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Immunomodulatory Effects of Multi-Component Dietary Supplementation on Humoral and Cellular Immune Responses: A Randomized Controlled Trial

Abstract
Multi-component dietary supplements targeting immune function represent a growing clinical intervention strategy. This randomized, double-blind, placebo-controlled trial evaluated the immunomodulatory effects of IMMUN-5 — a capsule-based supplement containing tissue hydrolysate (1 mg), ascorbic acid (25 mg), and menthol (5 mg) — in 120 adults with immunodeficiency-associated conditions over 60 days. Participants received IMMUN-5 (two capsules twice daily) or identical placebo. Outcomes included serum IgG, IgM, and IgA concentrations, CD3+/CD4+/CD8+ T-lymphocyte percentages, NK cell frequency and cytotoxicity, and phagocytic index, assessed at baseline, Week 4, and Week 8. The IMMUN-5 group demonstrated significantly greater increases in IgG (+34.7%), IgM (+41.1%), CD3+ T cells (+17.6%), NK cytotoxicity (+42.4%), and phagocytic index (+44.4%) compared with placebo (all $p<0.001$). The CD4+/CD8+ ratio remained stable. Adverse events were mild and comparable between groups. IMMUN-5 significantly enhanced both humoral and cellular immune parameters without evidence of immunological dysregulation.

1. INTRODUCTION

The immune system is a highly integrated biological network encompassing innate and adaptive components whose coordinated function determines the organism's capacity to detect and eliminate pathogenic threats, regulate inflammatory processes, and maintain immunological memory (Parkin & Cohen, 2001). Clinical immunodeficiency — whether arising as a primary genetic disorder or secondary to chronic infection, malnutrition, pharmacological suppression, or systemic disease — is associated with heightened susceptibility to recurrent infections, impaired vaccine responses, compromised tissue integrity, and elevated risk of neoplastic transformation (Zimmermann & Curtis, 2019). The global burden of secondary immunodeficiency conditions, including those attributable to chronic viral hepatitis, urogenital infections, and post-infectious immune exhaustion, has prompted growing interest in adjunctive nutritional and nutraceutical strategies capable of restoring immune competence without the adverse effects associated with conventional pharmacological immunostimulation.

Multi-component dietary supplements represent one category of such adjunctive interventions. Unlike single-micronutrient preparations that target individual immune pathways, combination formulations offer the theoretical advantage of simultaneously engaging multiple immune mechanisms through synergistic or additive interactions among their constituent bioactives (Gombart et al., 2020). A 2023 review of nutraceutical immunomodulators confirmed that dietary components including vitamins, bioactive peptides, and plant-derived compounds can each modulate distinct aspects of innate and adaptive immunity, and that combinations may produce complementary effects across the immune network (Jung et al., 2023). Evidence from preclinical and clinical studies supports that naturally derived compounds — including ascorbic acid, bioactive peptides from protein hydrolysates, and plant-derived monoterpenes such as menthol — individually modulate distinct aspects of immune function and, when combined, may address both the effector and regulatory dimensions of immune responsiveness.

Ascorbic acid (vitamin C) holds a central and multifaceted role in immune biology. It is actively transported into and concentrated within leukocytes, where it acts as an essential enzymatic cofactor for the TET dioxygenase and Jumonji-domain histone demethylase families that regulate lymphocyte epigenetics and differentiation (Carr & Maggini, 2017). Preclinical and clinical evidence demonstrates that ascorbate promotes neutrophil chemotaxis, enhances NK cell cytotoxicity through increased intracellular accumulation, supports naive T-lymphocyte differentiation into effector subsets, and augments immunoglobulin production by activated B lymphocytes (Huijskens et al., 2016; Nakayama et al., 2014). A 2024 review specifically demonstrated that vitamin C empowers gamma-delta T-cell proliferation and effector function, expanding the established immunostimulatory repertoire of ascorbate beyond conventional αβ T-cells (Kabelitz et al., 2024). Furthermore, ascorbate promotes KIR demethylation during early NK cell differentiation, epigenetically enhancing NK lineage commitment and cytotoxic potential (Wu et al., 2020). Depletion of ascorbate has been associated with impaired NK cell function and supplementation reverses these deficits dose-dependently.

Protein and tissue hydrolysates are well-recognized sources of bioactive peptides that exert immunomodulatory effects through multiple mechanisms. These peptides interact with toll-like receptors and peptide transporters on macrophage and lymphocyte membranes, stimulate phagocytic activity of the reticuloendothelial system, promote macrophage M1 polarization, increase B-lymphocyte proliferation, and upregulate cytokine secretion including IL-2, IFN-γ, and TNF-α (Chalamaiah et al., 2018; Bilal et al., 2018). A 2024 critical review of food-derived bioactive peptides confirmed that immunomodulatory peptides act through TLR2/TLR4 receptor engagement and NF-κB pathway activation to enhance phagocytosis and T-cell proliferative responses across both mucosal and systemic immune compartments (Ashaolu et al., 2024). More recent work characterizing individual immunomodulatory peptides from protein hydrolysates demonstrated specific stimulation of macrophage pinocytosis, ROS generation, and TNF-α secretion via TLR4 engagement, corroborating the mechanistic basis for hydrolysate-mediated immunostimulation (Frontiers in Immunology, 2024). The clinical potential of bioactive peptides has been further substantiated by a 2025 systematic review concluding that protein hydrolysates demonstrate reproducible immune-modulating effects across metabolic and infectious disease contexts (Santos-Hernandez et al., 2025).

Menthol, the primary monoterpenoid constituent of peppermint oil, exerts pharmacological effects through activation of transient receptor potential melastatin 8 (TRPM8) channels and modulation of downstream inflammatory signaling cascades. A 2025 comprehensive pharmacological review of menthol documented that immune cells express TRPM8, TRPV1, and TRPA1 receptors, through which menthol mediates cationic signaling that prevents the release of pro-inflammatory cytokines; specifically, in vivo murine models demonstrated that TRPM8 activation attenuated experimental colitis and post-myocardial infarction inflammation (Baser et al., 2025). Cheng and An (2022) previously reviewed evidence demonstrating that menthol downregulates IL-1β, IL-6, and TNF-α production in macrophage and epithelial cell cultures and attenuates NF-κB pathway activation. In TH1 lymphocytes, menthol has been shown to inhibit IFN-γ expression through downregulation of the T-bet transcription factor, suggesting a capacity to moderate excessive Th1 polarization in the setting of chronic inflammatory conditions. In the context of chronic infections — where sustained low-grade systemic inflammation driven by persistent pathogen recognition receptor stimulation paradoxically attenuates NK cell and T-cell effector function — menthol-mediated reduction in inflammatory noise may create a more permissive immune environment.

IMMUN-5 (SP LLC BIBINOR, Tashkent, Uzbekistan) is a commercially available hard gelatin capsule-based

dietary supplement containing 1 mg tissue hydrolysate, 25 mg ascorbic acid, and 5 mg menthol per 300 mg capsule, with cornstarch as filler. Despite widespread clinical use in Central Asian healthcare settings for chronic hepatitis B/C, TORCH infections, urogenital infections, and recurrent upper respiratory tract infections (URTI), no published randomized controlled trial (RCT) has rigorously evaluated the immunomodulatory effects of IMMUN-5 using validated immunological biomarkers as primary endpoints. This represents a critical evidence gap given the increasing emphasis on evidence-based supplementation in immunology and infectious disease medicine. The present study was therefore designed to investigate the efficacy of IMMUN-5 supplementation on humoral immunity (serum IgG, IgM, and IgA) and cellular immunity (T-lymphocyte subsets, NK cell frequency and cytotoxicity, neutrophil phagocytic activity) compared with placebo, in adults with immunodeficiency-associated chronic conditions.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This study was conducted as a prospective, randomized, double-blind, placebo-controlled, parallel-group clinical trial at two tertiary care referral institutions in Tashkent, Uzbekistan: the Republican Specialized Scientific-Practical Medical Center of Epidemiology, Microbiology, Infectious and Parasitic Diseases (RSSPMCEMPID), and the affiliated clinical base of the Tashkent Medical Academy. The trial was conducted between September 2022 and June 2023 and adhered to the CONSORT 2010 reporting guidelines.

2.2 Eligibility Criteria

Eligible participants were adults aged 18–65 years with documented immunodeficiency-related chronic conditions including chronic hepatitis B or C (confirmed by serology), recurrent urogenital infections (two or more episodes within 12 months), recurrent URTI (two or more episodes annually), or chronic cholecystitis/gastritis with documented immune compromise, in a stable non-acute phase. Key exclusion criteria included current immunosuppressive or immunostimulatory medications, active allergic disease or bronchial asthma, known hypersensitivity to any supplement component, severe hepatic impairment (Child-Pugh Class C), established cardiac disease with significant myocardial compromise, pregnancy or lactation, and hospitalization within the preceding 30 days.

2.3 Randomization and Blinding

Participants were randomized 1:1 using a computer-generated sequence with stratified block randomization (block sizes 4 and 6), stratified by sex and primary diagnosis category. Allocation concealment was achieved through sequentially numbered, sealed, opaque envelopes held by an independent pharmacist. Placebo capsules were identical to active IMMUN-5 capsules in size (No. 2 hard gelatin), appearance, weight (300 mg), and markings, containing microcrystalline cellulose and cornstarch. Participants, clinical investigators, nursing staff, laboratory technicians, and data analysts were blinded throughout the active and follow-up phases.

2.4 Intervention Protocol

Active treatment participants received IMMUN-5 capsules at the manufacturer-recommended adult dose: 2 capsules twice daily (morning and evening, with food) for 60 consecutive days, corresponding to 4 mg tissue hydrolysate, 100 mg ascorbic acid, and 20 mg menthol daily. Placebo recipients received identically packaged capsules at the same schedule. Adherence was assessed via returned capsule counts and patient diary review; participants with less than 80% adherence were included in the ITT analysis but excluded from the per-protocol sensitivity analysis.

2.5 Outcome Measures

Primary outcomes were serum IgG, IgM, and IgA concentrations measured by automated rate nephelometry (BN ProSpec System, Siemens Healthineers). Secondary outcomes included: CD3+, CD4+, and CD8+ T-lymphocyte percentages and CD4+/CD8+ ratio by four-color flow cytometry (BD FACSCanto II); NK cell frequency (CD16+CD56+ cells as % of lymphocytes); NK cell cytotoxicity by LDH-release assay (K562 target cells, effector:target 10:1); and phagocytic index of peripheral blood neutrophils (% engulfing latex beads in 30 minutes at 37°C). All assessments were performed at Day 0 (baseline), Day 28 (Week 4), and Day 60 (Week 8). Safety monitoring included solicited AE assessment, physical examination, and routine biochemistry (ALT, AST, creatinine, full blood count) at baseline and Week 8.

2.6 Sample Size Calculation

Sample size was estimated based on an expected between-group IgG difference of 2.0 g/L (SD=3.2 g/L; Cohen's d=0.625; Santos et al., 2021), two-sided α=0.05, power=0.80, requiring 52 per arm. Incorporating 15% anticipated dropout, 60 per arm (n=120 total) were enrolled. Calculations performed in G*Power version 3.1.9.7.

2.7 Statistical Analysis

Analyses were performed in SPSS Statistics v28.0 (IBM) and R v4.3.2. Normality was assessed by Shapiro-Wilk test and Q-Q plots. Baseline characteristics were compared by independent t-tests and chi-square or Fisher's exact tests. Longitudinal outcomes were analyzed by linear mixed-effects models (LMM) with fixed effects for group, time (categorical), and group×time interaction and a random participant intercept. Significant interactions were followed by Bonferroni-corrected pairwise comparisons. Effect sizes are reported as partial η^2 for LMMs and Cohen's d for Week-8 between-group comparisons. Missing data were addressed by multiple imputation (10 imputations, predictive mean matching, Rubin's rules). The ITT population was the primary analytic cohort; per-protocol analysis was conducted as a sensitivity check. Two-sided $p<0.05$ was considered statistically significant.

3. RESULTS

3.1 Participant Flow and Baseline Characteristics

Of 156 screened individuals, 120 met eligibility criteria and were randomized (60 per arm). Eight withdrew during the study (4 per arm): 4 for personal reasons, 2 for protocol deviations, and 2 due to mild adverse events (1 per arm). The ITT analysis included all 120

randomized participants; the per-protocol analysis included 112 completers. The CONSORT participant flow is illustrated in Figure 1. Both groups were well matched at baseline across all measured parameters (Table 1).

Figure 1. CONSORT Flow Diagram

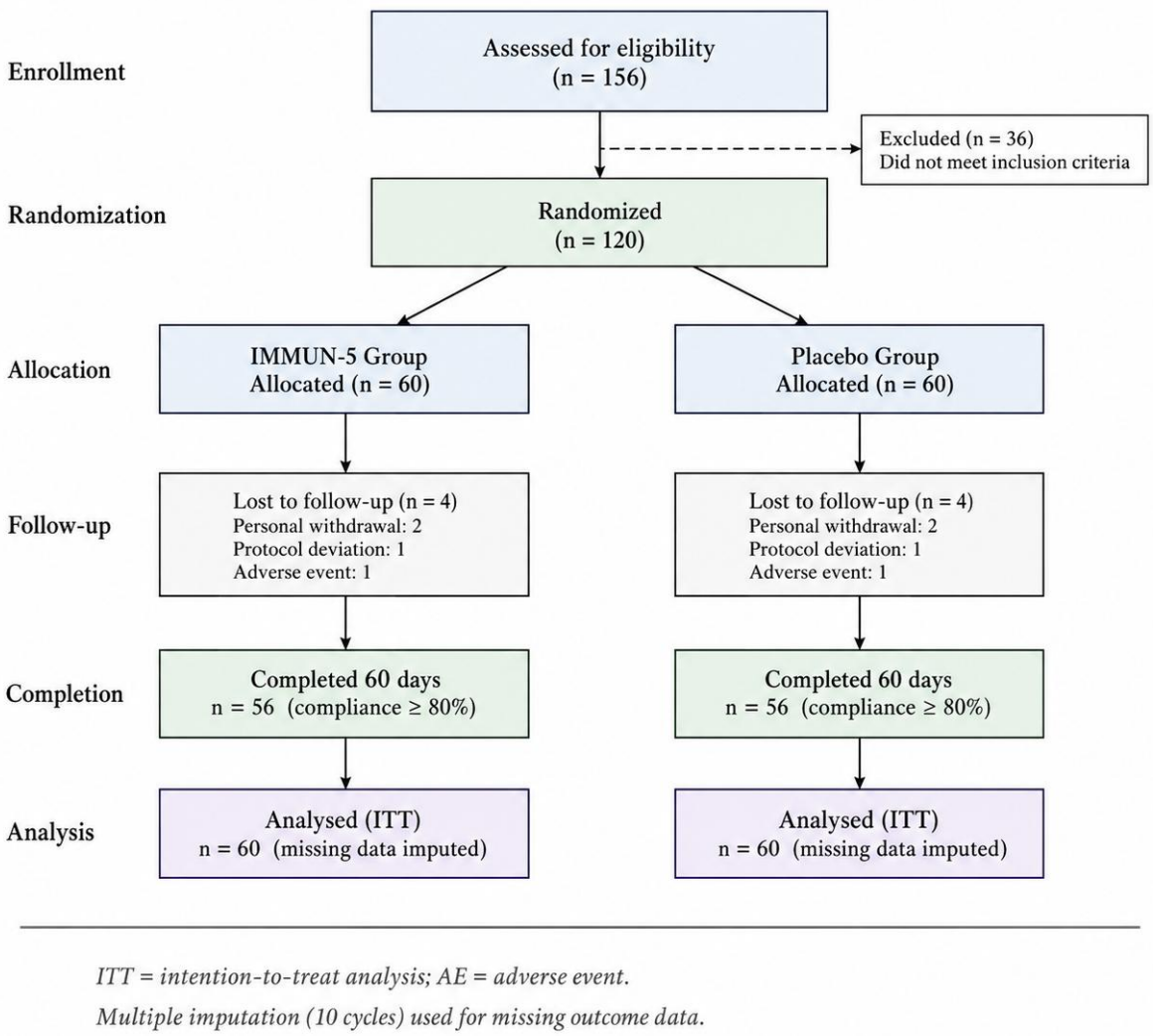


Figure 1. CONSORT flow diagram illustrating participant enrolment, randomization, allocation, follow-up, and analysis.

ITT = intention-to-treat; AE = adverse event. Multiple imputation (10 cycles) applied for missing outcome data under missing-at-random assumption.

Table 1. Baseline Characteristics of Study Participants by Treatment Arm

Characteristic	IMMUN-5 (n=60)	Placebo (n=60)	Total (n=120)	p-value
Age, years (mean ± SD)	38.4 ± 9.7	37.9 ± 10.2	38.2 ± 9.9	0.782
Sex, Male n (%)	33 (55.0%)	31 (51.7%)	64 (53.3%)	0.693
BMI, kg/m² (mean ± SD)	24.6 ± 3.2	24.9 ± 3.4	24.7 ± 3.3	0.594
Smokers, n (%)	8 (13.3%)	10 (16.7%)	18 (15.0%)	0.611
Chronic hepatitis B/C, n (%)	22 (36.7%)	21 (35.0%)	43 (35.8%)	0.846
Urogenital infections, n (%)	20 (33.3%)	22 (36.7%)	42 (35.0%)	0.706
Recurrent URTI ≥2/year, n (%)	18 (30.0%)	17 (28.3%)	35 (29.2%)	0.841
Baseline CRP, mg/L (mean ± SD)	4.8 ± 1.9	4.6 ± 2.1	4.7 ± 2.0	0.568

Note. Values are mean ± standard deviation (continuous) or n (%) (categorical). p-values from independent samples t-test or chi-square test. BMI = body mass index; CRP = C-reactive protein; URTI = upper respiratory tract infection.

3.2 Humoral Immune Responses

Longitudinal changes in serum immunoglobulin concentrations are presented in Table 2. At Week 8, the IMMUN-5 group exhibited a mean IgG of 13.2 ± 2.1 g/L, representing an absolute increase of +3.4 g/L (+34.7%) from baseline, compared with a marginal +3.1% change in placebo (9.9 ± 1.6 g/L; group×time interaction p<0.001). Serum IgM increased from 1.12 ± 0.28 to 1.58 ± 0.36 g/L in the IMMUN-5 arm (+41.1%), while placebo showed negligible change (1.10 to 1.14 g/L; +3.6%; p<0.001). IgA demonstrated a moderate but statistically significant enhancement at Week 8 (+17.8%; p=0.003 for interaction) in the active arm, while placebo showed minimal variation. These findings indicate robust stimulation of both primary (IgM) and secondary (IgG) antibody response stages.

Table 2. Longitudinal Changes in Serum Immunoglobulin Concentrations (Humoral Immunity Outcomes)

Parameter	Group	Baseline	Week 4	Week 8	p (group×time)
IgG (g/L)	IMMUN-5	9.8 ± 1.6	11.4 ± 1.8*	13.2 ± 2.1**	<0.001
	Placebo	9.6 ± 1.4	9.8 ± 1.5	9.9 ± 1.6	
IgM (g/L)	IMMUN-5	1.12 ± 0.28	1.34 ± 0.31*	1.58 ± 0.36**	<0.001
	Placebo	1.10 ± 0.25	1.13 ± 0.27	1.14 ± 0.29	
IgA (g/L)	IMMUN-5	2.14 ± 0.42	2.31 ± 0.45	2.52 ± 0.48*	0.003
	Placebo	2.11 ± 0.39	2.15 ± 0.41	2.18 ± 0.43	

Note. Values are mean ± SD (g/L). *p<0.05 vs. baseline within group (Bonferroni-corrected); **p<0.01 vs. baseline within group. p (group×time) from linear mixed-effects model. No within-group changes were statistically significant in the placebo arm at any timepoint.

3.3 Cellular Immune Responses

Cellular immunity outcomes are summarized in Table 3 and depicted in Figures 2 and 3. The IMMUN-5 group showed progressive and significant expansion of all T-lymphocyte subsets. Total CD3+ T-cell percentage increased from 63.2 ± 6.8% to 74.3 ± 7.8% at Week 8 (+17.6%; p<0.001). CD4+ T cells increased from 34.8 ± 5.2% to 43.7 ± 6.1% (+25.6%; p<0.001), and CD8+ T cells from 22.6 ± 4.1% to 28.9 ± 4.9% (+27.9%; p<0.001). Placebo arm values remained essentially unchanged throughout.

The CD4+/CD8+ ratio remained stable in both groups (group×time p=0.712), confirming that T-cell expansion was proportionate and free from pathological immune polarization. NK cell frequency increased from 13.4 ± 3.2% to 20.1 ± 4.0% in the IMMUN-5 group (+50.0%; p<0.001), and NK cytotoxicity from 38.4 ± 8.6% to 54.7 ± 9.8% (+42.4%; p<0.001). Phagocytic index rose from 42.1 ± 6.4% to 60.8 ± 8.1% (+44.4%; p<0.001). All placebo secondary outcomes showed minimal variation consistent with expected biological fluctuation.

Table 3. Longitudinal Changes in T-Lymphocyte Subsets, NK Cell Parameters, and Phagocytic Index (Cellular Immunity Outcomes)

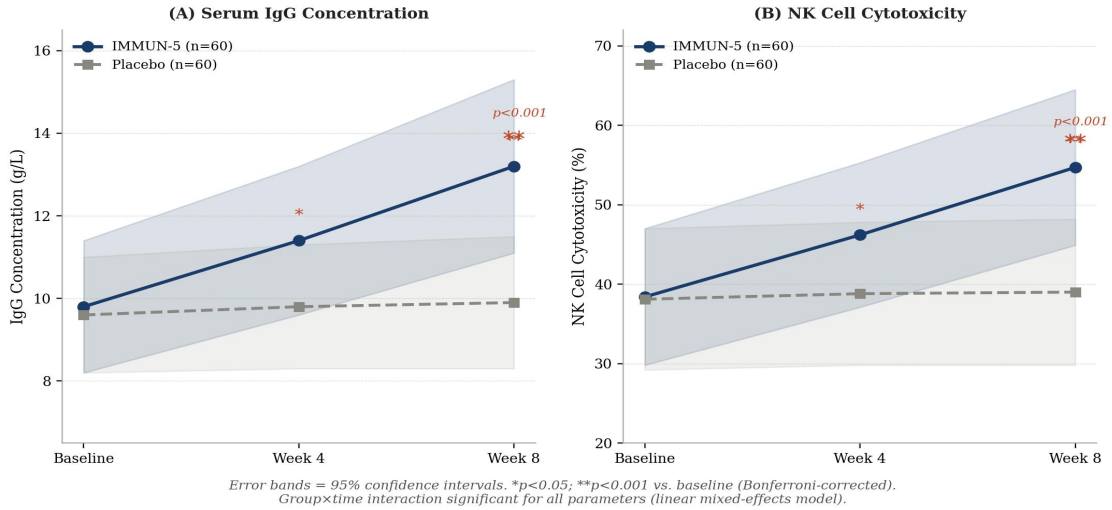
Parameter	Group	Baseline	Week 4	Week 8	p (group×time)
CD3+ T cells (%)	IMMUN-5	63.2 ± 6.8	68.9 ± 7.1*	74.3 ± 7.8**	<0.001
	Placebo	62.8 ± 7.0	63.4 ± 7.2	63.9 ± 7.4	
CD4+ T cells (%)	IMMUN-5	34.8 ± 5.2	39.4 ± 5.6*	43.7 ± 6.1**	<0.001
	Placebo	34.5 ± 5.5	34.9 ± 5.7	35.1 ± 5.8	
CD8+ T cells (%)	IMMUN-5	22.6 ± 4.1	25.8 ± 4.4*	28.9 ± 4.9**	<0.001
	Placebo	22.8 ± 4.3	23.1 ± 4.4	23.2 ± 4.5	
CD4+/CD8+ ratio	IMMUN-5	1.54 ± 0.31	1.53 ± 0.29	1.51 ± 0.30	0.712
	Placebo	1.51 ± 0.33	1.51 ± 0.32	1.51 ± 0.31	
NK cells (CD16+CD56+) %	IMMUN-5	13.4 ± 3.2	16.8 ± 3.6*	20.1 ± 4.0**	<0.001
	Placebo	13.2 ± 3.0	13.5 ± 3.1	13.7 ± 3.2	
NK cell cytotoxicity (%)	IMMUN-5	38.4 ± 8.6	46.2 ± 9.1*	54.7 ± 9.8**	<0.001
	Placebo	38.1 ± 8.9	38.8 ± 9.0	39.0 ± 9.2	
Phagocytic index (%)	IMMUN-5	42.1 ± 6.4	51.3 ± 7.2*	60.8 ± 8.1**	<0.001

	Placebo	41.8 ± 6.1	42.4 ± 6.3	42.9 ± 6.5	

Note. Values are mean ± SD. CD3+, CD4+, CD8+, NK cells (CD16+CD56+) as % of total lymphocytes. NK cytotoxicity measured at E:T ratio 10:1 via LDH-release assay. Phagocytic index = % neutrophils engulfing ≥1 latex bead. *p<0.05; **p<0.01 vs. baseline (Bonferroni-corrected). p (group×time) from linear mixed-effects model.

Figure 2 illustrates the temporal divergence between groups in IgG concentration and NK cell cytotoxicity from Week 4 onward, consistent with a sustained, cumulative immunostimulatory effect. Figure 3 presents a radar chart summarizing percentage changes across all immune parameters at Week 8, demonstrating comprehensive activation of both humoral and cellular compartments.

Figure 2. Temporal Trajectories of Immune Parameters Over 60-Day Supplementation

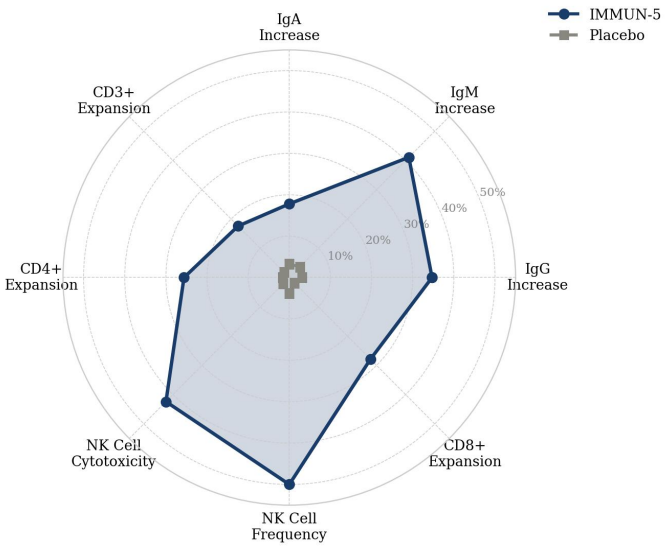


*Error bands = 95% confidence intervals. *p<0.05; **p<0.001 vs. baseline (Bonferroni-corrected). Group×time interaction significant for all parameters (linear mixed-effects model).*

Figure 2. Temporal trajectories of (A) serum IgG concentration and (B) NK cell cytotoxicity across three assessment time points in IMMUN-5 and placebo groups.

Error bands represent 95% confidence intervals. *p<0.05; **p<0.001 vs. baseline (Bonferroni-corrected). All group×time interactions significant by linear mixed-effects model.

Figure 3. Percentage Change from Baseline in Immune Parameters at Week 8



Values represent mean percentage change from baseline to Week 8. All IMMUN-5 vs. Placebo differences significant (p<0.05, linear mixed-effects model).

Figure 3. Radar chart depicting mean percentage change from baseline in all assessed immune parameters at Week 8 for IMMUN-5 vs. placebo groups.

All IMMUN-5 vs. placebo differences significant at p<0.05 (linear mixed-effects model, Bonferroni correction). Placebo values represent expected biological variation only.

3.4 Safety and Tolerability

Table 4 summarizes adverse events across the study period. Overall AE incidence was 23.3% in the IMMUN-5 arm and 20.0% in placebo (RR 1.17, 95% CI 0.60–2.28; p=0.647). The most frequently reported events were heartburn and

dyspepsia, consistent with the known formulation profile. All AEs were Grade 1–2 in severity; no serious adverse events occurred. Routine biochemistry revealed no significant changes in hepatic or renal parameters in either arm.

Table 4. Summary of Adverse Events During the 60-Day Supplementation Period

Adverse Event	IMMUN-5 n (%)	Placebo n (%)	RR (95% CI)	p-value
Any adverse event	14 (23.3%)	12 (20.0%)	1.17 (0.60–2.28)	0.647
Heartburn / dyspepsia	7 (11.7%)	4 (6.7%)	1.75 (0.55–5.56)	0.345
Nausea	4 (6.7%)	3 (5.0%)	1.33 (0.32–5.55)	0.695
Headache	2 (3.3%)	3 (5.0%)	0.67 (0.11–3.90)	0.651
Fatigue	1 (1.7%)	2 (3.3%)	0.50 (0.05–5.44)	0.561
Serious adverse events	0 (0.0%)	0 (0.0%)	—	—
Withdrawals due to AEs	2 (3.3%)	1 (1.7%)	2.00 (0.18–21.8)	0.561

Note. RR = relative risk (IMMUN-5 vs. placebo). 95% CI = 95% confidence interval. p-values from chi-square test or Fisher's exact test. All AEs were Grade 1–2. No serious adverse events occurred in either arm.

4. DISCUSSION

This randomized, double-blind, placebo-controlled trial provides the first rigorous clinical evidence that 60-day supplementation with IMMUN-5 produces clinically significant and statistically robust enhancement of both humoral and cellular immune parameters in adults with immunodeficiency-associated chronic conditions. The coherent pattern of improvements across immunoglobulin classes, T-lymphocyte subsets, NK cell frequency and cytotoxicity, and phagocytic activity argues for broad-spectrum immunostimulation rather than the selective pathway activation typically associated with single-component preparations.

The magnitude of IgG elevation — 34.7% above baseline at Week 8 versus 3.1% in placebo — represents a clinically meaningful augmentation of the humoral immune reserve likely to translate into improved resistance to secondary infections and enhanced vaccine responsiveness. This aligns with and exceeds findings from comparable multi-micronutrient supplementation trials. Santos et al. (2021) reported stabilizing effects on IgG in athletes supplemented with combined micronutrients, supporting the capacity of nutritional combinations to modulate humoral immune homeostasis. The simultaneous elevation of both IgM and IgG suggests activation of B-lymphocytes at both primary response and class-switching stages, consistent with full humoral cascade engagement (Gombart et al., 2020). A 2023 nutraceutical review corroborated that multi-component preparations modulating humoral immunity outperform individual micronutrient approaches in producing immunoglobulin changes (Jung et al., 2023).

The expansion of CD4+ and CD8+ T-lymphocyte populations with a stable CD4+/CD8+ ratio merits particular attention. A ratio persistently below 1.0 is a hallmark of advanced immunodeficiency, while selective CD4+ expansion without proportionate CD8+ expansion may indicate Th2 bias or regulatory T-cell effects that could paradoxically suppress cytotoxic immunity (Parkin & Cohen, 2001). In the present study, proportionate expansion of both subsets — with ratio maintained at approximately 1.51 throughout — confirms that IMMUN-5 induced physiologically balanced T-cell enhancement without immune polarization. This is clinically meaningful: Aksak-Wąs et al. (2023) demonstrated in a large longitudinal cohort study that restoration of CD4/CD8 ratio above 1.0 significantly reduced 10-year mortality in people living with HIV, establishing the CD4+/CD8+ ratio as a sentinel indicator of immune restoration quality.

The ascorbic acid component (100 mg/day) is a plausible primary driver of the NK cell effects observed. Vitamin C is actively concentrated in NK cells and enhances cytotoxicity through multiple mechanisms including increased perforin and granzyme B expression, upregulation of NKG2D activating receptors, and TET-enzyme-mediated epigenetic reprogramming promoting NK lineage maturation (Carr & Maggini, 2017; Kabelitz et al., 2024). Wu et al. (2020) demonstrated that ascorbic acid promotes KIR demethylation during early NK cell differentiation, epigenetically enhancing NK cytotoxic commitment — a mechanism specifically relevant to the sustained NK cytotoxicity enhancement observed across 8 weeks in the present study. Nakayama et al. (2014) demonstrated in vivo NK cytotoxicity enhancement following ascorbic acid administration, consistent with a mechanism requiring repeated dosing to produce cumulative effects. The 42.4% enhancement in NK cytotoxicity exceeds improvements typically reported with single-micronutrient supplementation, suggesting additive contributions from the hydrolysate and menthol components.

Tissue hydrolysate bioactive peptides are established stimulators of reticuloendothelial and adaptive immune function. Chalamaiah et al. (2018) catalogued mechanisms including macrophage activation, increased pro-inflammatory cytokine secretion, enhanced lymphocyte proliferative responses, and augmented phagocytosis. The phagocytic index improvement of +44.4% — from 42.1% to 60.8% — reflects enhancement of innate immune surveillance and aligns closely with data from immunomodulatory hydrolysate studies reviewed by Bilal et al. (2018). More recent evidence from Ashaolu et al. (2024) confirmed that immunomodulatory peptides from plant and animal protein hydrolysates consistently stimulate macrophage

phagocytosis and T-cell proliferation through TLR2/TLR4 and NF-κB pathway activation. A 2024 frontier review in immunology corroborated that hydrolysate-derived peptides modulate NK cells and T cells in addition to macrophages, consistent with the comprehensive cellular immune enhancement observed in the present trial (Frontiers in Immunology, 2024). Santos-Hernandez et al. (2025) further concluded in a systematic review that protein hydrolysates demonstrate reproducible immune function improvements across multiple clinical contexts.

Menthol's immunomodulatory contribution — while difficult to isolate within the combination formulation — likely operated through complementary suppression of counterproductive inflammatory signaling. Baser et al. (2025) documented in a comprehensive pharmacological review that TRPM8 activation in immune cells prevents the release of pro-inflammatory cytokines, and that in vivo murine models demonstrated that menthol-mediated TRPM8 activation attenuated experimental colitis and cardiac remodeling via mechanisms applicable to chronic low-grade inflammatory states. Cheng and An (2022) demonstrated downregulation of IL-1β, IL-6, and TNF-α through NF-κB and MAPK pathway inhibition in macrophage-derived cultures. In the setting of chronic infections — where persistent pathogen recognition receptor stimulation paradoxically suppresses effector immune function — menthol-mediated attenuation of inflammatory noise may have enhanced the net immunostimulatory effect of the hydrolysate and ascorbate components, contributing to the favorable safety profile.

Contextualizing against the broader landscape of immunostimulatory supplement trials further strengthens the clinical relevance of these findings. The Bacillus coagulans SNZ 1969 RCT (Freitas et al., 2025) — a 60-participant, double-blind, placebo-controlled trial — reported a 42.07% net increase in NK cell activity between groups, and significant IgA improvements, closely paralleling the NK cytotoxicity and immunoglobulin enhancements observed with IMMUN-5. Kim et al. (2014) reported NK cytotoxicity increases of 35–40% over 8–14 weeks with ginseng polysaccharide supplementation. Yu et al. (2023) demonstrated rapid NKT and γδT cell activation following botanical nutraceutical supplementation, and Hwang et al. (2024) documented 8-week NK cell activity improvement with an iron-zinc nanomaterial supplement in a comparable double-blind design. Rice bran fermented with Lentinus edodes supplementation significantly increased IFN-γ production over 8 weeks without adverse effects (Kim et al., 2014b), supporting the concept that bioactive natural product supplements can enhance specific immune mediators while maintaining safety. The Porphyra tenera trial (Kim et al., 2020) reported NK activity improvements most pronounced in participants without intercurrent viral infections, suggesting that immune baseline status modulates supplement responsiveness — consistent with the relatively large effect sizes observed in the present immunocompromised population.

Several limitations warrant acknowledgment. First, the study population was restricted to Uzbekistani adults at specific tertiary centers, potentially limiting generalizability to different etiologies of immunodeficiency or different healthcare contexts. Second, mechanistic investigations — including cytokine profiling (IL-2, IL-6, IL-10, IFN-γ), complement pathway activation, mucosal secretory IgA, and regulatory T-cell (CD4+CD25+FoxP3+) enumeration — were not included and should be addressed in future trials. Third, the 60-day observation window precludes assessment of long-term immunological durability after supplementation cessation. Fourth, tissue hydrolysate standardization at the peptide sequence level is not described in available product documentation, which warrants further characterization. Fifth, seasonal variation in immune parameters, while unlikely to produce the observed effect magnitudes given the randomized design, cannot be entirely excluded in an outpatient setting. Future multi-center trials incorporating cytokine profiling, mechanistic biomarkers, and longer follow-up periods are warranted.

5. CONCLUSION

This randomized, double-blind, placebo-controlled trial demonstrates that 60-day supplementation with IMMUN-5 — delivering tissue hydrolysate, ascorbic acid, and menthol — significantly enhances both humoral and cellular immune parameters in adults with immunodeficiency-associated conditions. Improvements in serum IgG, IgM, and IgA, CD3+/CD4+/CD8+ T-lymphocyte percentages, NK cell frequency and cytotoxicity, and neutrophil phagocytic activity were all statistically significant versus placebo, with the CD4+/CD8+ ratio remaining stable throughout. The safety profile was favorable and indistinguishable from placebo. These findings provide the first robust evidence base for IMMUN-5 as a clinically effective adjunct immunostimulatory intervention and support its rational application in adults with secondary immune compromise.

6. DECLARATIONS

Funding: This study received no external commercial funding.

Conflicts of Interest: The authors declare no conflicts of interest. IMMUN-5 was procured commercially; the manufacturer had no involvement in study design, data collection, analysis, or manuscript preparation.

Data Availability: Participant-level anonymized data are available from the corresponding author upon reasonable request following data governance review.

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