INFORMATICS JOURNALS

Research Review

Journal of Natural Remedies
DOI: 10.18311/jnr/2026/53886

eISSN: 2320-3358 | Vol 26(4), 2026 | pp. 1033-1049

Immunostimulatory Effects of a Fermented *Moringa oleifera* Extract in Cyclophosphamide-induced Immunodeficient Mice

Tung Thanh Tran[1], Phong Xuan Pham[2], Cu Duc Ngo[3], Ha Thi Pham[3], Hang Thi Thu Dinh[1], Quang Vinh Trinh[1], Phuong Chi Pham[1], Thuy Thi Nguyen[1], Lam Bui Tung Pham[1] and Van Anh Thi Pham[1*]

[1]Hanoi Medical University, Hanoi – 100000, Vietnam; phamvananh@hmu.edu.vn

[2]Military Institute of Traditional Medicine, Hanoi – 100000, Vietnam

[3]Moringa Joint Stock Company, Hanoi – 100000, Vietnam

Abstract

Background: Immunosuppression, characterised by impaired immune function, often results from chemotherapy, chronic infections, or autoimmune diseases, increasing susceptibility to infections and complicating disease management. **Aim:** This study evaluated the immunostimulatory effects of a fermented *Moringa oleifera* extract (MOE), with *M. oleifera* as the main constituent, combined with complementary herbs *Celastrus hindsii* and *Curcuma longa*, in cyclophosphamide (CY)-induced immunodeficient Swiss mice. **Methods:** Fifty mice were randomly assigned to five groups: normal control, model (CY-treated), positive control (thymomodulin), and MOE-treated (14.4 mL/kg/day or 28.8 mL/kg/day). Immunosuppression was induced via a single intraperitoneal CY injection (200 mg/kg) on day 4. Assessed parameters included body weight, relative spleen/thymus weights, total/differential leukocyte counts, delayed-type hypersensitivity (DTH) to ovalbumin (OA), serum IL-2, TNF-α, and IgG levels, and spleen/thymus histopathology. **Results:** MOE enhanced immune responses compared to the model group, showing a protective trend in DTH responses, cytokine levels (IL-2, TNF-α), and lymphoid organ histology. At 14.4 mL/kg, peripheral IL-2 rose significantly to 14.82 ± 5.32 pg/mL ($p < 0.05$), with marked histopathological improvements in spleen and thymus ($p < 0.01$ each). At 28.8 mL/kg, IL-2 increased to 14.05 ± 5.85 pg/mL ($p < 0.05$), with thymus ($p < 0.05$) and spleen ($p < 0.01$) histological gains. **Conclusion:** Although trends were observed, no significant differences ($p > 0.05$) were found in organ weights, leukocyte counts, IgG levels, or DTH responses.

Major Finding: These findings suggest that MOE exerts immunostimulatory effects, primarily by improving selected cytokines and histology endpoints, supporting its potential as a therapeutic agent for immunodeficiency.

Keywords: *Celastrus hindsii*, *Curcuma longa*, Cyclophosphamide, Fermentation, Immunostimulatory, *Moringa oleifera*

(Article Chronicle: Article Received on: 02.12.2025; Revised on: 09.03.2026; Accepted on: 16.03.2026)

1. Introduction

Immunology is currently one of the most rapidly advancing and promising fields in biomedical technology, with significant implications for human health and disease prevention[1]. A crucial and urgent research direction in contemporary immunology involves addressing complex issues related to preventing and treating immune dysfunction disorders[2]. These disorders encompass a broad spectrum of conditions, including primary and secondary immunodeficiency diseases, chronic inflammatory conditions, cancer-associated immunosuppression, and diseases caused by excessive immune responses, such as autoimmune disorders and systemic inflammatory diseases[3]. The growing prevalence of these conditions, particularly in ageing populations and those susceptible to viral

*Author for correspondence

infections, underscores the critical need for effective immunomodulatory interventions[4,5]. Consequently, immune modulation to restore and maintain optimal immune system balance has emerged as a therapeutic goal for current research and clinical practice.

Immunostimulants enhance the functional activity of immune cells, thereby strengthening the body's defence mechanisms against pathogens and malignant cells[6]. The clinical application of chemically synthesised or semi-synthesised immunostimulants has demonstrated significant efficacy in preventing and treating various diseases[7]. However, the widespread use of chemical-based immunomodulatory drugs is often limited by their associated adverse effects, including hepatotoxicity, nephrotoxicity, and systemic toxicity[8]. In addition, highly effective recombinant cytokines present considerable economic barriers due to their high production costs and complex manufacturing requirements, making them less accessible for widespread therapeutic use. In contrast, natural substances, particularly those derived from medicinal plants, offer distinct advantages, including enhanced safety profiles, reduced toxicity, cost-effectiveness, and greater accessibility for patients in developed and developing countries[9].

Plant-based therapeutics are increasingly preferred in many healthcare systems, as botanical materials are generally more affordable and accessible than synthetic pharmaceuticals. Vietnam, in particular, possesses substantial advantages due to its rich biodiversity of medicinal herbs and the country's extensive historical tradition of herbal medicine in traditional healthcare practices[10–12]. Many medicinal herbs and their isolated bioactive compounds have been scientifically demonstrated to stimulate various aspects of immune responses, including innate and adaptive immunity[8].

Moringa oleifera, commonly known as the drumstick tree or horseradish tree, is a plant species indigenous to the western and sub-Himalayan regions, with natural distribution extending across India, Asia Minor, Arabia, various regions of Africa, and Pakistan[13]. This remarkable plant has gained international recognition for its well-documented efficacy as an immunostimulant, primarily attributed to its diverse bioactive phytochemical constituents[13]. The *M. oleifera*-based fermentation extract (MOE) utilised in this study represents a complex botanical preparation that incorporates not only *M. oleifera* as the primary active ingredient, but also complementary medicinal herbs including *C. hindsii* and *C. longa*[13,14]. Scientific studies have well-documented the individual therapeutic effectiveness of these herbs in supporting immune system function, with each component contributing unique bioactive compounds and mechanisms of action[13–15].

Despite the extensive individual characterisation of these medicinal plants, there remains a significant gap in the scientific literature regarding the immunomodulatory effects of combining these specific herbal ingredients in a standardised fermentation extract formulation. The combined immunomodulatory effects of these herbs and their impact on immune system parameters have not been adequately evaluated. Therefore, we conducted this study to assess the immunostimulatory effects of this novel MOE in a well-established cyclophosphamide (CY)-induced immunosuppression model using Swiss mice. To ensure scientific rigour and clinical relevance, thymomodulin was employed as a positive control reference drug widely utilised in clinical settings to enhance human immune system function[16]. Our experimental design incorporated an evaluation of fundamental immunodeficiency pathological parameters, including detailed assessment of both humoral and cell-mediated immune systems, delayed-type hypersensitivity reactions, and histopathological evaluations of the spleen and thymus—two critical lymphoid organs that play essential roles in immune system function and regulation[17].

2. Materials and Methods

2.1 Plant Materials and Extract Preparation

The *M. oleifera*-based fermentation extract (MOE) used in this study was manufactured by Vietnam Medicinal Herbs Joint Stock Company (VIETMEC). Fresh plant materials were provided by Moringa Joint Stock Company, Vietnam, cultivated at the company's farm, and harvested at optimal maturity stages to ensure maximum bioactive compound content. All botanical materials were authenticated and identified according to the Vietnamese Pharmacopoeia 5th edition standards[18].To ensure the quality and consistency of

the starting materials, the raw herbal powders were chemically characterised before fermentation. The official certificate of analysis, detailing the quantification of key bioactive marker compounds for the specific batch used in this study (No. 08/ĐT-DL/2025), is provided as a Supplementary File. The standardised herbal formulation comprised the following botanical components with their respective quantities per 100 mL of final product: *Moringa oleifera* (7.0 g), *Celastrus hindsii* (1.0 g), *Curcuma longa* (0.5 g), excipients, and stabilisers.

The production of MOE employed a two-stage process. The initial fermentation stage involved cultivating beneficial bacterial strains, including *Bacillus* spp., *Lactobacillus* spp., and other lactic acid bacteria (developed and standardised by VIETMEC). The detailed composition of the microbial strains, including identification methods and culture conditions, is presented in Table 1. The microbial inoculum was maintained within 8-13% of the total fermentation volume. Fermentation was conducted at a controlled temperature of 35-37 °C. The second stage utilised the matured microbial consortium as a starter culture at 3-5% of the total extract and substrate weight. The plant materials underwent controlled bio-fermentation for 15-20 days under the following conditions: 34- 38 °C, 1 atm ± 0.1 bar (anaerobic conditions, no compression or vacuum). Upon completion of the bio-fermentation process, the final product was filtered, standardised, and packaged in sterile 100 mL amber bottles to protect from light degradation. Products were stored under controlled conditions (<25°C, <70% relative humidity) until use.

Following manufacturing, all batches of the investigational product underwent a quality examination in accordance with Good Manufacturing Practice (GMP) standards. After the preparation, the investigational product was subjected to a quality examination confirmed by the VIETMEC Legal Director (Registration number: 121/TCSP/DLVN). The quality examination conformed to the guidelines of the Vietnamese Pharmacopoeia 5th edition[18]. The MOE also underwent quality control testing by the National Institute for Food Control in Vietnam to determine its physicochemical and microbiological standards, as shown in Table 2.

Table 1. Microbial consortium composition used in *Moringa oleifera*-based fermentation extract (MOE)

Strain Code	Microbial Group	Species Name	Isolation Source	Culture Conditions	Identification Method
B01–2025	*Bacillus* spp.	*Bacillus subtilis*	Soil / Fermented herbal extract	37°C, pH 7.0, Nutrient Agar	16S rRNA gene sequencing
B02–2025	*Bacillus* spp.	*Bacillus amyloliquefaciens*	Fermented herbal extract	37°C, pH 6.8, Nutrient Agar	Gram staining + 16S rRNA sequencing
B03–2025	*Bacillus* spp.	*Bacillus licheniformis*	Soil	35°C, pH 7.2	Biochemical analysis
L01–2025	*Lactobacillus* spp.	*Lactobacillus plantarum*	Fermented herbal extract	30°C, pH 6.5, MRS broth	16S rRNA gene sequencing
L02–2025	*Lactobacillus* spp.	*Lactobacillus casei*	Probiotic environment	37°C, pH 6.2, MRS agar	MALDI-TOF MS
L03–2025	*Lactobacillus* spp.	*Lactobacillus rhamnosus*	Fermented dairy product	37°C, pH 6.0, MRS broth	Gram staining + Gene sequencing
LAB02–2025	Lactic Acid Bacteria (LAB)	*Leuconostoc mesenteroides*	Fermented herbal extract	30°C, pH 6.5, MRS broth	16S rRNA gene sequencing
LAB02–2025	Lactic Acid Bacteria (LAB)	*Pediococcus pentosaceus*	Fermented herbal extract	35°C, pH 6.0, MRS agar	Biochemical analysis + PCR
LAB03–2025	Lactic Acid Bacteria (LAB)	*Enterococcus faecium*	Animal intestine / Probiotic environment	37°C, pH 7.0, MRS broth	16S rRNA gene sequencing

MRS: de Man, Rogosa and Sharp; MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry; PCR: Polymerase Chain Reaction. All strains were authenticated and maintained by Vietnam Medicinal Herbs Joint Stock Company (VIETMEC) following Good Manufacturing Practice (GMP) standards.

Table 2. Physicochemical and microbiological composition of *Moringa oleifera*-based fermentation extract (MOE)

Indicator Name	Unit	Testing Method	Result
Lysine	mg/100mL	NIFC.05.M.101 (HPLC)	15.1
Methionine	mg/100mL	NIFC.05.M.101 (HPLC)	1.15
Phenylalanine	mg/100mL	NIFC.05.M.101 (HPLC)	6.09
Polysaccharide (Calculated as Glucose)	g/100 mL	NIFC.05.M.175 (UV-VIS)	0.35
Proline	mg/100mL	NIFC.05.M.101 (HPLC)	13.0
Serine	mg/100mL	NIFC.05.M.101 (HPLC)	7.88
Threonine	mg/100mL	NIFC.05.M.101 (HPLC)	8.58
Triterpenoid (Calculated as Oleanolic acid)	mg/100mL	NIFC.05.M.206 (UV-VIS)	374
Tryptophan	mg/100mL	NIFC.05.M.188 (HPLC)	8.30
Tyrosine	mg/100mL	NIFC.05.M.101 (HPLC)	19.1
Valine	mg/100mL	NIFC.05.M.101 (HPLC)	14.8
Glucid	g/100mL	NIFC.02.M.06	2.21
Polyphenol (Calculated as gallic acid)	mg/100mL	NIFC.02.M.44 (UV-VIS)	80.6
Protein	g/100mL	NIFC.02.M.03	0.66
Vitamin B6 (Pyridoxine.HCl)	mg/100mL	NIFC.02.M.15 (HPLC)	1.21
Vitamin C (Ascorbic acid)	mg/100mL	NIFC.02.M.14 (HPLC)	5.59
Iron	mg/100mL	NIFC.03.M.19 (ICP-OES)	2.93
Amino acid	mg/100mL	NIFC.05.M.101 (HPLC)	292
Aspartic acid	mg/100mL	NIFC.05.M.101 (HPLC)	107
Glutamic acid	mg/100mL	NIFC.05.M.101 (HPLC)	36.1
Alanine	mg/100mL	NIFC.05.M.101 (HPLC)	26.8
Arginine	mg/100mL	NIFC.05.M.101 (HPLC)	5.02
Chlorophyll	mg/100mL	NIFC.05.M.123 (Ref. AOAC 942.04) (UV-VIS)	0.45
Cystine	mg/100mL	NIFC.05.M.101 (HPLC)	4.57
Flavonoid (Calculated as Quercetin)	mg/100mL	NIFC.05.M.139 (UV-VIS)	8.72
Glycine	mg/100mL	NIFC.05.M.101 (HPLC)	3.32
Gymnemic acid	g/100mL	NIFC.05.M.250 (HPLC)	0.49
Hesperidin	mg/100mL	NIFC.05.M.115 (HPLC)	3.18
Histidine	mg/100mL	NIFC.05.M.101 (HPLC)	2.31
Isoleucine	mg/100mL	NIFC.05.M.101 (HPLC)	9.27
Leucine	mg/100mL	NIFC.05.M.101 (HPLC)	12.4
Total aerobic microorganisms	CFU/mL	TCVN 4884-1:2015	5.0 x 10^2
Calcium	mg/100mL	NIFC.03.M.19 (ICP-OES)	27.4
Copper	µg/100mL	NIFC.03.M.45 (ICP-MS)	8.26
Potassium	mg/100mL	NIFC.03.M.19 (ICP-OES)	177
Magnesium	mg/100mL	NIFC.03.M.19 (ICP-OES)	35.2
Manganese	µg/100mL	NIFC.03.M.45 (ICP-MS)	1142
Phosphorus	mg/100mL	NIFC.03.M.19 (ICP-OES)	40.3

NIFC: National Institute for Food Control; HPLC: High-Performance Liquid Chromatography; UV-VIS: Ultraviolet-Visible Spectrophotometry; ICP-OES: Inductively Coupled Plasma-Optical Emission Spectrometry; ICP-MS: Inductively Coupled Plasma-Mass Spectrometry; CFU: Colony-Forming Units. Each testing method was coded based on NIFC's internal standardization system and accompanied by the respective quantitative analytical approach used.

2.2 Experimental Animals

Healthy Swiss mice of both sexes, weighing 25 ± 5 g, were obtained from the National Institute of Hygiene and Epidemiology (NIHE), Vietnam. Animals were housed in cages (10 animals per cage) under standardised laboratory conditions: temperature 25 ± 2°C, relative humidity 60 ± 10%, and a 12:12-hour light/dark cycle. Mice had *ad libitum* access to standard laboratory chow (NIHE, Vietnam) and sterile water. Before experimental procedures, all animals underwent a 7-day acclimatisation period at the Department of Pharmacology, Hanoi Medical University.

2.3 Drugs and Chemicals

Cyclophosphamide (CY): Endoxan® (Baxter Oncology GmbH, Germany), 200 mg vials; stock solutions were prepared fresh in sterile saline (0.9% NaCl) immediately before administration. Thymomodulin: Semozine® capsules, 80 mg (Yoo Young Pharmaceutical Company Limited, South Korea), used as the positive control for immunostimulatory activity. Ovalbumin (OA) emulsion: Complete Freund's adjuvant containing ovalbumin and aluminium hydroxide ($Al(OH)_3$) at a 1:4 ratio (Sigma-Aldrich, USA), prepared by dissolving 100 mg $Al(OH)_3$ in 50 mL sterile saline (0.9% NaCl) and combining with ovalbumin to achieve the specified ratio. Sheep red blood cells (SRBC): Fresh venous blood collected aseptically from healthy sheep and preserved in Alsever's solution (glucose 24.6 g, sodium citrate 9.6 g, sodium chloride 5.05 g, distilled water to 1200 mL, pH 6.1). SRBC suspension was prepared at 5% concentration in sterile saline and stored at 4°C for 2 weeks (provided by Nam Khoa Pharmaceutical Company, Vietnam).

2.4 Study Methods

Fifty Swiss mice were randomly allocated into five experimental groups (n=10 per group) using a randomized block design: Group 1 (Normal control) and Group 2 (Model): Received distilled water orally (10 mL/kg/day); Group 3 (Thymomodulin - positive control): Received thymomodulin (38.4 mg/kg/day, p.o.); Group 4 (Low-dose MOE): Received MOE (14.4 mL/kg/day, p.o.); Group 5 (High-dose MOE): Received MOE (28.8 mL/kg/day, p.o.).

The selected doses were based on the MOE safety profile from previous toxicity studies, the recommended dosage for each herb in the Vietnamese Pharmacopoeia 5th edition guidelines, and traditional medicinal usage[18].

The experimental protocol spanned 8 days with the following timeline (Table 3):

2.4.1 Body Weight Monitoring

Individual body weights were recorded using a digital scale (Precisa®, Swiss, Model 321LX Type 2200 C) at baseline (day 0) and study termination (day 7).

2.4.2 Organ Weight Assessment

Following sacrifice by ketamine anaesthesia (100 mg/kg, i.p.) and cardiac puncture, spleen and thymus were carefully dissected, cleared of surrounding tissues, blotted dry, and weighed immediately. Relative organ weights were calculated using the formula:

$$Relative\ spleen\ or\ thymus\ weight\ (mg/g) = \frac{Weight\ of\ spleen\ or\ thymus(mg)}{Body\ weight\ of\ mouse(g)}$$

Table 3. Experimental protocol timeline for immunosuppression study in *Swiss* mice.

Timeline	Action items
Days 1-7	Daily oral administration of respective treatments
Day 2	Immunization with SRBC (5% suspension, 0.5 mL, i.p.) and OA antigen (0.1 mL, s.c.)
Day 4	A single intraperitoneal injection of cyclophosphamide at 200 mg/kg (except normal control group, using physiological saline (10 mL/kg) [10–12]
Day 7	Challenge injection with OA antigen (50 µL into right hind footpad) and saline control (50 µL into left hind footpad)
Day 8	Animal sacrifice and sample collection

*All animals were coded, and outcome assessors were blinded to group allocation to minimize bias.

2.4.3 Delayed-type Hypersensitivity (DTH) Response

Cell-mediated immunity was assessed through the DTH response to OA antigen. Footpad thickness was measured using digital callipers (INSIZE®, Type 2312-20) immediately before challenge injection (day 7) and 24 hours post-challenge (day 8). For each measurement, duplicate readings were taken, and the average was recorded as the final value. Delayed-type hypersensitivity (DTH) response was calculated as:

$$DTH\ response\ (\%) = \frac{Thickness_{24h} - Thickness_{0h}(mm)}{Thickness_{0h}(mm)} \times 100$$

Where measurements represent the difference between antigen-injected and saline-injected footpads.

2.4.4 Leukocyte Counts

Blood samples collected via cardiac puncture were analysed using an automated haematology analyser (Exigo®-VET, Boule Medical AB, Sweden) to determine the number of total white blood cells, lymphocytes, neutrophils, and monocytes.

2.4.5 Cytokine and Immunoglobulin Quantification

Serum samples were separated by centrifugation (3000 rpm, 10 minutes, 4°C) and stored at -80°C until analysis. Concentrations of interleukin-2 (IL-2), tumour necrosis factor-alpha (TNF-α), and immunoglobulin G (IgG) were determined using commercial ELISA kits (Cloud-Clone Corp., USA; Catalogue numbers: SEA073Mu, SEA133Mu, and SEA544Mu, respectively) according to the manufacturer's protocols. Each sample was assayed in duplicate. Optical density was measured using a microplate reader (BioTek® ELx808, USA), and the mean of the duplicate readings was used for analysis.

2.4.6 Histopathological Examination of the Spleen and Thymus

Spleen and thymus tissues from 6 randomly selected animals per group were fixed in 10% neutral buffered formalin for 24 hours, processed through graded alcohols, embedded in paraffin, and sectioned at a thickness of 5 µm. Sections were stained with hematoxylin and eosin (H and E) and examined under light microscopy (Olympus BX10, Japan) at 400x (for spleen) and at 100× magnification (for thymus). Digital images were captured using an Olympus DP12 camera.

Tissue damage was evaluated based on the following parameters. As for spleen: Reticular cell integrity, lymphocyte density, macrophage infiltration, degeneration, necrosis, and inflammation. About thymus: Cortical-medullary architecture, lymphocyte density, macrophage infiltration, degeneration, necrosis, and inflammation. A semi-quantitative scoring system based on the percentage of affected tissue was employed as follows: 0 = Normal histological architecture; 1 = Mild damage (1-30% of tissue affected); 2 = Moderate damage (31-70% of tissue affected); 3 = Severe damage (>70% of tissue affected).

To mitigate the risk of measurement bias, outcome assessors were blinded to the group allocations. The technicians measuring the DTH response and processing the coded samples for haematology and ELISA were unaware of the treatment conditions. To ensure precision and minimise error, all DTH measurements were performed by a single technician. The histopathological examination was scored by a single pathologist, who remained blinded to all treatment groups throughout the histological preparation and evaluation process.

Table 4. Effects of MOE on DTH response and relative organ weights in cyclophosphamide-treated mice

Group	Treatment (n = 10)	Dermal response (% increase in foot thickness)	Relative weight of spleen (mg/g)	Relative weight of thymus (mg/g)
I	Normal control	9,69 ± 2,01	4.92 ± 0.99	2.94 ± 1.13
II	Model	6,39 ± 2,18**	2.67 ± 0.71***	0.98 ± 0.34***
III	Thymomodulin	9,16 ± 3,00$^{\Delta}$	3.33 ± 0.65**	1.48 ± 0.49**
IV	Low-dose MOE	7,43 ± 2,42	2.99 ± 0.82***	1.50 ± 0.65**
V	High-dose MOE	8,19 ± 2,68	2.94 ± 0.94***	1.13 ± 0.80***

***, ***: $p < 0.01$, $p < 0.001$ significantly different from the normal control;*
Δ: $p < 0.05$, significantly different from the model group.

2.4.7 Statistical Analysis

Data were entered and analysed using SPSS Version 26.0 software (IBM Corp, New York, USA). Results are expressed as observed mean ± standard deviation (SD). Before parametric testing, the normality of the continuous data distributions was confirmed using the Shapiro-Wilk test, and the homogeneity of variances was verified using Levene's test. The one-way analyses of variance (ANOVA), followed by Tukey HSD post-hoc comparisons tests, were applied. For non-parametric data (histological grading), the Kruskal–Wallis test followed by Dunn's post-hoc test was used for pairwise comparisons. A p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1 General Observations Throughout the Study

Throughout the 8-day experimental period, all fifty mice remained alive and healthy. No adverse effects were observed in any treatment group, with no notable changes in vital signs, physical appearance (skin and fur condition), or daily behavioural patterns. All animals maintained normal activity levels and feeding behaviour until the time of sacrifice on Day 8.

3.2 Effects of MOE on Relative Organ Weights

All groups treated with CY exhibited significant reductions in relative spleen and thymus weights compared to the normal control group ($p < 0.01$ or $p < 0.001$). The thymomodulin-treated group and both MOE treatment groups (14.4 mL/kg and 28.8 mL/kg) demonstrated protective trends, with relative spleen and thymus weights showing slight improvement compared to the model group. However, these improvements did not reach statistical significance ($p > 0.05$) (Table 4).

3.3 Effects of MOE on Delayed-type Hypersensitivity (DTH) Response

Cell-mediated immune function, assessed through DTH response to ovalbumin antigen, was significantly impaired in the cyclophosphamide-treated model group compared to normal controls ($p < 0.01$). The thymomodulin positive control group demonstrated significant restoration of DTH response ($p < 0.05$ compared to the model group), indicating effective immune-stimulation. Both MOE treatment groups showed protective effects, maintaining DTH responses comparable to normal controls with no significant difference from normal controls ($p > 0.05$). While both MOE groups showed numerical improvements compared to the model group, these differences did not achieve statistical significance ($p > 0.05$) (Table 4).

3.4 Effects of MOE on Leukocyte Populations

Cyclophosphamide (CY) treatment induced severe leukopenia across all CY-treated groups. Total white blood cell counts were dramatically reduced compared to normal controls ($p < 0.001$). Lymphocyte counts followed similar patterns, with all CY-treated groups showing significant reductions compared to controls ($p < 0.001$). Neutrophil counts remained relatively stable across all groups, with no significant differences observed ($p > 0.05$). Monocyte populations were particularly affected, with significant decreases in the model group and both MOE-treated groups compared to normal controls ($p < 0.01$)(Table 5).

Table 5. Effects of MOE on leukocyte populations in cyclophosphamide-treated mice

Group	Treatment (n = 10)	Leukocyte Count (Mean ± SD)			
		WBC (G/L)	*LYM (G/L)*	*NEU (G/L)*	*MONO (G/L)*
I	Normal control	3,76 ± 0,94	2.89 ± 0.81	0.24 ± 0.12	0.63 ± 0.27
II	Model	1,47 ± 0,44***	1.09 ± 0.34***	0.22 ± 0.08	0.16 ± 0.13**
III	Thymomodulin	1,67 ± 0,53***	1.10 ± 0.38***	0.20 ± 0.07	0.37 ± 0.28
IV	Low-dose MOE	1,55 ± 0,49***	1.18 ± 0.46***	0.19 ± 0.07	0.18 ± 0.08**
V	High-dose MOE	1,35 ± 0,56***	0.98 ± 0.39***	0.16 ± 0.07	0.21 ± 0.16**

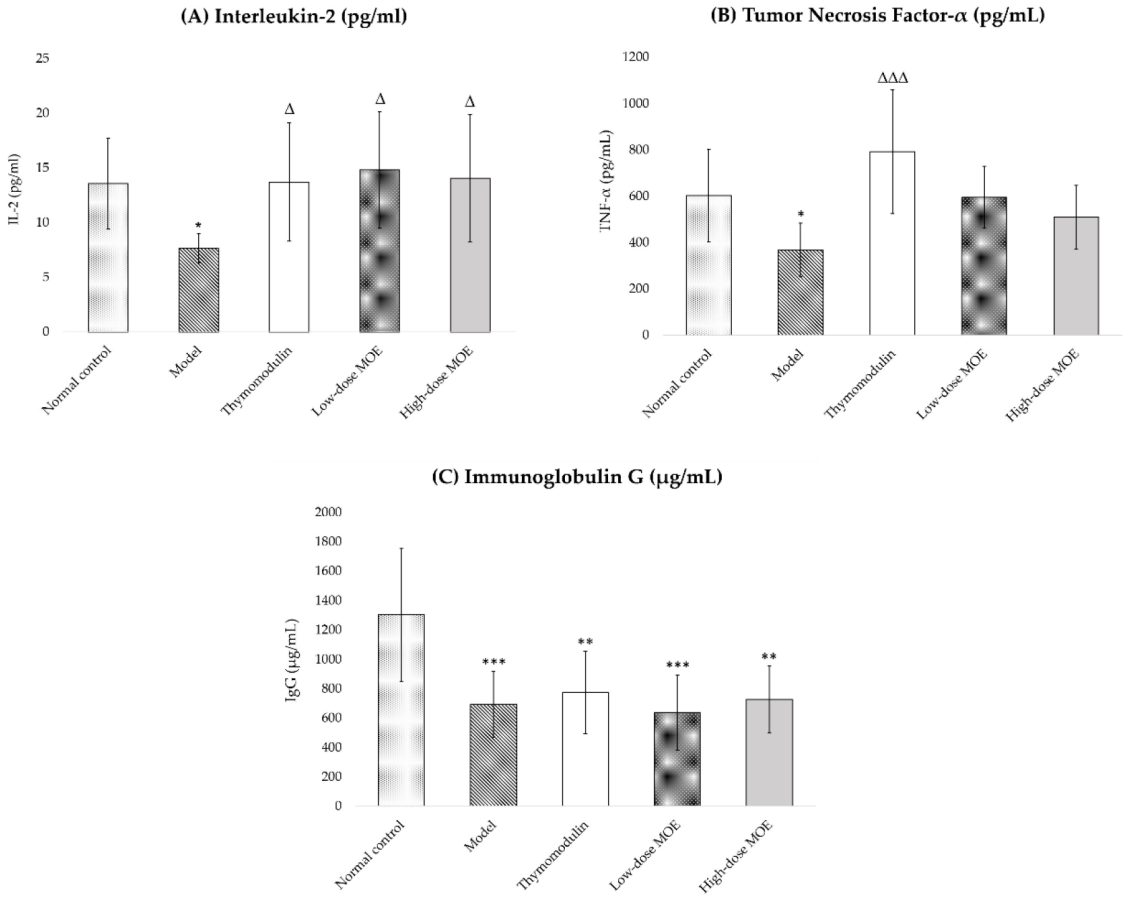
WBC: total white blood cell; LYM: lymphocyte; NEU: neutrophil; MONO: monocyte.
,: p < 0.01, p < 0.001 significantly different from the normal control.*

3.5 Effects of MOE on Serum Cytokines and Immunoglobulin Levels

Cyclophosphamide (CY) treatment significantly reduced serum TNF-α levels in the model group compared to normal controls ($p < 0.05$). The thymomodulin group showed robust restoration of TNF-α levels, significantly exceeding both normal controls and the model group ($p < 0.001$ compared to model). Both MOE treatment groups demonstrated protective effects on TNF-α levels, maintaining concentrations comparable to normal controls without significant differences ($p > 0.05$ compared to normal controls). IL-2 levels were significantly suppressed in the model group compared to normal controls ($p < 0.05$). The thymomodulin group showed significant restoration of IL-2 levels compared to the model group ($p < 0.05$). Both MOE treatment groups demonstrated significant improvements in IL-2 levels compared to the model group ($p < 0.05$), with levels comparable to or slightly exceeding normal controls. All cyclophosphamide-treated groups showed significant reductions in serum IgG levels compared to normal controls ($p < 0.01$ or $p < 0.001$)(Figure 1).

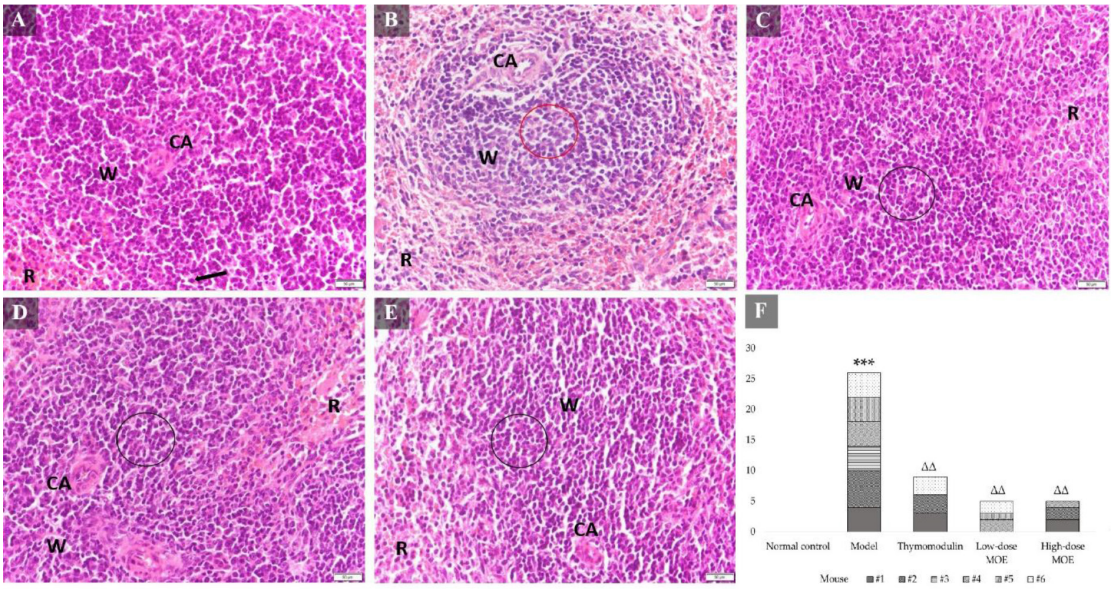
3.6 Histopathological Assessment of Lymphoid Organs

Microscopic examination revealed severe structural damage in the model group spleen compared to normal controls ($p < 0.001$). Both the thymomodulin and MOE treatment groups demonstrated significant protective effects, with substantially reduced damage scores compared to the model group ($p < 0.01$) (Figure 2F). Similar protective effects were observed in thymic tissue. The model group showed significant



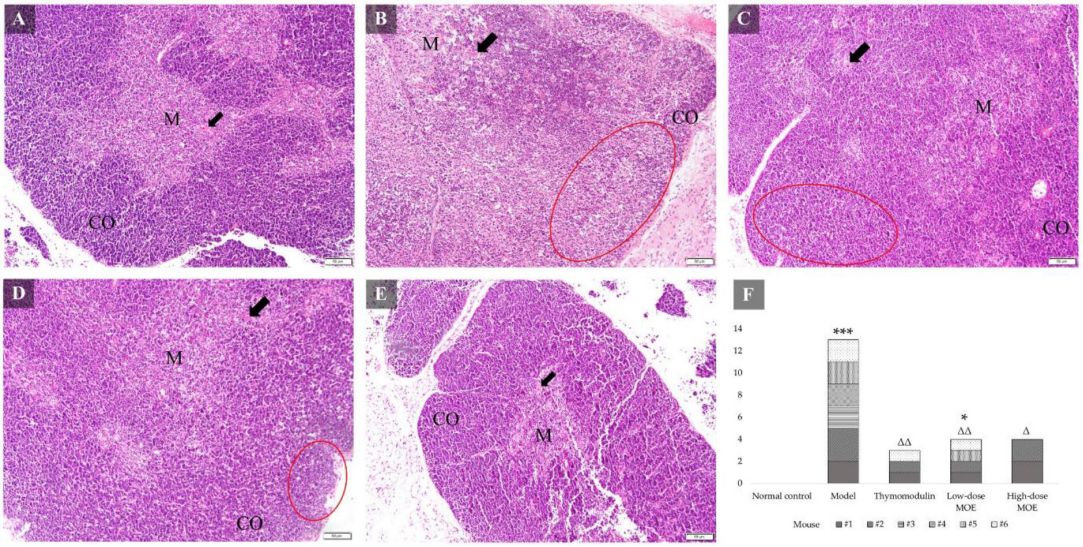
Quantification of (A) Interleukin-2 (IL-2, pg/mL), (B) Tumor Necrosis Factor-alpha (TNF-α, pg/mL), and (C) Immunoglobulin G (IgG, μg/mL) in serum across treatment groups (n=10 per group). **, **, ***: $p < 0.05$, $p < 0.01$, $p < 0.001$, significantly different from the normal control group;* $^{\Delta, \Delta\Delta\Delta}$*: $p < 0.05$, $p < 0.001$, significantly different from the model group.*

Figure 1. Serum levels of immunological markers in cyclophosphamide-treated mice.



H and E staining of spleen sections at 400x magnification. In the spleen, the low-dose MOE group exhibited a well-restored lymphocyte density in the white pulp region, nearly reaching normal levels, with no apparent signs of degeneration or necrosis. In contrast, the high-dose MOE group also showed improvement, but the lymphocyte density appeared less concentrated compared to the low-dose group. While the overall splenic architecture was recovered, some areas still displayed reduced cellularity. (A) Normal control: intact histological architecture with dense lymphocytes (black arrow). (B) Model: expanded red pulp, reduced white pulp with low lymphocyte density (red circle), and no degeneration or necrosis. (C) Thymomodulin: mildly reduced lymphocyte density in white pulp (black circle), no degeneration. (D) Low-dose MOE and (E) High-dose MOE: near-normal histological architecture with slightly reduced lymphocyte density. (F) Histological damage scores (n = 6 per group). ****: p < 0.001, significantly different from the normal control group; $^{\Delta\Delta}$: p < 0.01, significantly different from the model group. (W: White pulp; R: Red pulp; CA: Central artery).*

Figure 2(A-E, F). Histopathological analysis of spleen in cyclophosphamide-treated mice.



H and E staining of thymus sections at 100x magnification. In the thymus, the low-dose MOE group showed a marked restoration of lymphocyte density in both the cortex and medulla. Dense lymphocytic regions were clearly observed, accompanied by a significant reduction in signs of degeneration, necrosis, and inflammation. Similarly, the high-dose MOE group showed recovery in both regions; however, lymphocyte density in certain areas remained lower than in the low-dose group, with scattered regions of reduced cellularity still evident. (A) Normal control: intact cortical-medullary architecture. (B) Model: blurred cortical-medullary boundary, severely reduced lymphocyte density, no degeneration or necrosis. (C) Thymomodulin and (D) Low-dose MOE: clear cortical-medullary boundary, mildly reduced lymphocyte density, no degeneration. (E) High-dose MOE: near-normal histological architecture with lymphocyte density comparable to normal control. (F) Histological damage scores (n = 6 per group). **, ***: p < 0.05, p < 0.001, significantly different from the normal control group; $^{\Delta}$, $^{\Delta\Delta}$: p< 0.05, p < 0.01, significantly different from the model group. (M: Medulla; CO: Cortex; black arrows: Hassall's corpuscles; red circles: cortex with different levels of reduced lymphocyte density).*

Figure 3(A-E, F). Histopathological analysis of the thymus in cyclophosphamide-treated mice.

structural damage compared to controls ($p < 0.001$). All treatment groups demonstrated significant improvements: thymomodulin, low-dose MOE ($p < 0.01$), and high-dose MOE ($p < 0.05$) compared to the model group (Figure 3F). Histological analysis showed that cyclophosphamide treatment resulted in decreased lymphocyte density in both white and red pulp regions, along with disrupted splenic architecture. Treatment with thymomodulin and MOE significantly improved lymphocyte populations and restored normal splenic microarchitecture (Figure 2A-E). Moreover, the histopathological improvements correlated with the restoration of cortical-medullary architecture and increased lymphocyte density in the thymic cortex, indicating recovery of T-cell development and maturation processes (Figure 3A-E).

4. Discussion

Immunocompromised conditions, such as those induced by chemotherapy, chronic infections, or autoimmune diseases, significantly impair immune function, increasing susceptibility to infections and complicating disease management[19]. Given the limitations of synthetic immunostimulants, including high costs and adverse effects, there is a pressing need for safe, affordable, and effective alternatives[20]. This study evaluates the immunostimulatory potential of MOE in a CY-induced immunosuppression model in Swiss mice. Cyclophosphamide (CY), a cytotoxic alkylating agent and established prodrug, is metabolically activated by hepatic cytochrome P450 enzymes to produce phosphoramide mustard and acrolein[21]. Phosphoramide mustard forms covalent guanine–guanine crosslinks that inhibit DNA replication and transcription, while acrolein exerts cytotoxicity through free radical generation[22]. These cytotoxic effects preferentially target rapidly proliferating hematopoietic cells, leading to profound immunosuppression of both humoral and cell-mediated immune responses—making CY an ideal agent for creating reproducible immunodeficiency models that closely mimic clinical immunocompromised states[23–27].

Thymomodulin serves as an appropriate positive control in this study due to its well-characterised dual action on humoral and cell-mediated immunity, with particular emphasis on T-cell-mediated responses[28]. It enhances T-lymphocyte maturation in both *in vitro* and *in vivo* systems while simultaneously augmenting the functional capabilities of mature T-lymphocytes, creating cascading effects on B-cell activation and macrophage function[28,29]. The significant improvements in study parameters following thymomodulin treatment validate the experimental model and provide a benchmark for evaluating MOE efficacy.

Cell-mediated immunity was assessed through the DTH response to ovalbumin, a well-established method for measuring T-cell function[30]. Ovalbumin, a thymus-dependent antigen, requires processing by antigen-presenting cells before T-lymphocyte recognition, encompassing both direct cytotoxic responses and helper T-cell-mediated B-lymphocyte activation in a classic type IV hypersensitivity reaction[31]. While MOE treatment maintained a numerical protective trend in the DTH response, this did not reach statistical significance compared to the model group. The lack of robust DTH recovery is likely attributable to the timing of the ovalbumin challenge on day 7, which coincides with the nadir of CY-induced leukopenia. Without a sufficient population of circulating T-cells to traffic to the injection site, the localised delayed-type hypersensitivity response is inherently blunted regardless of supportive cytokine levels.

The humoral immune response is mediated primarily by B-lymphocyte activation, clonal expansion, and differentiation into plasma cells that synthesise antigen-specific antibodies[32]. In our experimental model, SRBC immunisation stimulates splenic B-lymphocytes to produce IgM initially, followed by IgG as the predominant antibody from approximately day 7 post-immunisation[33]. IgG levels in the MOE-treated groups showed no significant increase compared to the model group, likely owing to the severe depletion of B-lymphocytes by CY - B cells being up to 392 times more susceptible than T cells to CY-induced cytotoxicity[34], and to the short experimental window, which precluded optimal IgG class switching[35]. A notable limitation of the humoral assessment is the absence of IgM measurement; as the primary early-phase antibody isotype, IgM would have been a more sensitive marker within this timeframe, and its omission prevents firm conclusions regarding MOE's effect on B-cell function. Furthermore, although the statistically significant increase in IL-2 may stimulate

B-lymphocyte proliferation[36], IgG production depends on the coordinated action of additional cytokines (For example, IL-4, IL-5) and B-cell differentiation into plasma cells[37], none of which were directly assessed here. Future studies should incorporate IgM evaluation, additional cytokine profiling, and immunohistochemical analysis of B-cell markers (for example, CD19, CD20) to comprehensively characterise MOE's effect on humoral immunity.

Assessment of inflammatory markers and lymphocyte cytokines, including TNF-α and IL-2, provides insight into both pro-inflammatory and immunoregulatory pathways. TNF-α functions as a pivotal pro-inflammatory cytokine with pleiotropic effects on immune cell activation, while IL-2 serves as a critical T-cell growth factor essential for lymphocyte proliferation and effector function[38,39]. Notably, TNF-α levels, primarily produced by monocytes and macrophages, were highest in the thymomodulin group, which also exhibited the greatest preservation of monocyte counts[40]. MOE-treated groups showed moderate TNF-α increases, suggesting a less pronounced effect on monocyte-derived cytokine production. IL-2 levels in both thymomodulin- and MOE-treated groups were significantly improved compared to the model group ($p < 0.05$), despite persistently reduced peripheral lymphocyte counts ($p < 0.001$) across all CY-treated groups, indicating that MOE effectively supports key cytokine production pathways even when its ability to restore peripheral blood cell counts is limited.

A notable biological observation in this study is the significant restoration of IL-2 and TNF-α levels despite persistent, profound leukopenia in the MOE-treated groups. This apparent paradox can likely be explained by the acute temporal dynamics of CY toxicity and bone marrow recovery[41]. CY induces severe apoptosis in rapidly dividing haematopoietic stem cells, causing massive peripheral leukocyte depletion. MOE appears to rapidly stimulate surviving peripheral immune cells, including tissue-resident macrophages and lymphocytes, as evidenced by improved splenic architecture, to upregulate cytokine secretion per cell. The four-day recovery window post-CY is biologically insufficient for full bone marrow repopulation; thus, MOE initiates early functional cytokine production before systemic cellular proliferation.

The spleen, as the primary peripheral lymphoid organ, serves as a critical site for immune cell interactions and humoral immune responses[42,43], while the thymus functions as the central lymphoid organ responsible for T-lymphocyte education, selection, and differentiation[44]. The significant histological improvements in both organs strongly support MOE's efficacy in treating immunodeficiency, matching the effects of thymomodulin. The non-significant upward trend in relative organ weights likely reflects the short treatment duration and insufficient time for structural recovery, representing a study limitation. Future research should extend treatment periods, as chronic low-dose CY models have demonstrated notable improvements in organ weights[45,46], unlike the acute high-dose protocol employed here[10,11,47].

A notable pharmacological observation is that the lower MOE dose (14.4 mL/kg) demonstrated comparable or slightly superior efficacy to the higher dose (28.8 mL/kg). Non-linear dose-response relationships in botanical pharmacology have been attributed to gastrointestinal polyphenol absorption saturation at higher concentrations[48], or to paradoxical pro-oxidant activity of antioxidants at elevated doses[49]. However, these mechanisms are strictly theoretical in the present context, as no pharmacokinetic analyses or oxidative stress measurements were performed. Formal pharmacokinetic profiling and targeted biomarker analysis are essential priorities for future research to establish optimal clinical dosing.

Additional limitations of the study include the omission of regulatory cytokines such as IL-10 and TGF-β, which would have provided a more comprehensive assessment of the balance between pro-inflammatory and immunoregulatory networks. Regarding histopathological evaluation, the semi-quantitative scoring system was performed by a single blinded pathologist without inter-observer validation or objective digital quantification, limiting reproducibility. Furthermore, the absence of immunohistochemical markers (For example, CD3, CD19, Ki-67) precluded precise quantification of lymphocyte subpopulation dynamics and cellular proliferation rates within the lymphoid organs. Future mechanistic studies should incorporate these markers to confirm and expand upon the initial histological observations.

The study findings are consistent with the phytochemical basis of MOE's combined components. *Moringa oleifera* has well-documented immunostimulatory effects through multiple experimental approaches[50,51], including enhancement of macrophage pinocytosis and stimulation of nitric oxide, IL-6, and TNF-α production[52,53]. These effects are attributed to polyphenols, flavonoids (notably quercetin, kaempferol, and rutin), glucosinolates, and alkaloids[51]. *Celastrus hindsii* contains bioactive flavonoids, diterpenes, sesquiterpenes, alkaloids, and triterpenes with antioxidant, cytotoxic, and immunostimulatory properties[54–57]; the triterpenes celasdin-A, celasdin-B, and celasdin-C demonstrate immune enhancement through anti-HIV activity, while hindsiipropane B supports immune function through anti-inflammatory mechanisms[58,59]. *Curcuma longa*, particularly its active compound curcumin, interacts with macrophages, natural killer cells, dendritic cells, and B and T lymphocytes to modulate key cytokines and the Th1/Th2 balance[60–62], while additional curcuminoid derivatives further corroborate its immunostimulatory role[63].

The fermentation process likely contributes significantly to MOE's immunostimulatory properties by transforming raw plant materials into a more bioactive formulation[64]. Fermentation may generate beneficial metabolites, maintain viable probiotic bacteria that support gut-associated lymphoid tissue[65], and counteract CY-induced disruption of intestinal microbiota[66]. Fermented natural products enhance nutrient bioavailability, promote beneficial gut bacteria, exert antioxidant and anti-inflammatory effects[67–72], and have been shown in murine models to boost B- and T-cell proliferation, cytokine secretion, and NK-cell activity[73,74]. Collectively, the complex botanical matrix of the combined herbs and the fermentation process likely underlie MOE's immunostimulatory effects, while Table 2 details the rich physicochemical, amino acid, and polyphenol profile of the final MOE product, a direct comparative phytochemical analysis (For example, using HPLC/LC-MS) between the unfermented raw aqueous extract and the final fermented product was not conducted in the present study. Consequently, without side-by-side analytical profiling, the precise quantitative alterations in specific bioactive markers before and after fermentation remain unmapped. Profiling these specific biotransformations represents a critical next step for our future phytochemical research.

The results of this study, framed within an acute immunosuppression model, suggest potential therapeutic applications in immunodeficiency associated with acute viral infections—such as influenza or severe coronavirus infections—characterised by lymphopenia and dysregulated cytokine responses[75–77]. While this acute, high-dose model provides a valuable initial proof-of-concept for the extract's rapid immunostimulatory activity, it does not fully replicate the clinical reality of human patients undergoing prolonged chemotherapy or managing chronic autoimmune diseases. A chronic, low-dose CY model would undoubtedly provide a more clinically relevant evaluation of MOE's therapeutic potential in those specific long-term conditions. Therefore, we concur that further studies utilising chronic models of immunosuppression are essential before drawing definitive conclusions about MOE's efficacy in protecting against chemotherapy-induced or acquired chronic immunodeficiency states[78]. Notably, *in vitro* evidence suggests that *M. oleifera* leaf extract may enhance the cytotoxic effects of chemotherapy on cancer cells[79], raising the possibility that combining MOE with chemotherapy could improve treatment efficacy while allowing dose reduction to minimise adverse effects. MOE's antioxidant activity may also neutralise chemotherapy-generated free radicals, protecting healthy cells and reducing systemic inflammation to improve patient quality of life[80].

5. Conclusion

In this Swiss mouse model of acute CY-induced immunosuppression, MOE produced selective rather than broad immunostimulatory effects. Statistically significant improvements were limited to serum IL-2 levels and the histological architecture of the spleen and thymus. Several clinically relevant endpoints—including relative organ weights, peripheral leukocyte counts, IgG levels, and the DTH response—showed only non-significant protective trends, indicating that MOE did not achieve systemic immune restoration within this acute, short-duration model. These findings provide preliminary evidence that MOE may support specific immune recovery pathways, but are

insufficient to characterise it as a broadly effective immunostimulant. Further studies employing chronic immunosuppression models, extended treatment durations, expanded cytokine profiling, and rigorous dose-optimisation are required before conclusions about MOE's therapeutic utility can be drawn.

6. Acknowledgement

This study received support from the staff at the Department of Pharmacology, Hanoi Medical University (HMU). The authors, therefore, acknowledge with thanks the HMU staff for useful advice and technical support.

7. References

1. Varadé J, Magadán S, González-Fernández Á. Human immunology and immunotherapy: main achievements and challenges. Cell Mol Immunol. 2021; 18:805-828. https://doi.org/10.1038/s41423-020-00530-6 PMid: 32879472 PMCid: PMC7463107.
2. Seymour F, Carmichael J, Taylor C, Parrish C, Cook G. Immune senescence in multiple myeloma—a role for mitochondrial dysfunction? Leukemia. 2022; 36:2368-2373. https://doi.org/10.1038/s41375-022-01653-7 PMid: 35879358.
3. Barreto ICDP, Barreto BP, Cavalcante E, Neto AC. Immunological deficiencies: more frequent than they seem to be. J Pediatr. 2020; 97:S49-S58. https://doi.org/10.1016/j.jped.2020.10.009 PMid: 33238140 PMCid: PMC9432333.
4. Angel C, Furman D, Pham E, Fram BJ, Nguyen T, Ahmed A, *et al.* Profiling system-wide immune signaling in chronic viral infection and its response to viral clearance. J Immunol. 2016; 196:79.3. https://doi.org/10.4049/jimmunol.196.supp.79.3
5. Yu W, Yu Y, Sun S, Lu C, Zhai J, Lei Y, *et al.* Immune alterations with aging: mechanisms and intervention strategies. Nutrients. 2024; 16:3830. https://doi.org/10.3390/nu16223830 PMid: 39599617 PMCid: PMC11597283.
6. Mohamed SIA, Jantan I, Haque MdA. Naturally occurring immunomodulators with antitumor activity: an insight on their mechanisms of action. Int Immunopharmacol. 2017; 50:291-304. https://doi.org/10.1016/j.intimp.2017.07.010 PMid: 28734166.
7. Su S, Chen L, Yang M, Liang D, Ke B, Liu Z, *et al.* Design, synthesis and immunological evaluation of monophosphoryl lipid A derivatives as adjuvants for a RBD-hFc based SARS-CoV-2 vaccine. RSC Med Chem. 2023; 14:47-55. https://doi.org/10.1039/D2MD00298A PMid: 36760743 PMCid: PMC9890559.
8. Nikiema WA, Ouédraogo M, Ouédraogo WP, Fofana S, Ouédraogo BHA, Delma TE, *et al.* Systematic review of chemical compounds with immunomodulatory action isolated from African medicinal plants. Molecules. 2024; 29:2010. https://doi.org/10.3390/molecules29092010 PMid: 38731500 PMCid: PMC11085867.
9. Süntar I. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochem Rev. 2020; 19:1199-1209. https://doi.org/10.1007/s11101-019-09629-9
10. Pham BQ, Nguyen NKT, Pham SQ, Nguyen CD, Trinh LV, Dinh HTT, *et al.* Potential effects of Linh Loc Son hard capsule – a Vietnamese herbal combination in immunodeficiency induced by cyclophosphamide on mice. J Pharm Pharmacogn Res. 2024; 12:892-899. https://doi.org/10.56499/jppres23.1751_12.5.892
11. Van Pham AT, Luong MH, Dinh HTT, Mai TP, Trinh QV, Luong LH. Immunostimulatory effect of *Moringa oleifera* Extracts on cyclophosphamide-induced immunosuppressed mice. J Herbs Spices Med Plants. 2021; 27:377-385. https://doi.org/10.1080/10496475.2021.1934620
12. Bui H, Phan N, Pham L, Pham S, Nguyen H, Bui V, *et al.* Immunomodulatory effects of *Launaea sarmentosa* extract on cyclophosphamide-induced immunosuppressed mice. Trop J Nat Prod Res. 2025; 9:5406-5413. https://doi.org/10.26538/tjnpr/v9i11.21
13. Balasubramaniam M, Sapuan S, Hashim IF, Ismail NI, Yaakop AS, Kamaruzaman NA, *et al.* The properties and mechanism of action of plant immunomodulators in regulation of immune response - a narrative review focusing on *Curcuma longa* L., *Panax ginseng* C. A. Meyer and *Moringa oleifera* Lam. Heliyon. 2024; 10:e28261. https://doi.org/10.1016/j.heliyon.2024.e28261 PMid: 38586374 PMCid: PMC10998053.
14. Pham TL, Nguyen VH. An *in silico* approach for identification of potential therapeutic targets for cancer treatment from *Celastrus hindsii* Benth. Malay Appl Biol. 2024; 53:35-42. https://doi.org/10.55230/mabjournal.v53i1.2807
15. Mahato DK, Kargwal R, Kamle M, Sharma B, Pandhi S, Mishra S, *et al.* Ethnopharmacological properties and nutraceutical potential of *Moringa oleifera*. Phytomedicine Plus. 2022; 2:100168. https://doi.org/10.1016/j.phyplu.2021.100168
16. Fiocchi A, Borella E, Riva E, Arensi D, Travaglini P, Cazzola P, *et al.* A double-blind clinical trial for the evaluation of the therapeutical effectiveness of a calf thymus derivative (Thymomodulin) in children with recurrent respiratory infections. Thymus. 1986; 8:331-339.
17. Boehm T, Bleul CC. The evolutionary history of lymphoid organs. Nature Immunology. 2007; 8:131-135. https://doi.org/10.1038/ni1435 PMid: 17242686.
18. Vietnamese Ministry of Health. Vietnamese Pharmacopoeia. Fifth edition. Ha Noi: Medical Publishing House Co., Ltd. 2017.

19. Kreitmann L, Helms J, Martín-Loeches I, Salluh J, Poulakou G, Pène F, *et al*. ICU-acquired infections in immunocompromised patients. Intensive Care Med. 2024; 50:332-349. https://doi.org/10.1007/s00134-023-07295-2 PMid: 38197931 PMCid: PMC11078843.
20. Berber A, Del-Rio-Navarro BE. Cost-effectiveness analysis of OM-85 *vs* placebo in the prevention of acute respiratory tract infections (ARTIs) in children that attend day-care centers. Health Econ Rev. 2019; 9:1-9. https://doi.org/10.1186/s13561-019-0230-1 PMid: 31065886 PMCid: PMC6734393.
21. Ahmed RA, Alam MF, Alshahrani S, Jali AM, Qahl AM, Khalid M, *et al*. Capsaicin ameliorates the cyclophosphamide-induced cardiotoxicity by inhibiting free radicals generation, inflammatory cytokines, and apoptotic pathway in rats. Life. 2023; 13:786. https://doi.org/10.3390/life13030786 PMid: 36983940 PMCid: PMC10056591.
22. Karati D, Mahadik KR, Trivedi P, Kumar D. Alkylating agents, the road less traversed, changing anticancer therapy. Anti-Cancer Agents Med Chem. 2022; 22:1478-1495. https://doi.org/10.2174/1871520621666210811105344 PMid: 36983940 PMCid: PMC10056591.
23. Mohamed MA, El-Mleeh AA, Hamad RT, Abu-Alya IS, El-Hewaity MH, Elbestawy AR, *et al*. Immunostimulant potential of *Moringa oleifera* leaves alcoholic extract versus Oregano Essential Oil (OEO) against cyclophosphamide-induced immunosuppression in broilers chicks. Trop Anim Health Prod. 2023; 55:209. https://doi.org/10.1007/s11250-023-03620-5 PMid: 37202581 PMCid: PMC10195712.
24. Yan H, Lu J, Wang J, Chen L, Wang Y, Li L, *et al*. Prevention of cyclophosphamide-induced immunosuppression in mice with traditional Chinese medicine *Xuanfei Baidu* decoction. Front Pharmacol. 2021; 12:730567. https://doi.org/10.3389/fphar.2021.730567 PMid: 34737699 PMCid: PMC8560678.
25. Bagri P, Kumar V. Determination of genoprotection against cyclophosphamide induced toxicity in bone marrow of Swiss albino mice by *Moringa oleifera* leaves and *Tinospora cordifolia* stem. J Toxicol Environ Health. 2024; 87:647-661. https://doi.org/10.1080/15287394.2024.2356861 PMid: 38804873.
26. Mughal KS, Ikram M, Uddin Z, Rashid A, Rashid U, Khan M, *et al*. Syringic acid improves cyclophosphamide-induced immunosuppression in a mouse model. Biochem Biophys Res Commun. 2024; 734:150777. https://doi.org/https://doi.org/10.1016/j.bbrc.2024.150777 PMid: 39383831.
27. Luo W, Li X, Zhang C, Shen K, Li M, Zhuang Y, *et al*. Physicochemical characterization and protective effects of raw and nine-steamed *Polygonatum cyrtonema* polysaccharides on cyclophosphamide-induced immunosuppression in mice. Int J Biol Macromol. 2025; 307:141911. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2025.141911 PMid: 40068755.
28. Kouttab NM, Prada M, Cazzola P. Thymomodulin: biological properties and clinical applications. Med Oncol Tumor Pharmacother. 1989; 6:5-9. https://doi.org/10.1007/BF02985217 PMid: 40068755.
29. Diniz VLS, Alvares-Saraiva AM, Serdan TDA, dos Santos-Oliveira LC, Cruzat V, Lobato TB, *et al*. Essential metabolism required for T and B lymphocyte functions: an update. Clin Sci. 2023; 137:807-821. https://doi.org/10.1042/CS20220869 PMid: 37219940.
30. Lin CC, Chuang KC, Chen SW, Chao YH, Yen CC, Yang SH, *et al*. Lactoferrin ameliorates ovalbumin-induced asthma in mice through reducing dendritic-cell-derived Th2 cell responses. Int J Mol Sci. 2022; 23:14185. https://doi.org/10.3390/ijms232214185 PMid: 36430662 PMCid: PMC9696322.
31. Bai X, Chai Y, Shi W, Li Y, Zhang T, Liu P. *Lonicera japonica* polysaccharides attenuate ovalbumin-induced allergic rhinitis by regulation of Th17 cells in BALB/c mice. J Funct Foods. 2020; 65:103758. https://doi.org/10.1016/j.jff.2019.103758
32. Labeur-Iurman L, Harker JA. Mechanisms of antibody mediated immunity – distinct in early life. Int J Biochem Cell Biol. 2024; 172:106588. https://doi.org/10.1016/j.biocel.2024.106588 PMid: 38768890.
33. Alharbi KS, Alenezi SK, Alsahli T, Afzal M, Mantargi MJS, Kazmi I, *et al*. Effect of sakuranetin against cyclophosphamide-induced immunodeficiency mice: role of IFN-γ/TNF-α/IgG/IgM/interleukins. Naunyn-Schmiedeberg's Arch Pharmacol. 2025; 398:10951-10962. https://doi.org/10.1007/s00210-025-03988-1 PMid: 40056204.
34. Wilmer JL, Colvin OM, Bloom SE. Cytogenetic mechanisms in the selective toxicity of cyclophosphamide analogs and metabolites towards avian embryonic B lymphocytes *in vivo*. Mutat Res Fundam Mol Mech Mutagen. 1992; 268:115-130. https://doi.org/10.1016/0027-5107(92)90089-K PMid: 1378178.
35. Anh BTV, Thuý NT, Tùng BT, Khanh NV, Huyền NT, Khánh ĐTH, *et al*. Tác dụng kích thích miễn dịch của cao chiết N-Hexan hy thiêm (*Siegesbeckia orientalis* L.) trên thực nghiệm. J Med Res. 2025; 191:344-352. https://doi.org/10.52852/tcncyh.v191i6.3309
36. Rump JA, Jahreis A, Schlesier M, Dräger R, Melchers I, Peter HH. Possible role of IL-2 deficiency for hypogammaglobulinaemia in patients with common variable immunodeficiency. Clin Exp Immunol. 1992; 89:204-210. https://doi.org/10.1111/j.1365-2249.1992.tb06933.x PMid: 1638764 PMCid: PMC1554446.
37. Moens L, Tangye SG. Cytokine-mediated regulation of plasma cell generation: IL-21 takes center stage. Front Immunol. 2014; 5:65. https://doi.org/10.3389/fimmu.2014.00065 PMid: 24600453 PMCid: PMC3927127.
38. Guo Y, Hu K, Li Y, Lu C, Ling K, Cai C, *et al*. Targeting TNF-α for COVID-19: recent advanced and controversies.

Front Public Health. 2022; 10. https://doi.org/10.3389/fpubh.2022.833967 PMid: 35223745 PMCid: PMC8873570.
39. Lykhopiy V, Malviya V, Humblet-Baron S, Schlenner SM. IL-2 immunotherapy for targeting regulatory T cells in autoimmunity. Genes Immun. 2023; 24:248-262. https://doi.org/10.1038/s41435-023-00221-y PMid: 37741949 PMCid: PMC10575774.
40. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007; 449:819-826. https://doi.org/10.1038/nature06246 PMid: 17943118.
41. Zhong W, Huang H, Yang Z, Chang P. rhCNB improves cyclophosphamide-induced immunodeficiency in BALB/c mice. Evid Based Complement Alternat Med. 2022; 2022:4891399. https://doi.org/10.1155/2022/4891399 PMid: 36204132 PMCid: PMC9532092.
42. Kawaguchi Y, Shimizu T, Ando H, Ishima Y, Ishida T. Development of a nanocarrier-based splenic B cell-targeting system for loading antigens *in vitro*. Biol Pharm Bull. 2022; 45:926-933. https://doi.org/10.1248/bpb.b22-00222 PMid: 35786600.
43. Lewis SM, Williams A, Eisenbarth SC. Structure and function of the immune system in the spleen. Sci Immunol. 2019; 4:eaau6085. https://doi.org/10.1126/sciimmunol.aau6085 PMid: 30824527 PMCid: PMC6495537.
44. Wang H, Zúñiga-Pflücker JC. Thymic microenvironment: interactions between innate immune cells and developing thymocytes. Front Immunol. 2022; 13:885280. https://doi.org/10.3389/fimmu.2022.885280 PMid: 35464404 PMCid: PMC9024034.
45. Kim H, Hong JY, Lee J, Yeo C, Jeon WJ, Lee YJ, *et al*. Immune-boosting effect of Yookgong-dan against cyclophosphamide-induced immunosuppression in mice. Heliyon. 2024; 10. https://doi.org/10.1016/j.heliyon.2024.e24033 PMid: 38293434 PMCid: PMC10826668.
46. Xie H, Fang J, Farag MA, Li Z, Sun P, Shao P. *Dendrobium officinale* leaf polysaccharides regulation of immune response and gut microbiota composition in cyclophosphamide-treated mice. Food Chemistry: X. 2022; 13:100235. https://doi.org/10.1016/j.fochx.2022.100235 PMid: 35499019 PMCid: PMC9039934.
47. Mayumi H. A review of cyclophosphamide-induced transplantation tolerance in mice and its relationship with the HLA-haploidentical bone marrow transplantation/post-transplantation cyclophosphamide platform. Front Immunol. 2021; 12. https://doi.org/10.3389/fimmu.2021.744430 PMid: 34659242 PMCid: PMC8513786.
48. Silberberg M, Morand C, Mathevon T, Besson C, Manach C, Scalbert A, *et al*. The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. Eur J Nutr. 2006; 45:88-96. https://doi.org/10.1007/S00394-005-0568-5 PMid: 15981077.
49. Nowak M, Tryniszewski W, Sarniak A, Wlodarczyk A, Nowak PJ, Nowak D. Concentration dependence of anti- and pro-oxidant activity of polyphenols as evaluated with a light-emitting Fe2+-Egta-H_2O_2 system. Molecules. 2022; 27:3453. https://doi.org/10.3390/molecules27113453 PMid: 35684391 PMCid: PMC9182469.
50. Gupta S, Jain R, Kachhwaha S, Kothari SL. Nutritional and medicinal applications of *Moringa oleifera* Lam.—review of current status and future possibilities. J Herb Med. 2018; 11:1-11. https://doi.org/10.1016/j.hermed.2017.07.003
51. Stohs SJ, Hartman MJ. Review of the safety and efficacy of *Moringa oleifera*. Phytother Res. 2015; 29:796-804. https://doi.org/10.1002/ptr.5325 PMid: 25808883 PMCid: PMC6680322.
52. Dong Z, Li C, Huang Q, Zhang B, Fu X, Liu RH. Characterization of a novel polysaccharide from the leaves of *Moringa oleifera* and its immunostimulatory activity. J Funct Foods. 2018; 49:391-400. https://doi.org/10.1016/j.jff.2018.09.002
53. Li C, Dong Z, Zhang B, Huang Q, Liu G, Fu X. Structural characterization and immune enhancement activity of a novel polysaccharide from *Moringa oleifera* leaves. Carbohydr Polym. 2020; 234:115897. https://doi.org/10.1016/j.carbpol.2020.115897 PMid: 32070517.
54. Duyen BTT, Hung VM, Tung BT. Cytotoxicity and antioxidant effects of *Celastrus hindsii* Benth. leaf extract. VNU J Sci: Med Pharm Sci. 2020; 36. https://doi.org/10.25073/2588-1132/vnumps.4203
55. Viet TD, Xuan TD, Anh LH. α-Amyrin and β-Amyrin isolated from *Celastrus hindsii* leaves and their antioxidant, anti-xanthine oxidase, and anti-tyrosinase potentials. Molecules. 2021; 26:7248. https://doi.org/10.3390/molecules26237248 PMid: 34885832 PMCid: PMC8658892.
56. Gan N, Chen G, Zhang W, Zhou J. Oleanen induces apoptosis of cervical cancer cells by up-regulation of Bim. Int J Gynecol Cancer. 2012; 22:38-42. https://doi.org/10.1097/IGC.0b013e31822b62be PMid: 21897270.
57. Do Dat T, Hai ND, Nam NTH, Thanh NM, Huyen NTT, Duong LTT, *et al*. Flavonoid content and antifungal activity of *Celastrus hindsii* leaf extract obtained by supercritical carbon dioxide using ethanol as co-solvent. Biocatal Agric Biotechnol. 2023; 52:102824. https://doi.org/10.1016/j.bcab.2023.102824
58. Kuo YH, Kuo LM. Antitumour and anti-AIDS triterpenes from *Celastrus hindsii*. Phytochemistry. 1997; 44:1275-1281. https://doi.org/10.1016/s0031-9422(96)00719-4 PMid: 9115698.
59. Jo H, Jang HY, Youn GS, Kim D, Lee CY, Jang JH, *et al*. Hindsiipropane B alleviates HIV-1 Tat-induced inflammatory responses by suppressing HDAC6-NADPH oxidase-ROS axis in astrocytes. BMB Rep. 2018; 51:394-399. https://doi.org/10.5483/bmbrep.2018.51.8.061 PMid: 29699604 PMCid: PMC6130829.

60. Memarzia A, Khazdair MR, Behrouz S, Gholamnezhad Z, Jafarnezhad M, Saadat S, *et al*. Experimental and clinical reports on anti-inflammatory, antioxidant, and immunomodulatory effects of *Curcuma longa* and curcumin, an updated and comprehensive review. Biofactors. 2021; 47:311-350. https://doi.org/10.1002/biof.1716 PMid: 33606322.
61. Allegra A, Mirabile G, Ettari R, Pioggia G, Gangemi S. The impact of curcumin on immune response: an immunomodulatory strategy to treat sepsis. Int J Mol Sci. 2022; 23:14710. https://doi.org/10.3390/ijms232314710 PMid: 36499036 PMCid: PMC9738113.
62. Momtazi-Borojeni AA, Haftcheshmeh SM, Esmaeili SA, Johnston TP, Abdollahi E, Sahebkar A. Curcumin: a natural modulator of immune cells in systemic lupus erythematosus. Autoimmun Rev. 2018; 17:125-135. https://doi.org/10.1016/j.autrev.2017.11.016 PMid: 29180127.
63. Yuandani, Jantan I, Rohani AS, Sumantri IB. Immunomodulatory effects and mechanisms of curcuma species and their bioactive compounds: a review. Front Pharmacol. 2021; 12:643119. https://doi.org/10.3389/fphar.2021.643119 PMid: 33995049 PMCid: PMC8120430.
64. Li Y, Lei Z, Guo Y, Liu Y, Guo X, Wang X, *et al*. Fermentation of *Ganoderma lucidum* and *Raphani Semen* with a probiotic mixture attenuates cyclophosphamide-induced immunosuppression through microbiota-dependent or -independent regulation of intestinal mucosal barrier and immune responses. Phytomedicine. 2023; 121:155082. https://doi.org/10.1016/j.phymed.2023.155082 PMid: 37722243.
65. Takayama H, Fukatsu K, Takahashi K, Noguchi M, Watkins A, Matsumoto N, *et al*. Influences of a fermented milk with *Lactobacillus bulgaricus* and *Streptococcus thermophiles* on gut associated lymphoid tissue, mucosal IgA, and gut flora in mice. Clin Nutr Open Sci. 2022; 42:36-48. https://doi.org/10.1016/j.nutos.2022.01.007
66. Park JH, Son SU, Kim KH, Jung US, Shin KS. Immunostimulatory effects of rhamnogalacturonan-I fraction purified from *Glycyrrhiza glabra* roots on cyclophosphamide-induced immunosuppressed mice. Int J Biol Macromol. 2025; 288:138687. https://doi.org/10.1016/j.ijbiomac.2024.138687 PMid: 39672444.
67. Das D, Sarkar S, Borsingh Wann S, Kalita J, Manna P. Current perspectives on the anti-inflammatory potential of fermented soy foods. Food Res Int. 2022; 152:110922. https://doi.org/10.1016/j.foodres.2021.110922 PMid: 35181093.
68. Shahbazi R, Sharifzad F, Bagheri R, Alsadi N, Yasavoli-Sharahi H, Matar C. Anti-inflammatory and immunomodulatory properties of fermented plant foods. Nutrients. 2021; 13:1516. https://doi.org/10.3390/nu13051516 PMid: 33946303 PMCid: PMC8147091.
69. Li C, He J, Yang Y, Gou Y, Wang Z, Chen H, *et al*. White tip silver needle (slightly fermented white tea) flavonoids help prevent aging via antioxidative and anti-inflammatory effects. Drug Des Devel Ther. 2021; 15:1441-1457. https://doi.org/10.2147/DDDT.S304885 PMid: 33833503 PMCid: PMC8020812.
70. Hwang HM, Park YM, Lee HY, Shin DY, Kim JG, Bae JS, *et al*. Immunostimulatory effects of red beet ferment extract on cyclophosphamide-induced immunosuppression in Wistar rats. Appl Sci. 2024; 14:11013. https://doi.org/10.3390/app142311013
71. Adebo JA, Njobeh P, Gbashi S, Oyedeji A, Ogundele O, Oyeyinka S, *et al*. Fermentation of cereals and legumes: impact on nutritional constituents and nutrient bioavailability. Fermentation. 2022; 8(2):63. https://doi.org/10.3390/fermentation8020063
72. Samtiya M, Aluko RE, Puniya AK, Dhewa T. Enhancing micronutrients bioavailability through fermentation of plant-based foods: a concise review. Fermentation. 2021; 7:63. https://doi.org/10.3390/fermentation7020063
73. Choi SI, Men X, Oh G, Im JH, Choi YE, Yang JM, *et al*. Immunomodulatory effect of fermented *Benincasa hispida* Cong. extracts on BALB/c mice. J Funct Foods. 2024; 112:105942. https://doi.org/10.1016/j.jff.2023.105942
74. Barisone GA, O'Donnell RT, Ma Y, Abuhay MW, Lundeberg K, Gowda S, *et al*. A purified, fermented, extract of *Triticum aestivum* has lymphomacidal activity mediated via natural killer cell activation. PLoS One. 2018; 13:e0190860. https://doi.org/10.1371/journal.pone.0190860 PMid: 29304125 PMCid: PMC5755921.
75. Lee S, Channappanavar R, Kanneganti TD. Coronaviruses: innate immunity, inflammasome activation, inflammatory cell death, and cytokines. Trends Immunol. 2020; 41:1083-1099. https://doi.org/10.1016/j.it.2020.10.005 PMid: 33153908 PMCid: PMC7561287.
76. Cao X. COVID-19: immunopathology and its implications for therapy. Nat Rev Immunol. 2020; 20:269-270. https://doi.org/10.1038/s41577-020-0308-3 PMid: 32273594 PMCid: PMC7143200.
77. Clementi N, Ghosh S, De Santis M, Castelli M, Criscuolo E, Zanoni I, *et al*. Viral respiratory pathogens and lung injury. Clin Microbiol Rev. 2021; 34:10.1128/cmr.00103-20. https://doi.org/10.1128/cmr.00103-20 PMid: 33789928 PMCid: PMC8142519.
78. Singh J, Gautam DNS, Sourav S, Sharma R. Role of *Moringa oleifera* Lam. in cancer: phytochemistry and pharmacological insights. Food Front. 2023; 4:164-206. https://doi.org/10.1002/fft2.181
79. Ege H, Enguven G, Celebi A, Orhan C, Buyru AN, Gunduz O, *et al*. *In vitro* evaluation of the therapeutic efficacy of *Moringa oleifera* and oxaliplatin in 3D printed

polycaprolactone/gelatin implantable patches: a potential strategy for overcoming drug resistance in colorectal cancer treatment. Int J Biol Macromol. 2025; 316:144129. https://doi.org/10.1016/j.ijbiomac.2025.144129 PMid: 40368208.

80. Moremane MM, Abrahams B, Tiloke C. *Moringa oleifera*: a review on the antiproliferative potential in breast cancer cells. Curr Issues Mol Biol. 2023; 45:6880-6902. https://doi.org/10.3390/cimb45080434 PMid: 37623253 PMCid: PMC10453312.

Author Contribution

Tung Thanh Tran: Supervision, methodology, project administration, investigation, and formal analysis. Phong Xuan Pham: Visualisation, validation, writing-original draft. Cu Duc Ngo: Conceptualisation, data curation, investigation. Ha Thi Pham: Resources, methodology, investigation. Hang Thi Thu Dinh: Methodology, investigation, data curation, Writing-review and editing. Quang Vinh Trinh: Resources, software, data curation, writing review and editing. Phuong Chi Pham: Resources, validation, writing original draft. Thuy Thi Nguyen: Investigation, formal analysis, writing-original draft. Lam Bui Tung Pham: Investigation, software. Van Anh Thi Pham: Conceptualisation, investigation, project administration, supervision, validation, visualisation, writing review and editing. All authors have read and agreed to the published version of the manuscript.