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Original Research Article

An observational study on the efficacy and safety of Enzotein plus in post-surgical recovery in critically ill patients by improving nutritional indices

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ABSTRACT

Background: Critically ill patients, especially those requiring mechanical ventilation and suffering from sepsis or multiorgan dysfunction, are at increased risk of malnutrition, muscle wasting, and prolonged recovery. Protein supplementation is essential, but enteral feeding via Ryle's or jejunal tubes may impair proteolytic enzyme activity, limiting protein absorption. Thus, this study aimed to evaluate Enzotein plus, a formulation containing whey protein, proteolytic enzymes, and essential micronutrients for its clinical and biochemical benefits in such patients.
Methods: This observational study involved 100 post-surgical critically ill patients conducted at Department of General Surgery, Goa Medical College, Bambolim, Tiswadi, Goa from March-2022 to October 2022. Patients received Enzotein Plus (one sachet containing 68 g/dose) for 14 days via oral or RT/JT administration. Primary outcomes included serum albumin, haemoglobin, packed cell volume (PCV), body weight, and cumulative organ failure-free days. Secondary outcomes included ICU stay, mortality, infection rate, pressure sores, and diarrhoea severity. Statistical analysis used SPSS v21, employing repeated measures ANOVA, Tukey's post hoc, and chi-square tests ($p<0.05$).
Results: Significant improvements were observed in serum albumin ($p<0.001$), haemoglobin ($p=0.022$), PCV ($p<0.001$), and body weight ($p<0.05$). Wound healing increased from 50% to 97% over 14 days. Pressure sores and diarrhoea incidence decreased. Mortality was 2%, infection rate 24%, and mean ICU stay 4.6±8.1 days. Organ failure-free days averaged 8.9±9.2.
Conclusions: Enzotein Plus demonstrated significant clinical and nutritional benefits with a favourable safety profile. Its use may enhance recovery in critically ill, post-surgical patients. Larger randomized controlled trials are recommended for validation.

Keywords: Whey proteins, Proteolytic enzymes, Nutritional support, Enteral nutrition, Serum albumin, Wound healing

INTRODUCTION

Critically ill patients receiving mechanical ventilation are at high risk of dying or developing complications that delay their recovery. Patients who develop sepsis, have multiorgan failure, or require prolonged mechanical ventilation or immobility are at particular risk for developing weakness and other neuromuscular abnormalities.[1,2] These impairments are associated with delayed liberation from mechanical ventilation, extended

intensive care unit (ICU) and hospital stays, more healthcare related hospital costs, a higher risk of death, and impaired physical functioning and quality of life in the months following an ICU admission.[1-3] These limitations emphasize the importance of developing new strategies to aid in the physical recovery of critically ill patients. Emerging evidence suggests that exogenous protein/amino acid supplementation has the potential to favorably affect protein balance and improve the recovery of critically ill patients. After a careful review of the published evidence, experts concluded that critically ill patients should receive up to 2.0-2.5 g/kg/d of protein, and receiving at least 80% of the protein that is prescribed is associated with optimal outcomes.[4,5]

Beneficial outcomes of critical illness are positively associated with the patients' muscle mass on ICU admission, the predominant endogenous source of amino acids.[6] Moreover, the catabolic response leads to reductions in muscle mass up to 1 kg/day during the first 10 days of ICU stay in patients with MODS.[7] ICU survivors are also challenged by persistent catabolism and hypermetabolism for months to years. The HP-ONS trial and another recent review emphasize that anabolic and anticatabolic interventions, such as propranolol, oxandrolone, and other agents targeted at restoring lean muscle mass may be essential components to allow for meaningful recovery of QoL and survival post-ICU.[8,9] Targeted nutrition that includes adequate protein delivery and "muscle-recovery targeted" anabolic/anticatabolic agents combined with exercise potentially lead to meaningful improvements in QoL.[10]

Malnutrition in critically ill patients results in 2% muscle protein loss per day and is a predisposition factor to sepsis.[11] American Society of Parenteral Nutrition recommends protein 2-2.5 gm/kg/bw in critically ill patients admitted in the ICU exploring the effect of combining adequate protein delivery with early mobility and/or resistance exercise in the ICU setting for improving the functional outcomes of survivors of critical illness. In addition patient on RT/JT feed the cephalic phase deprives achieving protein target of 2-2.5 gm/kg/bw, due to improper secretion of proteolytic enzyme to enable protein breakdown and assimilation in the small intestine within 90 minutes. This warrants a novel solution which enhances protein breakdown into di and tri peptides and maximise the absorption in the small intestine.

Enzotein plus (Protinace 98®,68 g/dose) is a combination of Whey protein 20gm and proteolytic enzymes 0.455mn USP, fortified with 13 vitamins, 15 micro and macro nutrients. Proteolytic enzymes 0.455mnUSP can effectively breakdown 20 gm protein in to di and tri peptides within 90minutes of administration. The proteolytic enzymes (Patent of Mylin Biotech India Pvt Ltd, Bengaluru) and, whey protein used in this trial is imported from USA. The efficacy and safety of proteolytic enzyme is well documented in using preotelytic enzyme in combination with egg white in Peritoneal Dialysis patients published by Singh et al.[11] Gharia et al studied the combination effect of Whey protein with Proteolytic enzyme and established the significant improvement of Albumin turnover by 18 times more with Enzotein therapy compared with Whey protein alone 1.5%.[12] In addition the study also established the significant decrease in hsCRP level in the combination therapy. With this background, considering the safety and efficacy profile of Enzotein plus this observational study was conducted in critically ill post-surgical patients.

METHODS

This study was conducted at Department of General Surgery, Goa Medical College, Bambolim, Tiswadi, Goa from March-2022 to October 2022. The study was conducted in accordance with the ethical principle that has their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirements. The protocol was approved by institutional review board (IRB)/independent ethics committee (IEC). Investigational product was manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP) and used in accordance with approved protocol.

Informed consent was obtained from every subject prior to study participation for eligible participants. Patients of either gender above age of 18 years post-surgically were included in the study. Those with autoimmune diseases e.g. SLE/ vasculitis or pregnant or lactating were excluded. All the 100 patients enrolled in the study were given unique id.

Enzotein plus was given as an adjuvant therapy without changing the current protocol in managing post-surgical critically ill patients. Sixty-eight grams of Enzotein Plus powder was thoroughly mixed in 200 mL of previously boiled and cooled water, and the prepared solution was administered orally or through a Ryle's tube or jejunal tube.

Vital like blood pressure, pulse rate, height and weight were recorded. The primary endpoint included cumulative organ failure-free days, assessing cardiovascular, respiratory, renal, bone marrow, and liver functions. Biochemical parameters such as serum albumin, hemoglobin, PCV, and body weight loss were also evaluated. The secondary endpoint measured mortality, the number of days in the ICU, infection rate, pressure sores (categorized as mild, moderate, and severe on a scale of 1-10) and diarrhea (assessed as mild, moderate, and severe on a scale of 1-10). The safety endpoint focused on treatment-emergent adverse events, including their incidence and severity.

Statistical analysis was conducted using IBM SPSS Statistics version 21, while Microsoft excel was used for data documentation. The primary endpoint involved comparing laboratory and clinical outcomes across all

groups at baseline and after 7 days and 14 days intervention. Data are presented as mean±standard deviation (SD) and frequency and percentage appropriately. Repeated measures ANOVA was used to compare means followed by Tukey's post hoc test and chi square test was used to compare proportions. A p value of less than 0.05 was considered statistically significant.

RESULTS

A progressive and statistically significant improvement was observed in several clinical and laboratory parameters over the 14-day period. Serum albumin levels increased markedly from a baseline mean of 2.96±0.23 g/dL on Day 1 to 3.45±0.28 g/dl on day 7 and 3.79±0.24 g/dl by day 14 (Figure 1), with all comparisons between time points showing high statistical significance ($p<0.001$). Haemoglobin levels rose from 10.2±3.1 g/dl at baseline to 10.9±1.6 g/dl on day 7 and 12.7±4.3 g/dl on day 14 (Figure 1), although statistical significance was reached only in the comparison between day 1 and day 14 ($p=0.022$). PCV also showed a consistent and significant rise from 30.46±4.32% to 35.68±4.19% on day 7 and 38.83±5.66% on day 14 (Figure 1), with $p<0.001$, 0.030, and <0.001 respectively for comparisons between the three time points.

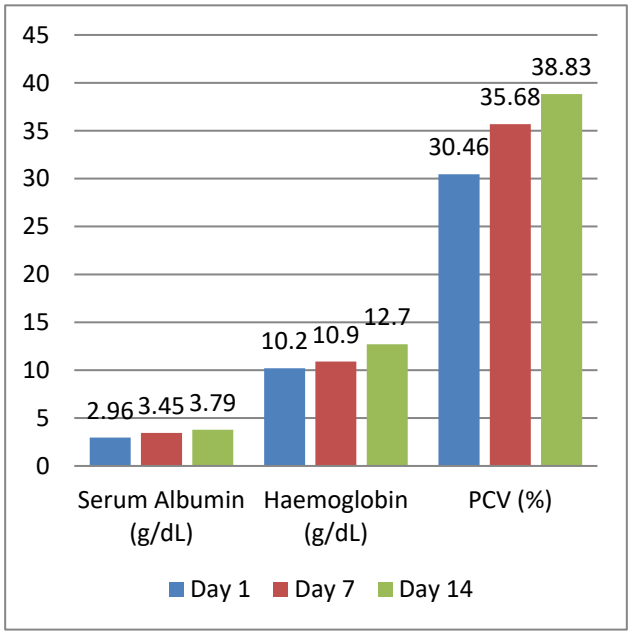


Figure 1: Comparison of means of blood parameters at various time points.

Body weight showed statistically significant increase in both males and females (Figure 2). Male participants' mean body weight rose from 48.59±4.88 kg on day 1 to 51.34±3.92 kg on day 7 and 59.31±4.26 kg by day 14 ($p=0.032$, <0.001, and <0.001). Female participants showed a similar trend with body weight increasing from 41.62±3.45 kg to 44.87±4.25 kg and 49.22±3.16 kg, also with all comparisons statistically significant ($p=0.004$, <0.001, and <0.001).

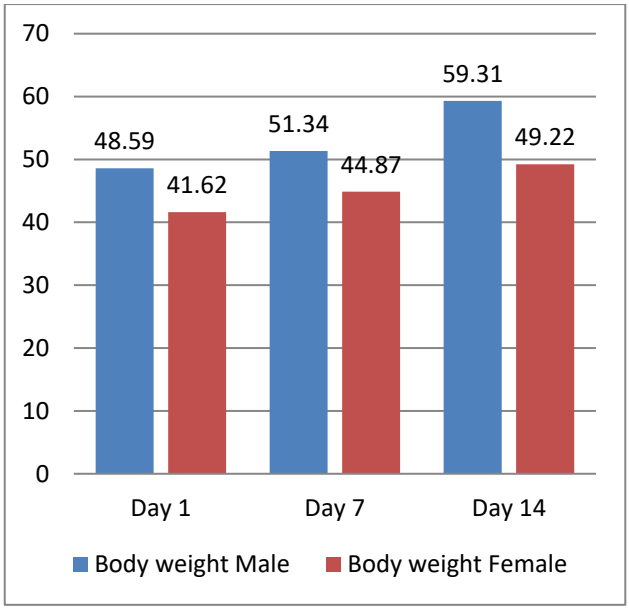


Figure 2: Comparison of mean body weights at various time points.

Regarding clinical outcomes, on day 1, 50% of patients demonstrated evidence of wound healing, which progressively increased to 78% by day 7 and further to 97% by day 14 (Figure 3). This gradual and statistically significant improvement over time ($p<0.05$) suggests a potential role of Enzotein Plus in enhancing and accelerating the wound healing process.

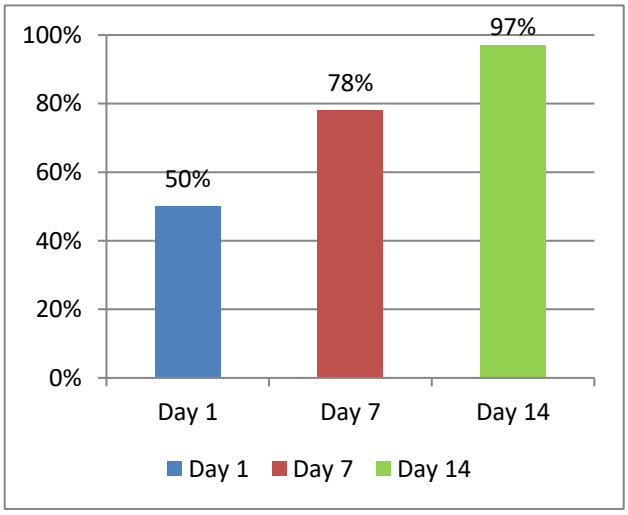


Figure 3: Comparison of percentage of wound healing at various time points.

The prevalence of pressure sores decreased over time (Figure 4). Mild cases reduced from 6% on day 1 to 4% on day 7 and 2% on day 14. Moderate pressure sores declined from 2% on day 1 to 1% on day 7 and were absent by day 14. Severe cases decreased from 4% to 2% between day 1 and day 7, remaining stable through day 14. Diarrhoea was present in 2% of patients as mild on day 1 but was absent from day 7 onward (Figure 4).

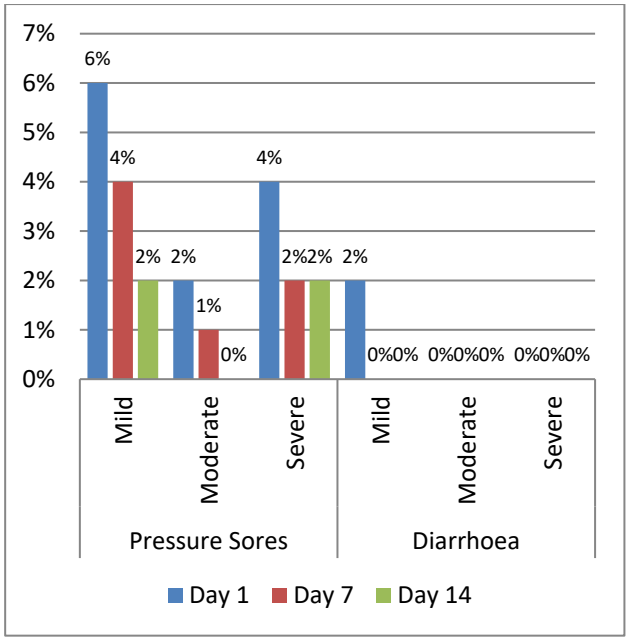


Figure 4: Comparison of percentage of pressure sores and diarrhoea at various time points.

Table 1 shows that mortality during the study period was recorded at 2%, while the infection rate was 24%. Furthermore, the mean duration of ICU stay was 4.6±8.1 days, and the mean number of cumulative organ failure-free days was 8.9±9.2.

Table 1: Comparison of clinical outcomes at the end of 14 days.

Parameters	Value
Mortality	2 (2%)
Infection rate	24 (24%)
Number of days in ICU (Mean±SD)	4.6±8.1
Cumulative organ failure free days (Mean±SD)	8.9±9.2

DISCUSSION

This observational study evaluated the clinical and biochemical impact of Enzotein Plus, a nutraceutical formulation. Administered to critically ill post-surgical patients, Enzotein Plus demonstrated a significant improvement in key nutritional and clinical parameters over a 14-day period. The mechanism of action of whey protein and proteolytic enzymes in post-surgical recovery in critically ill patients is illustrated in Figure 5.

A marked and statistically significant increase in serum albumin levels was observed from day 1 to day 14. This finding is consistent with the study by Gharia et al which reported an 18-fold enhancement in albumin turnover when whey protein was combined with proteolytic enzymes compared to whey protein alone (1.5% improvement).[12] The rapid enzymatic hydrolysis of protein into absorbable di- and tri-peptides within 90 minutes of administration may have facilitated faster protein assimilation and synthesis, thus enhancing albumin restoration.

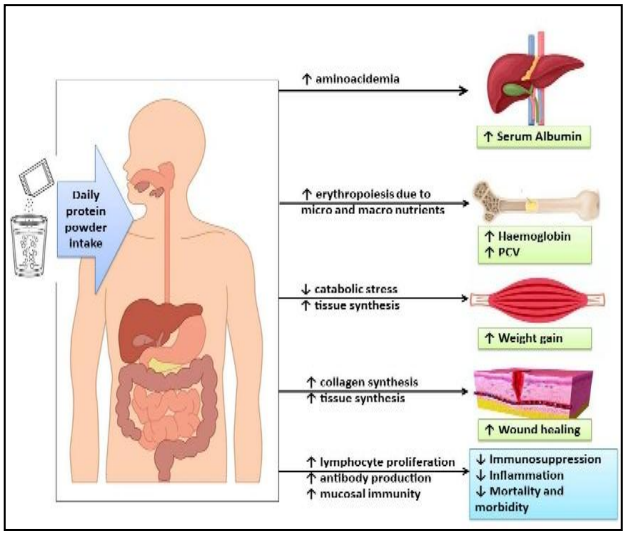


Figure 5: Mechanism of action of Enzotein Plus in post-surgical recovery in critically ill patients.

Another study by Hassan et al conducted on hypoalbuminemic peritoneal dialysis (PD) patients over a 12-week period demonstrated that whey protein supplementation led to a significant improvement in serum albumin levels. In this randomized trial involving 36 stable PD patients, those who received whey protein as part of their daily protein intake not only achieved higher serum albumin levels but also showed better blood pressure regulation compared to the control group. These findings support the role of whey protein in enhancing nutritional status and managing complications in hypoalbuminemic patients, aligning with our study's observation of albumin improvement.[13]

The increase in serum albumin can be attributed to the bioactive components present in whey protein, including β-lactoglobulin, α-lactalbumin, glycomacropeptide, peptone-3, immunoglobulins, and serum albumin. These constituents actively enhance intestinal absorption, stimulate hepatic albumin synthesis, reduce systemic inflammation, and modulate the stress response. Collectively, these effects lead to improved protein metabolism and directly contribute to the elevation of serum albumin levels in patients receiving whey protein supplementation.[14]

A preclinical study by Santhosh Kumar et al reported that the addition of a proprietary protease enzyme blend to whey protein resulted in weight gain in male albino Wistar rats.[15] In another study where addition of proteolytic enzymes with whey protein resulted in a significantly earlier and more pronounced rise in plasma amino acid levels (aminoacidemia) compared to whey protein alone or a control drink in resistance-trained males. At 30 minutes post-ingestion, the whey protein group without enzymes

showed no significant difference from the control, whereas the group receiving whey protein with Proteolytic enzyme exhibited enhanced amino acid availability. This suggests that proteolytic enzymes accelerate the digestion and absorption of whey protein, thereby enhancing its anabolic potential and supporting more efficient protein utilization and synthesis, which may contribute to increased serum albumin production in clinical populations.[12] Together, these findings support that proteolytic enzymes accelerate digestion and absorption of whey protein-derived amino acids, supplying the liver with an optimized substrate pool to synthesize albumin more effectively. Clinically, this manifests as greater increases in serum albumin levels compared to whey protein alone-a response likely reflected in the significant albumin gains seen with Enzotein Plus.

Haemoglobin and PCV showed significant upward trends in this study, with haemoglobin levels increasing from day 1 to day 14 ($p=0.022$) and PCV improving consistently across all measured intervals ($p<0.001$ to 0.030). These improvements can be attributed to enhanced erythropoiesis facilitated by the micronutrient-rich formulation of Enzotein Plus, which contains essential hematinic nutrients such as iron, folic acid, and vitamin B12. These micronutrients play a central role in hemoglobin synthesis and erythrocyte maturation. In critically ill or malnourished patients, deficiencies in these components are commonly observed and are associated with anemia and impaired recovery.[16,17] Supplementation with these nutrients has been shown to correct anemia and improve hematological indices, particularly in the postoperative or intensive care setting.[18,19] Thus, the observed increases in hemoglobin and PCV likely reflect the repletion of these essential nutrients and a restoration of effective erythropoietic function.

In our study, significant improvements in body weight were observed in both male and female participants over the 14-day intervention period, with all comparisons reaching statistical significance ($p<0.05$). This consistent weight gain reflects the anabolic effect of whey protein, which is well-documented for its high biological value, rich essential amino acid profile, and rapid digestibility that supports lean tissue accretion and overall nutritional recovery in catabolic states.[20,21] Furthermore, the incorporation of proteolytic enzymes in Enzotein Plus likely enhanced protein digestion and amino acid availability, as shown in prior studies where enzyme-treated whey led to more rapid and pronounced aminoacidemia.[15] The additional micronutrient support, including zinc, magnesium, and B-complex vitamins, further contributed to metabolic efficiency and tissue synthesis, thereby supporting weight restoration in nutritionally depleted patients. These findings suggest a synergistic effect between whey protein, enzymatic hydrolysis, and micronutrient fortification in promoting anabolic recovery in critically ill or post-surgical individuals.

Wound healing is a complex biological process involving overlapping phases of hemostasis, inflammation, proliferation, and remodeling. Nutrition plays a crucial role in supporting these processes by providing essential substrates and cofactors. In the context of our study on Enzotein Plus, the observed progressive improvement in wound healing-from 50% on day 1 to 97% on day 14-can be attributed not only to the proteolytic activity of the formulation but also to its potential nutritional support in modulating inflammatory and reparative responses. Micronutrients such as calcium and vitamin K are essential for the coagulation cascade and fibrin clot formation during inflammatory phase. These nutrients facilitate initial wound stabilization, which may be indirectly supported in patients supplemented with enzyme-nutrient formulations like Enzotein Plus. Moreover, vitamin A supports monocyte and macrophage migration, a critical component in clearing debris and orchestrating tissue repair, while vitamin E contributes to membrane stability and provides antioxidative, anti-inflammatory effects.[22-26]

Importantly, zinc plays a regulatory role in immune responses and is essential for the activity of many wound-related enzymes. Adequate zinc levels are associated with enhanced epithelialization and reduced infection risk. The healing acceleration observed in the Enzotein Plus group may reflect such immunonutritional synergy. From a macronutrient perspective, proteins and amino acids such as arginine and glutamine are pivotal. Arginine supports cellular proliferation and collagen deposition, while glutamine acts as a fuel for rapidly dividing cells and contributes to the synthesis of glutathione, a potent antioxidant that reduces oxidative stress in wounds.[27-29] Enzotein Plus, by supporting proteolytic debridement, may also enhance local nutrient delivery and utilization by promoting microcirculatory perfusion and reducing inflammatory load. Lipids, particularly omega-3 fatty acids, modulate inflammatory cytokine production and stabilize cell membranes. While Enzotein Plus is primarily recognized for its enzymatic activity, any indirect support to lipid metabolism or anti-inflammatory signaling could contribute to the favorable wound healing outcomes observed in our study. Overall, the nutritional components described in the literature complement the enzymatic actions of Enzotein Plus. The formulation's therapeutic effect likely arises from the synergistic interplay between enzymatic debridement and systemic support for immune modulation, oxidative stress reduction, and tissue regeneration. This dual-action mechanism may explain the significant and rapid progression of wound healing observed in our patient cohort.

From a clinical standpoint, the observed reduction in pressure sores and resolution of mild diarrhoea during the study period suggests improved skin integrity, enhanced wound healing, and better gastrointestinal tolerance. By day 14, moderate pressure sores had completely resolved, and the frequency of mild cases significantly decreased, reflecting improved nutritional and metabolic support. Adequate protein intake, particularly from high-quality

sources like whey, plays a critical role in collagen synthesis, tissue repair, and maintaining skin integrity-key factors in the prevention and healing of pressure ulcers.[30,31] The complete resolution of mild diarrhoea by day 7 also reflects good gastrointestinal tolerance of the Enzotein Plus formulation, despite its high protein and micronutrient content. This is crucial in critically ill patients, where gastrointestinal symptoms can compromise nutritional therapy and recovery. Collectively, these findings highlight the clinical benefit of targeted nutritional supplementation in reducing morbidity in critical care.

In our study, the low mortality rate of 2% and a relatively moderate infection rate of 24% underscore the potential role of targeted nutritional therapy in enhancing immune resilience and supporting clinical recovery in critically ill post-surgical patients. Adequate protein intake, especially from high-quality sources like whey, has been shown to support immune function by enhancing lymphocyte proliferation, antibody production, and mucosal immunity.[32,33] The immunomodulatory effects of specific amino acids such as cysteine and glutamine-both abundant in whey protein-are particularly important in critically ill patients, where malnutrition is a major contributor to immunosuppression and infection risk.

The relatively short mean ICU stay of 4.6±8.1 days and a favorable mean of 8.9±9.2 cumulative organ failure-free days in our cohort suggest that Enzotein Plus may have supported faster clinical stabilization, possibly by improving nutritional status, enhancing protein utilization, and reducing catabolic stress. These findings align with evidence that early and appropriate nutritional support can significantly improve clinical outcomes in critically ill patients.[34]

The safety and efficacy of proteolytic enzymes in combination with protein-based therapy have been previously documented by Singh et al in a study involving peritoneal dialysis patients, where improved albumin levels and reduced inflammation were noted.[11] These prior studies, in combination with the present findings, reinforce the therapeutic benefit of targeted nutritional supplementation in critically ill populations.

CONCLUSION

In conclusion, Enzotein Plus appears to be an effective and safe adjunct in the nutritional management of post-surgical critically ill patients. Its use was associated with significant improvements in biochemical and clinical outcomes. However, further randomized controlled trials are necessary to confirm these findings and evaluate long-term benefits.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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