**SUPPLEMENT FILE**

**Efficacy and Safety of *Bacillus coagulans* LBSC in Irritable Bowel Syndrome: A Prospective, Interventional, Randomized, Double-Blind, Placebo-Controlled Clinical Study [CONSORT Compliant]**

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**Methods**

Method of Analysis

The investigational product (IP) containing active ingredient, *Bacillus coagulans* LBSC [2000 million active spores per gram per sachet (2000×106 active spore/g/sachet) which is equivalent to 2 billion active spores per gram per sachet (2×109 active spore/g/sachet)], was supplied by Advanced Enzyme Technologies Ltd., Thane, India. The viable spore count of *B. coagulans* LBSC was determined by pour plate technique. Briefly, one sachet of IP powder (1.00 g) was suspended in tween peptone water (%, compositions: protease peptone, 1.0 %; sodium chloride, 0.5 %; disodium phosphate, 0.35 %; monosodium phosphate, 0.15; tween 80, 0.2 %) and serially diluted. The diluted samples were given heat shock in a water bath for 30 min at 75°C, followed by immediate cooling to below 45°C. 1.0 ml of thus obtained treated spore suspension was dispensed in petriplate and presterilized molten GYE agar (M963, HiMedia, Mumbai, India) was added to the plates. The plates were allowed to solidify and invertedly incubated at 37 °C for 48–72 h. The activity was expressed in colony forming units of viable spores per gram (cfu/g) of powder by taking the average mean of results. The placebo contained only the excipient, maltodextrin. Both the investigational and placebo product had stringently passed through other required specifications like physical appearance, microscopic appearance, strength, and microbial limit [other aerobic microbial count, yeast & mold count, *E. coli*, *Salmonella* spp., *S. aureus* and *P. aeruginosa*]. All microbial limits were analysed following standard Indian Pharmacopeia guidelines or other international standard guidelines. All the analyses were performed in triplicate, for three times.

Details of Exclusion and Withdrawal Criteria

Exclusion Criteria

The subjects who had following criteria were excluded for the study

* On antibiotics or laxatives within the preceding 6 weeks.
* Presence of inflammatory bowel disease
* Presence of acute GI tract infection
* Presence of fever, abdominal mass, signs of bowel obstruction
* History of colon cancer or diverticulitis
* Infection by human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus
* Patients with celiac disease defined by biopsy of the duodenal mucosa.
* History of scleroderma and gastroparesis
* Hypothyroidism

### *Withdrawal Criteria*

The patient can be withdrawn from the study by the investigator for any of the following:

* Occurrence of an adverse event, associated with the administration of the IP and requiring its cancellation.
* Emergence of any diseases or conditions during the study that worsen the prognosis of the patient, as well as make it impossible for the patient to continue his/her participation in the clinical study.
* The need for a forbidden concomitant therapy.
* Pregnancy of the patient.
* Violation of the study protocol, like

Improper inclusion of the patient who did not meet the inclusion criteria and/or met the relevant exclusion criteria; other violations of the protocol, which, according to the investigators, are significant.

* Withdrawal of the informed consent by the patient

**Supplement Table 1** Results of vital examinations at different visits (visit 01 – visit 05) from both Test-G and Placebo-H arm. Values are expressed as mean ± SD.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study arms** | **Parameters** | **Visit 01** | **Visit 02** | **Visit 03** | **Visit 04** | **Visit 05** |
| **Test-G** | Pulse (per minute) | 73.1±3.34 | 72.25±3.37 | 70.63±2.49 | 72.79±4.81 | 71.15±3.85 |
| Respiratory rate (per minute) | 19.00±1.77 | 19.70±1.62 | 19.47±2.19 | 22.47±13.56 | 20.10±2.05 |
| Systolic blood pressure (mm/Hg) | 118.10±9.64 | 117.90±28.97 | 117.84±7.78 | 117.32±6.63 | 119.79±5.12 |
| Diastolic blood pressure (mm/Hg) | 77.40±7.71 | 77.90±8.19 | 77.16±7.81 | 77.47±7.65 | 75.58±7.23 |
| Temperature (°F) | 98.18±0.46 | 98.21±0.47 | 98.23±0.47 | 98.21±0.46 | 98.23±0.41 |
| **Placebo-H** | Pulse (per minute) | 71.30±4.78 | 70.75±4.34 | 70.84±3.85 | 70.58±4.10 | 71.47±3.02 |
| Respiratory rate (per minute) | 19.75±1.99 | 19.70±2.54 | 19.58±2.36 | 19.89±1.41 | 19.42±1.38 |
| Systolic blood pressure (mm/Hg) | 116.00±8.02 | 119.70±6.75 | 115.79±8.46 | 115.58±7.70 | 117.58±5.11 |
| Diastolic blood pressure (mm/Hg) | 76.70±7.66 | 76.80±7.35 | 75.68±8.52 | 73.79±9.28 | 74.95±6.94 |
| Temperature (°F) | 98.05±0.49 | 98.26±0.36 | 98.26±0.49 | 98.22±0.51 | 98.26±0.39 |
| **Group mean difference (Test-G to Placebo-H) by one-way ANOVA** |
|  |  | **F ratio**  | **P value (p<0.05)** |  |
| Pulse (per minute) | 3.913 | 0.083 |  |
| Respiratory rate (per minute) | 0.614 | 0.455 |  |
| Systolic blood pressure (mm/Hg) | 2.033 | 0.191 |  |
| Diastolic blood pressure (mm/Hg) | 4.833 | 0.059 |  |
| Temperature (°F) | 0.002 | 0.962 |  |

**Supplement Table 2** Results of hematological and biochemical parameters at baseline and at the end of treatment (EOT) in Test-G and Placebo-H group. Values expressed as mean ± S.D.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Visit** | **Test-G** | **Placebo-H** | **P value (p<0.05)**  | **95% CI** | **Normal range** |
| *Hemoglobin (g%)* | Baseline | 14.2±1.80 | 14.3±1.60 | 0.8574 | -1.02 - 1.22 | 13-16 (Male)12-15 (Female) |
| EOT | 14.00±1.70 | 13.9±1.40 | 0.8442 | -1.12 - 0.92 |
| *SGOT (AST) (U/L)* | Baseline | 28.74±11.66 | 25.31±11.10 | 0.3592 | -10.92 - 4.06 | 15-37 |
| EOT | 26.68±10.34 | 26.63±6.22 | 0.9857 | 5.66 - 5.56 |
| *SGPT (ALT) (U/L)* | Baseline | 37.63±20.11 | 35.84±17.50 | 0.7714 | -14.19 - 10.61 | 30-65 |
| EOT | 35.31±17.13 | 37.79±13.32 | 0.6214 | -7.61 - 12.58 |
| *Creatinine (mg/dl)* | Baseline | 0.83±0.17 | 1.31±2.06 | 0.3182 | -0.48 - 1.44 | 0.5-1.4 |
| EOT | 0.80±0.16 | 0.84±0.17 | 0.4600 | -0.07 - 0.15 |
| *BUN (mg/dl)* | Baseline | 9.71±3.86 | 8.39±3.44 | 0.2732 | -3.72 - 1.08 | 14-36 |
| EOT | 10.88±3.98 | 9.82±3.18 | 0.3705 | -3.43 - 1.31 |
| *RBC (million/cmm)* | Baseline | 5.12±0.50 | 5.02±0.39 | 0.4962 | -0.39 - 0.19 | 4.60-6.00 (Male)4.00-5.40 (Female) |
| EOT | 5.10±0.42 | 4.96±0.38 | 0.2885 | -0.40 - 0.12 |
| *Total leukocyte count**(Cells cu. mm−1)* | Baseline | 7879.74±2042.25 | 8014.74±2180.7 | 0.8450 | -1255.09 - 1525.09 | 4,000.00-11,000.00 |
| EOT | 8030.00±2252.75 | 7878.95±1871.59 | 0.8234 | -1513.74 -1211.64 |
| *Eosinophils (%)* | Baseline | 3.54±1.98 | 2.99±2.03 | 0.4035 | -1.87 -0.77 | 1-7 |
| EOT | 4.47±2.35 | 3.11±1.63 | 0.0454 | -2.69 - -0.03 |
| *Basophils (%)* | Baseline | 0.36±0.31 | 0.77±0.96 | 0.0849 | -0.06 - 0.88 | 0-1 |
| EOT | 0.42±0.41 | 0.44±0.36 | 0.8739 | -0.23 - 0.27 |
| *Neutrophils (%)* | Baseline | 59.54±9.45 | 60.82±9.43 | 0.6785 | -4.93 - 7.49 | 40-75 |
| EOT | 57.96±8.37 | 58.17±10.89 | 0.9472 | -6.18 - 6.60 |
| *Lymphocytes (%)* | Baseline | 30.24±8.61 | 28.99±8.17 | 0.6490 | -6.77 - 4.27 | 20-42 |
| EOT | 30.85±8.17 | 32.02±9.21 | 0.6812 | -4.56 - 6.89 |
| *Monocytes (%)* | Baseline | 6.33±1.68 | 6.44±1.29 | 0.8222 | -0.87 - 1.09 | 2-10 |
| EOT | 6.34±1.68 | 7.20±2.21 | 0.1853 | -0.43 - 2.15 |
| *Hematocrit (PCV %)*  | Baseline | 42.81±4.87 | 42.89±3.83 | 0.9554 | -2.80 - 2.96 | 40-50 (Male)36-46 (Female) |
| EOT | 42.80±4.74 | 41.82±3.93 | 0.4923 | -3.84 - 1.88 |
| *ESR (mm/hr)*  | Baseline | 21.66±15.85 | 17.16±15.15 | 0.3769 | -14.70 - 5.70 | 0-20 (Male)0-30 (Female) |
| EOT | 14.84±10.21 | 15.42±10.93 | 0.8667 | -6.37 - 7.53 |
| *Platelet count (Million/cmm)* | Baseline | 0.31±0.11 | 0.27±0.09 | 0.2279 | -0.11 - 0.03 | 0.15-0.45 |
| EOT | 0.29±0.09 | 0.25±0.10 | 0.2032 | -0.10 - 0.02 |