.87±0.72	< 0.0001	0.76±0.67	-0.74±0.80	< 0.0001	0.4066
Males	**ETB-F01 (*n* = 13)**			**Placebo (*n* = 19)**			
BCSS total score	2.31±1.65	-2.69±1.70	< 0.0001	2.11±2.47	-2.21±2.10	< 0.0001	0.5827
BCSS breathlessness score	0.62±0.65	-0.69±0.85	0.0128	0.47±0.51	-0.53±0.70	0.0040	0.5419
BCSS cough score	0.85±0.69	-1.23±0.73	< 0.0001	0.84±0.69	-0.89±0.99	0.0010	0.4108
BCSS sputum score	0.85±0.69	-0.77±0.83	0.0060	0.79±0.63	-0.79±0.79	0.0004	0.8432

The values are presented as the mean ± SD.
[1] Compared within groups; P-value for paired t-test (P).
[2] Compared between groups; P-value for two-sample t-test (T).