# Supplemental therapy to attenuate the acute respiratory disorder by exogenous administration of ketone monoester: A single-arm clinical trial study

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***Abstract***

***Background:*** *Acute respiratory Disorder (ARD) has a significant fatality rate due to airway inflammation and immune system response accompanied by a cytokine storm. This could be described at the molecular level by decreased energy metabolism, reduced oxidation changes, oxidative damage, and cell death. BHB stimulates anti-inflammatory markers and suppresses NLRP3 inflammasome activity. This study aimed to assess the efficacy of exogenous ketone monoesters beta-hydroxybutyrate as an adjuvant treatment for ARD patients.* ***Methods:*** *Fifteen ARD patients enrolled in the single treatment arm study; all subjects were verified by RT-PCR and administered the product orally twice daily on an empty stomach for five days, along with standard treatment. The efficacy of beta-hydroxybutyrate in addressing mild respiratory stress in patients was determined by comparing, from day 0 to 5, forced expiratory volume, forced vital capacity, blood gas analysis, muscle weakness, and biomarkers, including tumour necrosis factor-alpha, interleukin-1β and interleukin-6.* ***Results:*** *Beta-hydroxybutyrate considerably reduced muscle weakness, as evidenced by reduced serum creatine kinase levels ($p \leq 0.05$). Clinically, the medication reduced respiratory discomfort to some extent, decreased interleukin levels and showed a significant shift in blood oxygen saturation from the initial day ($p=0.014$) while improving clinical symptoms.* ***Conclusion:*** *Oral intake of beta-hydroxybutyrate is safe and may positively influence ARD*

***Trial registration:*** *Indian Clinical Trial Registry on October 5, 2020, http://ctri.nic.in. [Registration No. CTRI/2020/10/028231]*

***Keywords:*** *Beta-Hydroxybutyrate, ARD, Interleukin-1β, Interleuin-6, Tumor Necrosis Factor-α.*

## 1. Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection in humans has an acute phase and is associated with a higher fatality rate than regular influenza infections. In the initial phase, there can be significant changes in innate immunity, biochemical reactions and acute respiratory distress syndrome (ARDS) in certain patients. A ketogenic diet or exogenous administration of ketones can help to restore altered NAD+/NADH, which helps to generate energy, and NADP+/NADPH helps to reduce oxidative stress. 3-hydroxybutyric acid (BHB), also known as beta-hydroxybutyrate or -hydroxybutyric acid (BHB), can inhibit histone deacetylases and the NLRP3 inflammasome while activating anti-inflammatory GPR109A signalling [1]. In addition, electron transport chain (ETC) gene expression can be increased by ketogenic diet intake. Thus, exogenous ketone delivery may be helpful during the early stages of respiratory distress. BHB mitigates oxidative stress and restores coenzyme ratios and redox reactions. [2]

Many clinical and non-clinical investigations have targeted cytokines or their receptors to capture the cytokine storm while tackling ARDS. For example, tocilizumab, an Interleukin-6 receptor monoclonal antibody (mAb) antagonist, has been approved for treatment. [3-5]

Although many sceptics of IL-6's participation in the cytokine storm syndrome or cytokine release syndrome (CRS), a large amount of evidence suggests that targeting IL-6 will reduce CRS and hence alleviate respiratory discomfort in ARDS patients [6].

Exogenous administration of BHB or similar derivatives increases ketone molecules in the blood and changes the ratios of the regulating coenzyme without following a high-calorie diet. As a metabolic therapy, BHB exerts various anti-inflammatory signalling effects and modulates metabolism to restore cellular redox function through an epigenetic modifier by initiating a gene expression programme that considerably governs metabolic flux through major metabolic pathways [7].

When virus-induced alterations in enzymatic activities may alter the metabolism of glucose [8] or fatty acids [9, 10], BHB-derived molecules assist in reviving the flux through oxidative phosphorylation. When BHB levels increase in the body, the fatty acid synthesis will rise, and as a result, NADH will increase, which helps to provide energy. The escalation of NADPH helps to raise oxyhemoglobin levels [11].

In this study, patients participated in a clinical trial of a formulation that included BHB. The goal was to learn more about the level of respiratory distress and how beta-hydroxybutyrate helped patients feel less tired.

## 2. Methods

The trial was carried out in a hospital where the critical care unit was established with the approval of the institutional ethics committee [IEC approval no. BPLS/KTJ/2020] was registered with the Indian Clinical Trial Registry on October 5, 2020 [Registration No. CTRI/2020/10/028231], with the first enrollment on October 12, 2020. The ethical principles of the Helsinki Declaration and as specified in the ICH-GCP and ICMR-New Delhi were followed for conducting the clinical study.

None of the subjects were recruited without their informed consent.

The BHB was administered twice daily for five days to all enrolled subjects, and ten days follow-up was done to find better support of efficacy or any adverse effect (Figure 1). Before administering the formulation to volunteers, medical history and demographic data were collected.

On screening day (day 0), cardiovascular parameters, body temperature, pulmonary function tests (forced expiratory volume (FEV1) and forced vital capacity (FVC) by spirometer), and partial pressures of blood gases ($pO_2$ and $pCO_2$) were recorded. 3 mL venous blood samples were collected at 0 hours and 2 hours for biochemical estimations such as interleukin-1beta (IL-1β) and interleukin-6 (IL-6) levels, tumour necrosis factor-alpha (TNF-α), serum creatine kinase, serum lactate, and serum BHB.

Subjects received the investigational product (BHB) as two bottles of 60 ml each in equally divided doses (i.e., twice a day, at least 8 hours apart) for five consecutive days in the fasting stage. The investigational product (60 mL) contained 25 g of beta-hydroxybutyrate per bottle.

On day 3, vital signs and pulmonary function test results were obtained using a spirometer, and blood oxygen saturation was recorded.

On day 5, Subjects were assessed for the same efficacy parameters, viz., vital signs, pulmonary function tests (FEV1 and FVC), blood gas analysis ($pO_2$, $pCO_2$), oxygen supply/ventilator support, and blood sampling for specific Interleukins (IL-1β, IL-6, TNF-α, serum creatine kinase and serum lactate). Also, BHB levels were measured and analyzed before (0 h) and after (2 h) product intake. The subjects were contacted by phone at least ten days after the fifth day to inquire about their overall well-being. The daily dietary intake of the subjects was recorded in the patient diaries. A standard diet menu was provided to nearly all 15 patients.

### 2.1 Statistics

The data generated from the 15 patients were compared and analyzed using SPSS version 20.0. Descriptive statistics for various patient characteristics were obtained at screening and on the $5^{th}$ day. All efficacy and safety parameters were analyzed in the study using a student t-test' and p-$\leq$0.05 was considered statistically significant.

## 3. Results

### *3.1 Demographic data:*

The total duration of this trial was 15 days (5 days of treatment and ten days of follow-up). The most prevalent dominant variations in this investigation were Alpha and Delta; however, since the variants were not detected, it is impossible to determine which variant affected each patient. The mean age of the patients included in the study was 46.47 years. The youngest and oldest participants were 26 and 71, respectively. Males accounted for 66.7% of the population, while females accounted for 33.3%. The patients' mean BMI at the screening was 23.97±7.58 $kg/m^2$, which remained stable on follow-up days. During the trial treatment period, there were no changes in the participants' demographic data, such as height, weight, or BMI. The mean values for the demographic parameters were within the normal range (Figure 2).

***3.2 Vital parameters:***

The body temperature, systolic blood pressure, diastolic blood pressure, mean heart rate, mean pulse rate, and mean respiratory rate were analyzed at screening, and on day 3rd and 5th were normal (Figure 3).

***3.3 Concentration of blood beta-hydroxybutyrate (BHB):***

The assessment of BHB at screening (prior dose was 0.08 mmol/L) before and after the dose (post-dose value was 0.18 mmol/L) revealed a statistically significant increase (p=0.029). In comparison, on day 5 (prior dose was 0.15 mmol/L and post-dose, 0.39 mmol/L), the change in the mean product was statistically insignificant (p=0.057), which indicates that BHB quantification should have been performed immediately after administration instead of 2 h post-dose (Figure 4).

Oxygen and mechanical support were not required for patients at any of the three-time points. Most VAS questionnaires reported relief from muscle fatigue over time using supplementary file 1.

***3.4 Hematological parameters:***

A total of 20 ml of venous blood was collected from each of the 15 volunteers at screening and on day 5. The comparison of haematological parameters between screening and the 5th day revealed that the total leukocyte and platelet counts showed a statistically significant increase (p=0.001 and p=0.008, respectively). The erythrocyte sedimentation rate exhibited a statistically significant drop in the mean values (p=0.037) (Table 1).

***3.5 Biochemical parameters:***

The result showed a significant decrease in serum alanine transaminase (ALT), serum aspartate transaminase (AST), serum creatine kinase and serum lactate from screening day to 5th day (Figure 5).

***3.6 Immunological marker:***

IL-1β, IL-6 and TNF-α decreased on day five compared to the screening day; however, the difference was not statistically significant (figure 6).

***3.7 Pulmonary function tests:***

The mean FEV1 and FVC showed no significant change from screening to day 5 (p>0.05). The increase in the mean partial pressure of $CO_2$ was not statistically significant; however, the difference in the partial pressure of $O_2$ was statistically significant (p = 0.014). Additionally, the P/F ratio differed significantly over time (p=0.045). The results of pulmonary function tests are shown in Figure 7.

## 4. Discussion

Nuclear factor kappa-B (NF-kB) and hydroxycarboxylic acid receptor 2 (HCAR2) are essential factors in the proinflammatory mechanism that NF-kB will induce through many signalling passages. NF-kB may get actuated during coronavirus infection [12, 13]. After activation, NF-kB induces proinflammatory markers like IL-1β, IL-6 and TNF-α and activates inflammasome, which may cause inflammation [14-17]. The outcome of this study indicates that BHB can reduce proinflammatory factors and thus show significant anti-inflammatory effects mediated by HCAR-2 and NF-kB.

ARDS occurs when the immune responses are insufficient to create a defence against microbial infections and can also be due to airway endothelial damage, alveolar oedema, and interstitial fibrosis [18]. Proinflammatory markers and mitochondrial dysfunction lead to damage of epithelial cells and cellular death, which may result in ARDS. [19]. Iatrogenic injury during treatment (ventilation) can be a reason for inflammation and mechanical damage [20]. Current therapy has focused on supportive care that maintains enough gas exchange to minimize the injury [21].

BHB may activate innate immunity, inhibit NLRP3 in peripheral macrophages to reduce inflammatory disorders, and also inhibit NF-κB-mediated inflammation by binding to HCAR2; in response to microbial infection or cellular injury, the NLRP3 inflammasome promotes the activation of caspase-1 and the release of proinflammatory cytokines such as IL-1β [22, 23]. Figure 8 demonstrates the hypothetical pathway of the BHB in prevention to reduce inflammation.

Oxidation of the ketones by Kupffer cells decreases fibrosis in high-fat induced hepatic injury [24]. The exertion of the innate immune response extends to the alveoli and lungs. Similarly, other effects of BHB include enhanced mitochondrial energy production, decreased oxidative stress, improved resistance to ischemia, and hypoxia, which may be relevant in reducing alveolar injury in ARDS. Ketosis promotes insulin sensitivity, which has previously been reported [25, 26]. Insulin increases the activity of the pyruvate dehydrogenase multi-enzyme complex, resulting in more acetyl-CoA being available for utilization in the Krebs cycle. Insulin or ketone bodies boosted acetyl-CoA production, suggesting that ketosis might mimic insulin metabolic effects [25, 27, 28].

BHB may raise the content of γ-aminobutyric acid (GABA) in the epileptic brain by preventing astrocytic GABA breakdown, which might explain its anti-epileptic actions [29]. Compared to seizures induced by a ketogenic diet, BHB is better at treating epilepsy connected with metabolic problems.

BHB has also been discovered to protect neurons from harm caused by glutamate-mediated lipid oxidation and glycolysis inhibition [30, 31]. BHB treatment enhances glutamate transport in the brain and has anti-convulsant properties [32].

In this study, none of the patients progressed to severe symptoms. Vitals parameters were under control on day five and were measured and recorded during all visits. None of the enrolled subjects had serious disorders or diseases. The safety laboratory parameters, haematology, and serum chemistry were within the normal limits on day 5. No clinically significant abnormalities were observed in vital signs, laboratory parameters, or adverse effects, indicating that the active product was safe for administration. The subjects were not permitted to take any prescription medicines or over-the-counter (OTC) products (including vitamins and products of natural origin) during the study.

## 5. Conclusion

At the end of the study, the patients were relieved of muscle fatigue, as they responded positively to questions regarding the VAS score for muscle fatigue. None of the patients experienced adverse effects during the study period or the post-study follow-up; anyhow, in addition to safety, the present data needs another clinical trial with a larger sample size for

better support. Oxygen pressure increased; out of this, we can conclude that BHB may reduce respiratory stress and might be the reason for lactate level reduction. Hence, it was supposed that BHB might affect muscle fatigue, ARDS symptoms, and interleukin levels and improve patients' symptoms. Further, a clinical trial study with more participants will give a better understanding and support for BHB effectiveness in Symptom management of ARDS.

**List of abbreviations**

| | |
|---|---|
| SEVERE ACUTE RESPIRATORY SYNDROME-CORONAVIRUS-2 | SARS-CoV-2 |
| ACUTE RESPIRATORY DISTRESS SYNDROME | ARDS |
| BETA-HYDROXYBUTYRATE | BHB |
| ELECTRON TRANSPORT CHAIN | ETC |
| CYTOKINE RELEASE SYNDROME | CRS |
| FORCED EXPIRATORY VOLUME | FEV1 |
| FORCED VITAL CAPACITY | FVC |
| INTERLEUKIN-1BETA | IL-1β |
| INTERLEUKIN-6 | IL-6 |
| TUMOUR NECROSIS FACTOR-ALPHA | TNF-α |
| CREATINE KINASE | Cr K |
| ALANINE TRANSAMINASE | ALT |
| ASPARTATE TRANSAMINASE | AST |
| NUCLEAR FACTOR KAPPA-B | NF-kB |
| HYDROXYCARBOXYLIC ACID RECEPTOR 2 | HCAR2 |
| OVER-THE-COUNTER | OTC |

**Declaration**

**Ethics Approval and Consent to Participate:** The trial was conducted in a hospital where the critical care unit was established with the approval from the Institutional Ethics Committee [IEC approval no. BPLS/KTJ/2020] and registered under Indian Clinical Trial Registry [Registration No. CTRI/2020/10/028231]. The ethical principles of the Helsinki Declaration and as specified in the ICH-GCP and ICMR-New Delhi were followed for conducting the clinical study. None of the subjects were recruited without informed consent.

**Consent for publication:** Not applicable

**Availability of data and materials:** This article includes all the data generated or analyzed during this study. Request for Raw data and material should be made to the corresponding author.

**Competing Interest:** All the authors have no relevant financial or non-financial interests to disclose.

**Funding:** The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

**Author Contributions**

"**Navid Reza Shahtaghi**, **Samira Bigdelitabar** and **Subhet Kumar Jain** contributed to the study conception and design. **Navid Reza Shahtaghi** and **Samira Bigdelitabar** performed material preparation, data collection, and analysis. **Navid Reza Shahtaghi** wrote

the first draft of the manuscript**,** and then **Subhet Kumar Jain** and **Samira Bigdelitabar** commented on previous versions. All authors read and approved the final manuscript."

**Acknowledgements:** We would like to thank Bio-plus Life Science Company for providing the chemical materials ex-Gracia to conduct this study. The researchers and investigators would also like to thank all the doctors and nurses at the hospital who helped us during this study.

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**Table 1.** Changes in various haematological parameters on screening and day 5.

| Haematological parameters | Screening | Day 5 |
|---|---|---|
| | Mean± SD | |
| Haemoglobin (g/dl) | 13.62±2.16 | 14.04±2.41 |
| RBC count (M/µl) | 4.85±0.53 | 5.01±0.69 |
| MCV (fl) | 86.07±7.68 | 85.87±6.58 |
| MCH (pg) | 28.07±3.28 | 28.01±2.74 |
| MCHC (g/dl) | 32.50±1.11 | 33.42±1.08 |
| Total leukocyte count | 5935.71±421.82 | 9357.14±295.06 |
| Neutrophil (%) | 70.26±16.75 | 89.60±12.05 |
| Lymphocyte (%) | 22.89±13.32 | 20.40±11.22 |
| Monocyte (%) | 5.36±3.88 | 5.27±1.78 |
| Eosinophil (%) | 1.19±0.71 | 1.55±1.30 |
| Basophil (%) | 0.13±0.15 | 0.09±0.07 |
| Erythrocyte sedimentation rate (mm/hr) | 19.93±5.62 | 10.50±4.07 |
| Platelet count (k/µl) | 200.80±20.92 | 266.33±33.48 |

**p<0.05, significant change in total leukocyte count, ESR, and platelet count on day 5.*

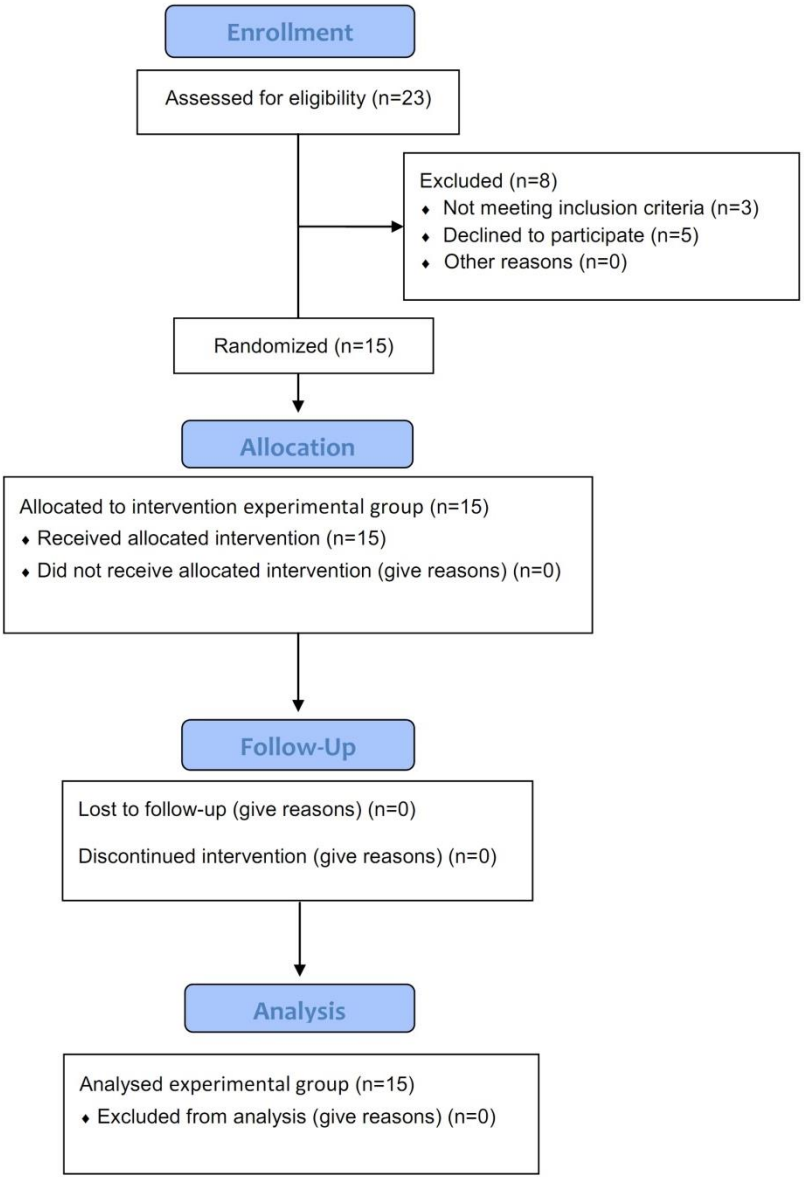
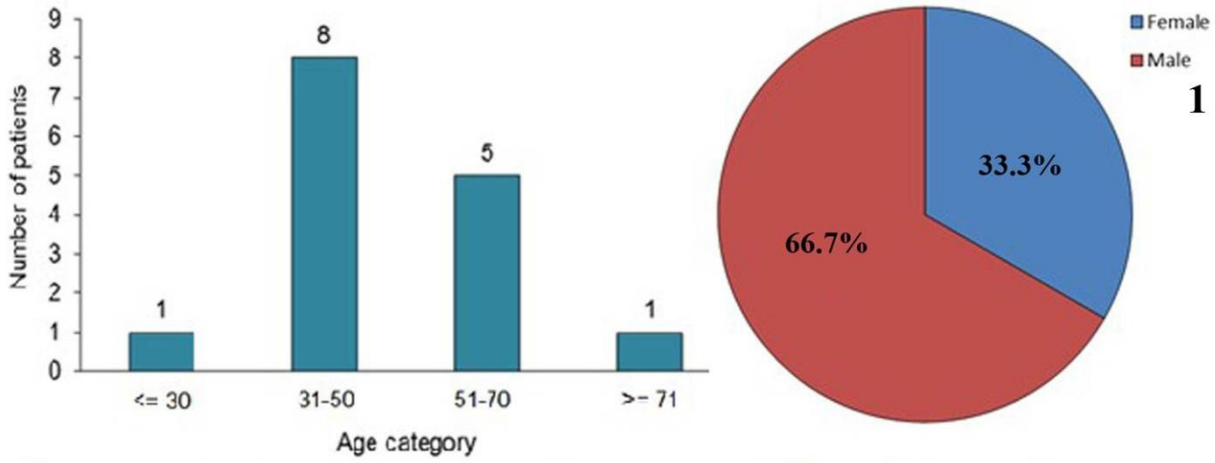



**Figure 1.** CONSORT diagram.




2

| Parameters | Time | Mean | Standard deviation |
|---|---|---|---|
| **Height (cms)** | Screening day | 161.53 | 6.58 |
| | Day 3 | 161.53 | 6.58 |
| | Day 5 | 161.53 | 6.58 |
| **Weight (kgs)** | Screening day | 63.27 | 24.69 |
| | Day 3 | 63.27 | 24.69 |
| | Day 5 | 63.27 | 24.69 |
| **BMI (kg/m2)** | Screening day | 23.97 | 7.58 |
| | Day 3 | 23.97 | 7.58 |
| | Day 5 | 23.97 | 7.58 |

**Figure 2.** (1) Age and gender distribution of volunteers who participated in the study. (2) Height, weight and BMI of volunteers.

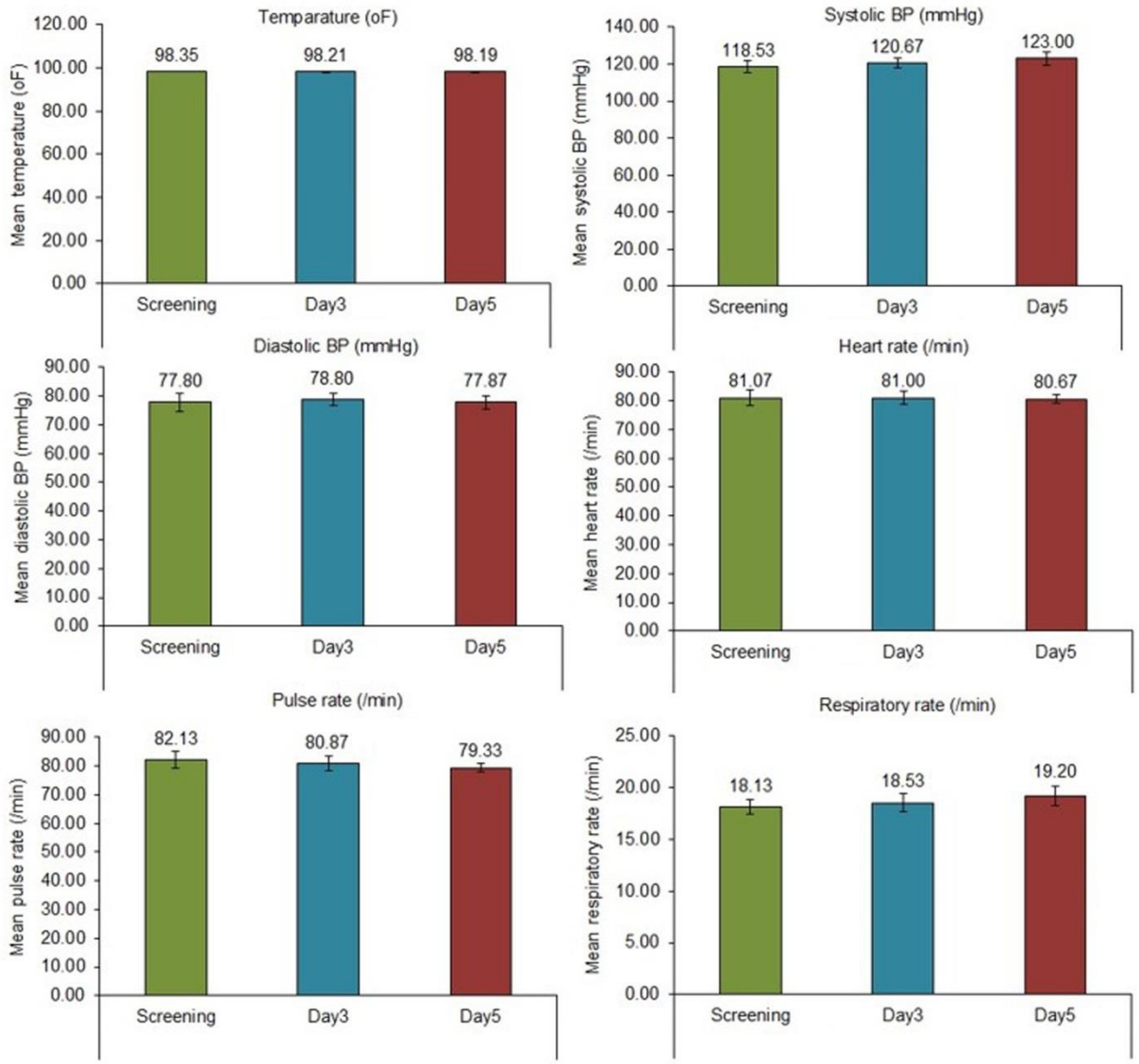



**Figure 3.** Vital parameters of the volunteers who participated in the study.

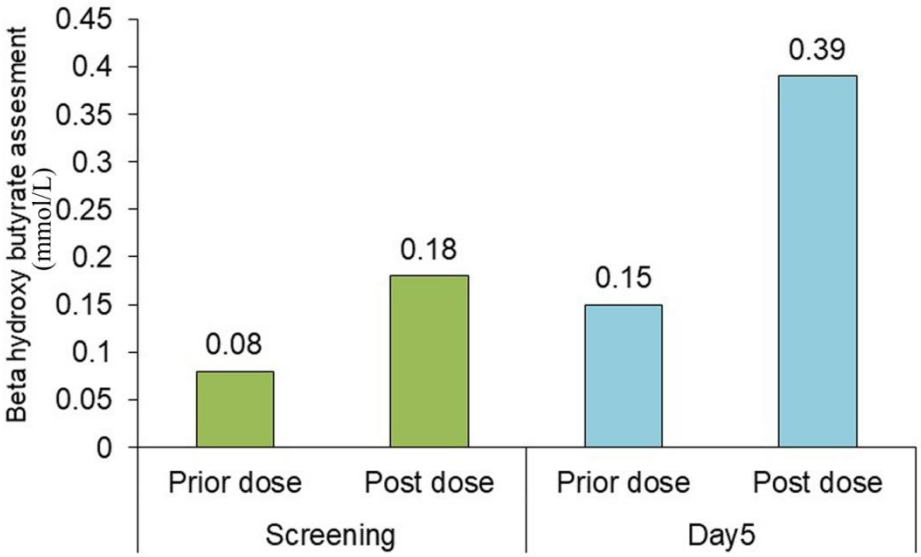



**Figure 4.** The mean Beta-hydroxybutyrate value at screening and on day 5.

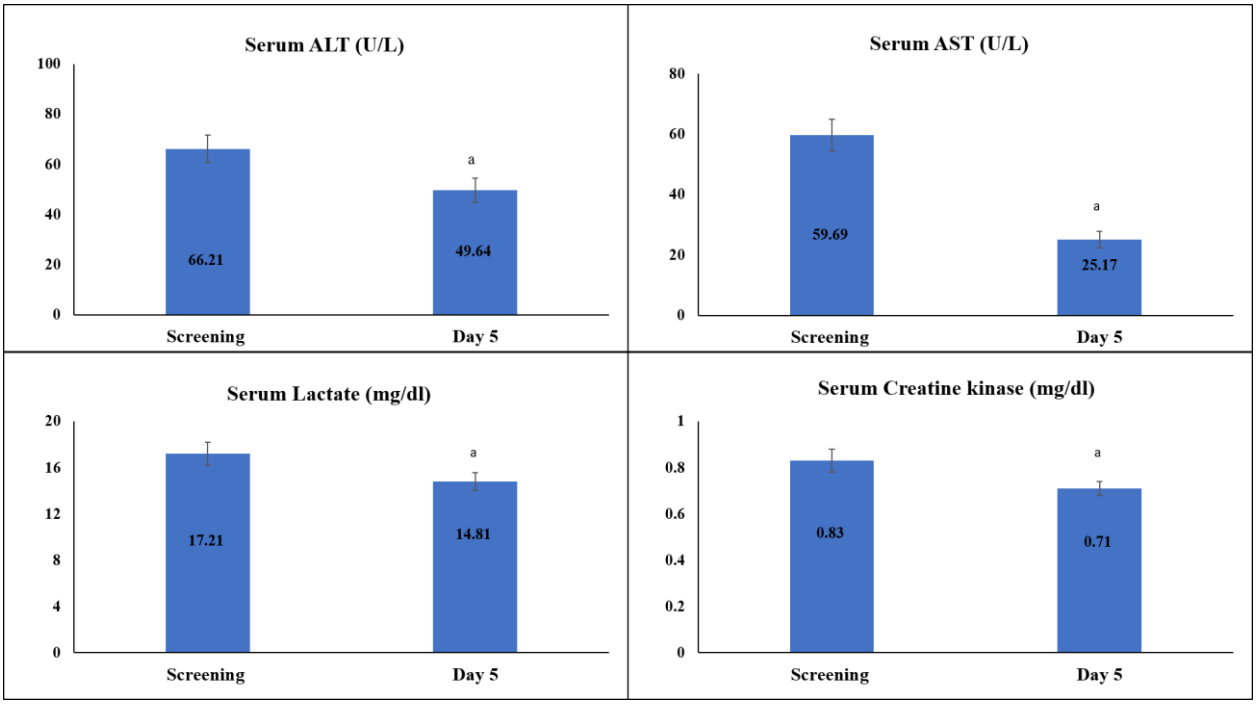



**Figure 5.** Serum biochemical parameters at screening and on day 5.

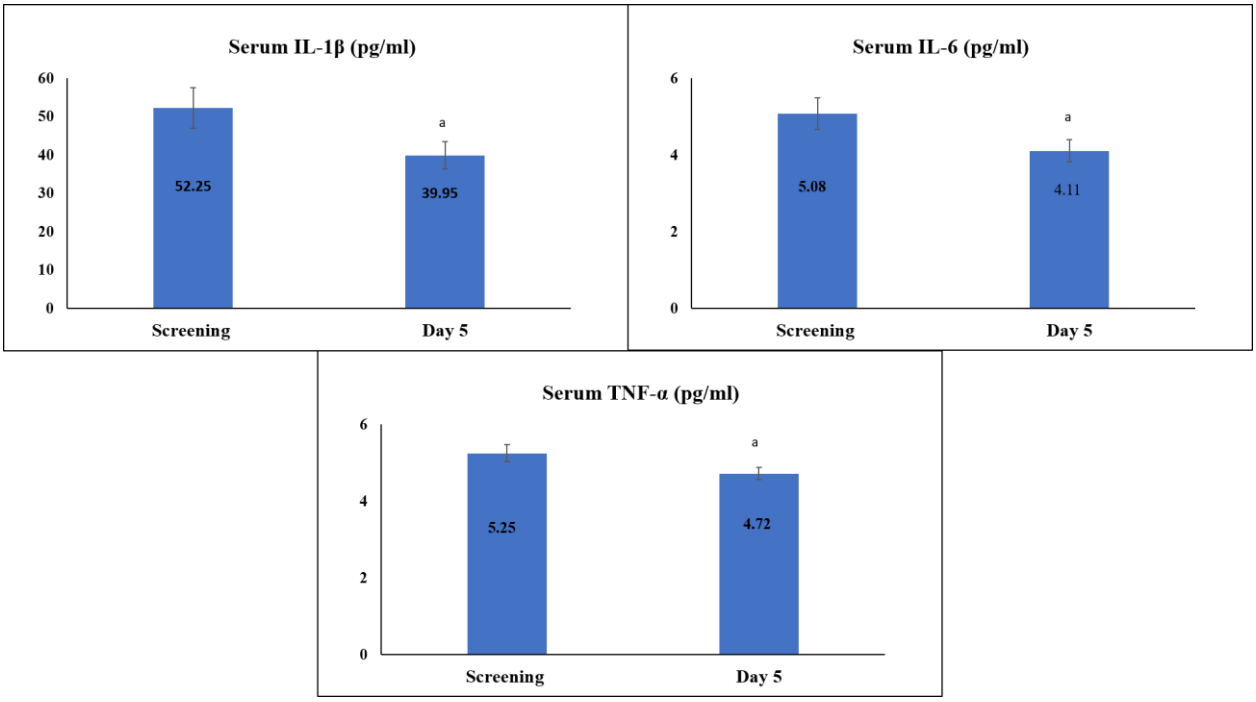



**Figure 6.** The mean value of proinflammatory markers (IL-1β, IL-6 and TNF-α) in serum at screening and day 5.

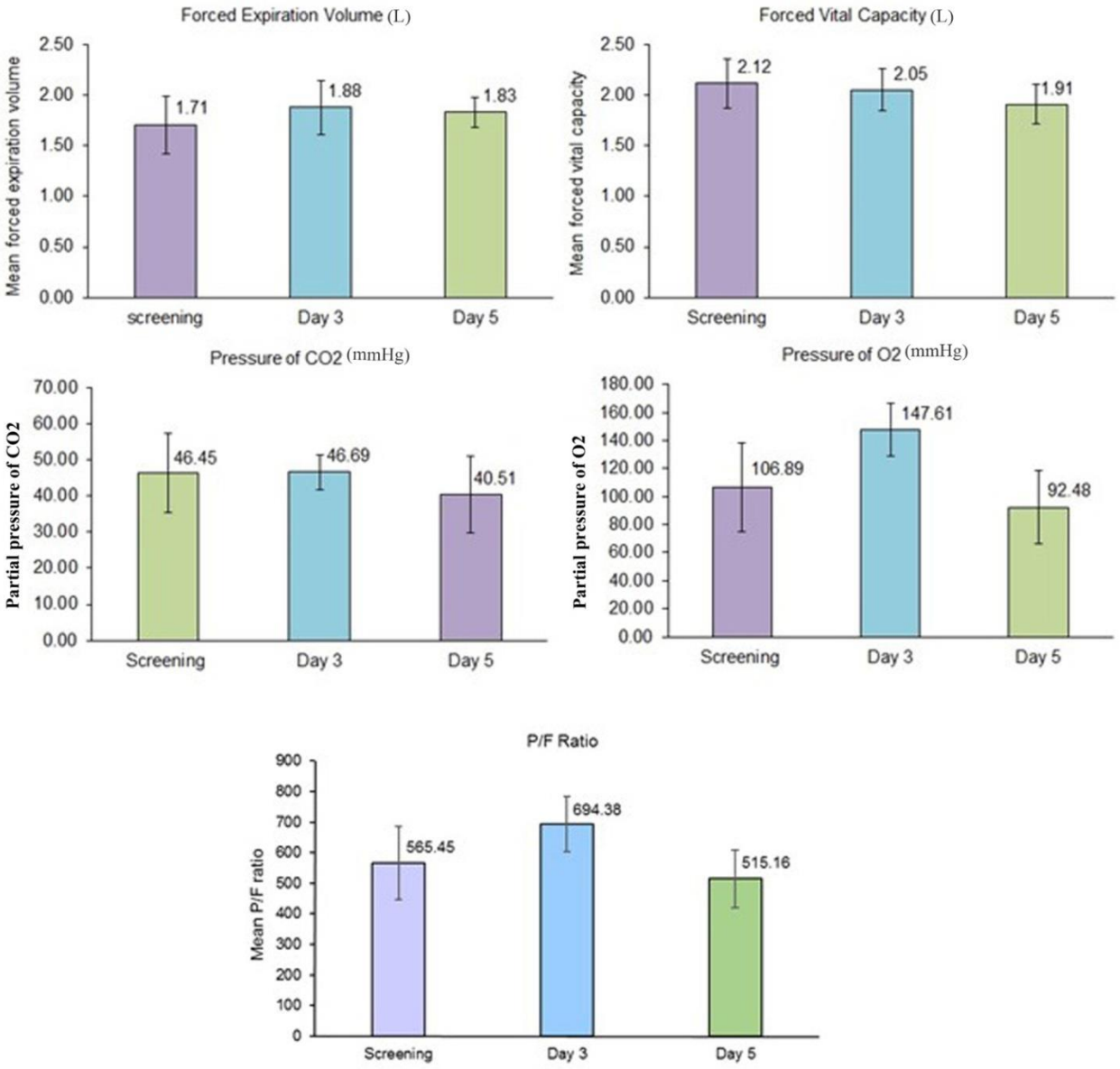



**Figure 7.** The mean value of pulmonary function test (PFT) parameters at screening, 3rd and 5th day.

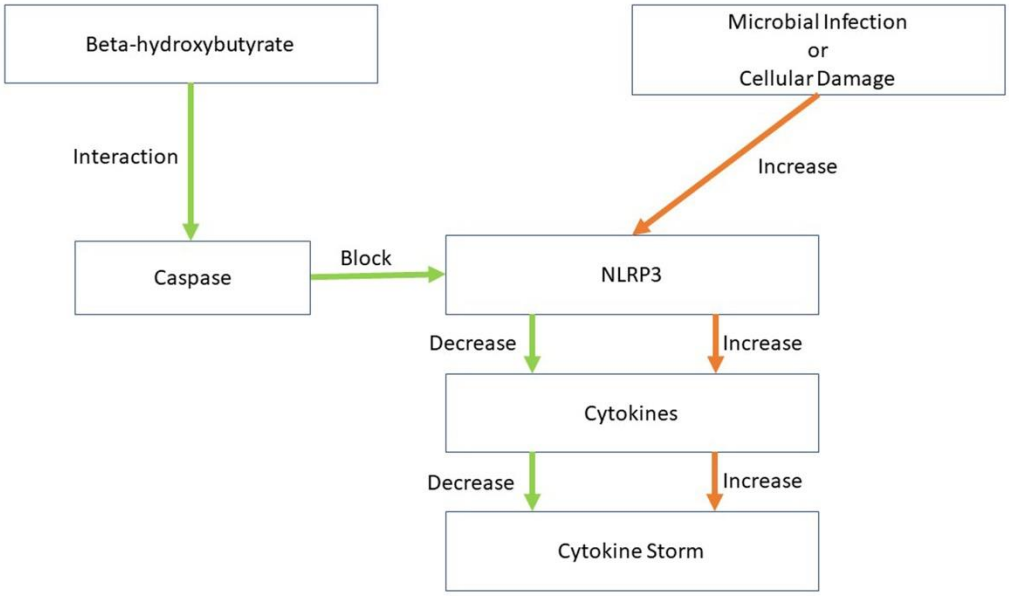



**Figure 8:** BHB pathway for reducing inflammation.

**Supplementary file:** Patients Feedback Questionnaire

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## SUBJECT FEEDBACK QUESTIONNAIRE

### Visual Analogue Scale to Evaluate Fatigue Severity (VAS-F)

**DIRECTIONS:**
Subject shall be asked each of the following lines to indicate how they are feeling RIGHT NOW.

For example, suppose the subject has not eaten since yesterday.
What number would the subject give as scoring?

| Not at all Hungry | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 hungry | 9 | 10 | Extremely |
|---|---|---|---|---|---|---|---|---|---|---|---|---|

Circle the number if the subject gives extremely hungry, as below

| Not at all Hungry | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely hungry |
|---|---|---|---|---|---|---|---|---|---|---|---|---|

.....

**Please complete the following items:**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Not at all **tired** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely **tired** |
| 2 | Not at all **sleepy** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely **sleepy** |
| 3 | Not at all **drowsy** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely **drowsy** |
| 4 | Not at all **fatigued** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely **fatigued** |
| 5 | Not at all worn out | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely **worn out** |
| 6 | Not at all **energetic** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely **energetic** |
| 7 | Not at all **active** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely **active** |
| 8 | Not at all **vigorous** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Extremely **vigorous** |

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## SUBJECT FEEDBACK QUESTIONNAIRE

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | Not at all **efficient** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Extremely **efficient** |
| 10 | Not at all **lively** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Extremely **lively** |
| 11 | Not at all **bushed** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Extremely **bushed** |
| 12 | Not at all **exhausted** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Extremely **exhausted** |
| 13 | Keeping my eyes open **Is no effort at all** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Keeping my eyes open **is a tremendous chore** |
| 14 | Moving my body **Is no effort at all** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Moving my body **is a tremendous chore** |
| 15 | Concentrating Is no effort at all | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Concentrating **is a tremendous chore** |
| 16 | Carrying on a conversation **Is no effort at all** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Carrying on a conversation **is a tremendous chore** |
| 17 | I have absolutely **no desire to close my eyes** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 I have a tremendous **desire to close my eyes** |
| 18 | I have absolutely **no desire to lie down** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 I have a tremendous **desire to lie down** |