Effects of Triphala on Immune Response in Elderly Volunteers: A Preliminary Randomized, Placebo-Controlled Trial

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Abstract

Immunosenescence is the progressive immunological dysfunction that results from aging. Immunosenescence decreases immune response ability, increases vulnerability to infections, and develops chronic diseases. Triphala was reported to have immunomodulatory effects in healthy adults by raising the proportion of B and T lymphocytes and natural killer (NK cells). However, no prior research has been done on the use of Triphala in the elderly. The objective of this preliminary study is to examine the immunomodulatory effects of Triphala in older participants by measuring the proportion of leukocyte subpopulations and cytokine levels in the blood. Participants were randomized to receive either 3,232 mg of Triphala daily or placebo for four weeks. No serious adverse events occurred. The study found no significant changes in the proportion of leukocyte subsets and cytokine levels in the Triphala group when compared to placebo. These unexpected results may be due to a lower diversity of commensal gut bacteria in the elderly, which has led to a lower ability to convert Triphala into active compounds. Additionally, the power of statistical tests is low due to the small sample size. In conclusion, 3,232 mg of Triphala daily for four weeks is safe but did not show a significant impact on the immune response in the elderly. Further studies should be done to evaluate the effect of Triphala on leukocyte function with a larger number of participants.

Keywords**:** *Triphala, Elderly, Aging, Immunosenescence, Immunomodulatory effect*

1. Introduction

An immune system is a complex biological process of an organism's cooperation in the human body to defend against and respond to a wide variety of pathogens. These mechanisms are to protect an organism from diseases. Generally, sufficient immune responses in healthy people can protect the host from microbial infections. However, there are endogenous factors that affect the immune response and decrease its function. Aging is the inevitable endogenous factor that regresses the immune responses in the elderly, termed immunosenescence. Immunosenescence is reflected in the increased susceptibility to infectious diseases, low efficacy of vaccination, and increased prevalence of cancer, autoimmune diseases, and other chronic diseases characterized by a pro-inflammatory state (Montecino-Rodriguez, Berent-Mao, & Dorshkind, 2013). It involves alteration of the quantity and declination in the cell-mediated immune function. Weiskopf, Weinberger, and Grubeck-Loebenstein (2009) reviewed age-related defects in T- and B-cell function. In the elderly, the decrease in naive T cells is accompanied by an increase in memory and effector cells that have reduced expression of co-stimulatory molecules. In addition, the number of naive B cells and diversity of the B cell repertoire decrease while increasing in memory B cells. Consequently, this leads to a decrease in interleukin (IL)-2 production. Besides, phagocytic cells such as neutrophils and macrophages have shown a significant reduction in phagocytic ability in the elderly as a result of decreased induction by interferon (IFN)-

γ. When compared to young adults, circulating monocytes in the elderly seem to be increased, and also increased in the expression of inflammation markers (tumor necrotic factor (TNF)-α and IL-6, for example) (Weiskopf et al., 2009; Cao et al., 2022). The total number of natural killer (NK) cells increases with age due to the rise of $CD56^{dim}$ NK cells. However, the elderly express $CD56^{Bright}$ NK cells lower than $CD56^{Dim}$ NK cells, which leads to a regression of cytokine production such as IFN-γ and interferon gamma-induced protein 10 (IP-10) and a reduced NK cytotoxicity response (Ventura, Casciaro, Gangemi, & Buquicchio, 2017; Gounder et al., 2018; Zhang et al., 2014). Contrary to children or adults, the elderly respond to infection without increased interferon (IFN)-λ1 expressions due to impaired IFN-λ signaling. Consequently, the incidence of viral infection in the elderly has risen (Gilbert et al., 2021). Type III interferon (IFN-λ1 and IFN-λ4) is known as anti-viral cytokines. IFN-λ cooperates stimulation and suppressive function with monocytes, macrophages, dendritic cells (DCs), neutrophils, NK cells, and also T- and B-cells. IFN-λ production is induced when a viral infection occurs at barrier surfaces of the body, such as the gut and respiratory epithelial cells (Feng, Balint, Poznanski, Ashkar, & Loeb, 2021).

The National Drug System Development Committee (2023) has included Triphala, a traditional Ayurvedic herbal formulation, as one of 50 Thai traditional medicines on the Thailand National List of Essential Medicines (NLEM) for reducing cough. Triphala consists of three dried fruits of *Terminalia chebula* Retz., *Terminalia bellirica* (Gaertn.) Roxb., and *Phyllanthus emblica* L. in equal proportion (1:1:1). It contains major active constituents such as ellagic acid, gallic acid, chebulinic acid, and chebulagic acid, etc. These polyphenol compounds have been claimed for health benefits (Peterson, Denniston, & Chopra, 2017; Belapurkar, Goyal, & Tiwari-Barua, 2014; Wang et al., 2023). In folk medicine, Triphala has been used for laxatives, detoxification, rejuvenation, and balancing the elements of the body (Phetkate, Rinthong, Kietinun, & Sriyakul, 2020). In traditional medicine, an imbalance of the elements, or the three major doshas (Pitta, Vata, and Kapha), causes illness and disease. Each plant of the Triphala remedy; *T. chebula*, *T. bellirica*, and *P. emblica*, stands for each dosha, Vata, Pitta, and Kapha, respectively (Foundation for the Restoration of Traditional Thai Medicine, 2011). Thus, we can use Triphala to balance the elements and improve health conditions.

Previous Triphala studies have demonstrated pharmacological activities including antibacterial, antiviral, antifungal, antipyretic, analgesic, anti-inflammatory, antimutagenic, anticancer, antioxidant, antidiabetic, and hypolipidaemic properties. It also possesses adaptogenic and immunomodulatory activities (Kumar, Nair, & Murali, 2016; Belapurkar et al., 2014). Phetkate, Kummalue, U-pratya, and Kietinun (2012) reported that ethanol extracts of Triphala 1,050 mg daily significantly increased the absolute number of lymphocyte subpopulations: cytotoxic T cells ($CD3^+CD8^+$) and NK cells ($CD3^-CD56^+$). The study also showed an increasing trend in the absolute number of B lymphocytes ($CD19^+CD45^+$) after the 2^{nd} week. However, cytokine secretion assessment (IL-6, IFN-γ, and TNF-α) found no significant elevation.
Triphala's immunomodulatory effects may have an impact on age-related changes in the immune response. Therefore, the present study aimed to evaluate the immunomodulatory effect and safety of Triphala 3,232 mg daily for 28 days in older participants by assessing the percentage of white blood cells and cytokine levels in the blood.

2. Objectives

1) To determine the immunomodulatory effect of Triphala in elderly participants.
2) To determine the safety of Triphala in elderly participants.

3. Materials and Methods

3.1 Drug and placebo preparations

Triphala capsules (500 mg/capsule containing 404 mg of herbal powder per capsule) consisting of *T. chebula, T. bellirica*, and *P. emblica* with a ratio of 1:1:1 were provided from World Happy Co., Ltd. (Reg. No. 24-2-01350-5-0052). Placebo capsules, which had an external appearance that looked like Triphala, were prepared by World Happy Co., Ltd.

3.2 High-performance liquid chromatography (HPLC), chemicals, and reagents

Gallic acid-certified reference material, ellagic acid, chebulagic acid, and chebulinic acid analytical standards were purchased from Sigma-Andrich (USA). Analysis of the solution was performed using the HPLC technique (Infinity 1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) (Pompimon et al., 2020). The mobile phase flow rate was 1 mL/min. The injection volume was 10 µL. The quantitation wavelength was set at 260 nm and 280 nm. The mobile phase was composed of mobile phase A (1% acetic acid in distilled water) and mobile phase B (MeOH) under isocratic conditions. The solutions were filtered through a nylon membrane (0.45 µm) and then analyzed with Agilent OpenLab CDS 2.6 Version Software following the HPLC procedure.

3.3 Research design and participants

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University (No. 278/2022). This is a phase 2 randomized, double-blind, placebo-controlled, parallel study.

The inclusion criteria were male or female aged between 51 and 70 years; no serious/uncontrolled underlying disease; a BMI 18.5-29.9 kg/m^2; being able to read and write in Thai; and all participants were informed both verbally and in writing of the possible risks and adverse events that may occur during the study and must sign the written consent forms to participate in the study. Exclusion criteria were: physical examination revealed clinically significant abnormalities; receiving any vaccination or immunosuppressants such as corticosteroids, cyclosporin, tacrolimus, methotrexate, or monoclonal antibodies within the past 3 months; receiving antiplatelet drugs (such as clopidogrel or aspirin) or anticoagulants such as warfarin; having any infection within the last 2 weeks; having a history of diarrhea-predominant irritable bowel syndrome (IBS) or alternating constipation and diarrhea IBS; having a history of autoimmune diseases; having a history of diabetes mellitus; having a history of hepatic or renal disease; having a history of uncontrolled hypertension, uncontrolled hyperlipidemia and/or cardiovascular disease or thrombosis; having a history of cancer; having a history of severe allergy to herbal medicine/product; smoking; pregnant, lactating; or clinically significant abnormalities in blood chemistry and hematology. Two weeks before the study period and during the 4-week study period, participants who enrolled in the study were informed to avoid all herbal medicines and immunomodulatory drugs such as corticosteroids, thalidomide, lenalidomide, etc. In addition, any vaccination during the study period was also not allowed.

Twenty-four eligible participants were randomly divided into two groups, with an allocation ratio of 1:1 (twelve participants per group). The randomization was stratified by age and gender. One group received Triphala (TP), 3,232 mg per day, while the other group received placebo (PL) capsules for four weeks. From day 0 to day 2, participants were instructed to take TP or PL 2 capsules before breakfast, and if there were no adverse effects, they could take 2 capsules before lunch, dinner, and bedtime. From day 3 to day 28, participants took TP or PL 4 capsules before breakfast and bedtime. The study design is demonstrated in Figure. 1.

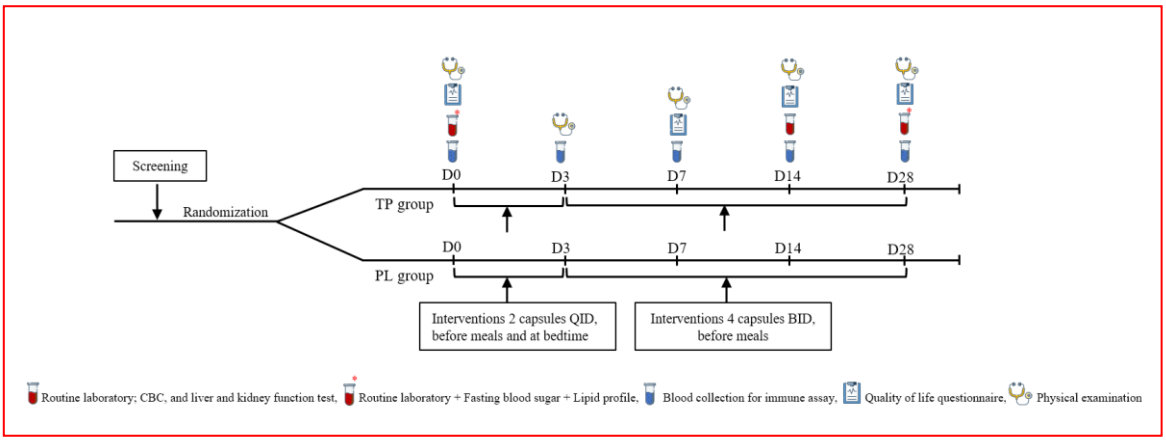


Figure 1: Study design. After screening, the participants were stratified, randomized by age and sex, into the TP group or PL group. D0: On the first day of drug administration, complete physical examinations, including routine laboratory analysis and immunological studies, were first performed on this day. D3, D7, D14, D28: the follow-up days, complete physical examinations, including routine laboratory analysis, immunological studies, drug compliance, and adverse event evaluation, were performed on these days.

3.4 Efficacy assessment: Immunological measurement for surface marker expression

DuraClone IM Phenotyping BASIC Tube, 25 Tests, RUO (B53309) was purchased from Beckman Coulter India PvT. Ltd., it contains 25 tests of eight color DuraClone IM Phenotyping BASIC Tube (i.e., a single tube is a single test containing 8-monoclonal antibody reagent that allows the identification of common extracellular markers of different subpopulations of lymphocytes, present in whole blood specimens) and 3Compensation Kits, each kit containing eight fluorescent reagent tubes, each of a single labeled color; CD4-FITC; CD4-PE; CD19-ECD; CD14-PC7; CD4-APC; CD8-A700; CD3-APC-A750; CD8-Krome Orange. The compensation kit was set up following standard procedures and instrument manufacturer instructions. The reagent tubes and compensation kit tubes were stored between 20 and 30℃ at room temperature, in a dry place, and protected from direct exposure to light and moisture. Initially, the $CD45^{+}CD14^{+}$ expression cells representing $CD14^{+}$monocytes were excluded from $CD45^{+}$ leukocytes, the $CD45^{+}CD19^{+}$ have differentiated apart from leukocytes; and it was also identified for $CD19^{+}$B cells. $CD45^{+}CD3^{+}$ is the marker excluded to identify T cells, encompassing $CD45^{+}CD3^{+}CD4^{+}$ T cells and $CD45^{+}CD3^{+}CD8^{+}$ T cells. $CD45^{+}CD3^{-}CD56^{+}$ identifies natural killer cells (NKs) (Gounder et al., 2018). These surface markers were detected from blood samples step by step following the manufacturer's instructions and then analyzed by CytExpert Software for a complex flow cytometric application (DxFLEX) provided by the manufacturer to determine the percentage of leukocyte subpopulations.

3.5 Efficacy assessment: Immunological measurement for cytokine level

Serum was collected from participants and kept at -50˚C before usage. IL-2, IL-6, IFN-λ1, IFN-γ, and IP-10 were detected in blood samples by enzyme-linked immunosorbent assay (ELISA) as described in the manufacturer's instructions. BioLegend's ELISA MAX™ Deluxe Set Human IL-2 (Lot no. B355727), BioLegend's ELISA MAX™ Deluxe Set Human IL-6 (Lot no. B338614), BioLegend's ELISA MAX™ Deluxe Set Human IFN-gamma (Lot no. B348077), BioLegend's LEGEND MAX Human IL-29 (IFN-lambda1) ELISA Kit (Lot no. B337919), and BioLegend's ELISA MAX™ Deluxe Set Human CXCL10 (IP-10) (Lot no. B349757) were purchased from Advanced Medical Science Co., Ltd. and stored between 2˚C to 8˚C.

3.6 Efficacy assessment: The quality-of-life questionnaire

The EQ-5D-5L Paper Self-Complete and EQ-5D VAS scores obtained from the EuroQol Group (Pattanaphesaj et al., 2018) with permission were used to assess the quality of life of enrolled participants. Participants were asked to respond to five dimensions: mobility, self-care, usual activities, pain or discomfort, and anxiety or depression. Each dimension has 5 levels of perceived problems: no problems (1 score), slight problems (2 scores), moderate problems (3 scores), severe problems (4 scores), and extreme problems (5 scores). The levels of perceived problems were converted to a single summary number, or 'index value'. An EQ-5D-5L index (utility) score ranges from -0.42 to 1.00 (-0.42 is the worst health state, while 1.00 is the full health state). The EQ VAS score, rated on a scale of 0-100 (0 is the worst health/extreme problems and 100 is the best health/no problems), indicated a health condition on the visited day.

3.7 Safety assessment

A completed physical examination and non-directive questioning for adverse events were performed at every visit. Participants were asked to record any adverse effects and report them to the investigators. Moreover, the laboratory tests, including the complete blood count, blood urea nitrogen (BUN),creatinine level, and liver function test, were evaluated at the end of weeks 2 and 4 after treatment.

3.8 Statistical analysis

The data were analyzed by a non-parametric test using SPSS software and presented as the median (min-max). The differences within the group analysis were analyzed using Friedman's two-way analysis of variance by rank test. The differences between the groups were analyzed using an independent-samples Mann-Whitney U test. Additionally, Fisher's exact test was used to determine the difference in the proportion of adverse events that occurred in the two groups.

4. Results and Discussion

4.1 Results

4.1.1 HPLC chemical profile

The HPLC fingerprint chromatogram of the TP powder showed the major peak of gallic acid, chebulagic acid, chebulinic acid, and ellagic acid, which were present at retention times of 7.639, 21.553, 25.290, and 28.318 min, respectively. For quantitative analysis, the concentrations of gallic acid, chebulagic acid, chebulinic acid, and ellagic acid were 14.76, 21.34, 11.32, and 26.64 mg per 1 g of TP, respectively.

4.1.2 Demographics and baseline characteristics

Thirty-nine participants were recruited to the study (9 males and 30 females), fourteen of them could not meet inclusion/exclusion criteria, and one was unable to participate in the study. Twenty-four participants were enrolled. Fourteen of them were middle-aged (58.3%), and 10 were old (41.7%). In this preliminary report, the data of 10 participants in the old-aged group has already been analyzed and shown. There were 4 males (40%) and 6 females (60%), and the median (min-max) age was 63 (61-67) and 67 (63-69) years old in the TP and PL groups, respectively (Table 1). In summary, none of the demographic parameters showed significant differences between groups.

Table 1: The participants' demographic data

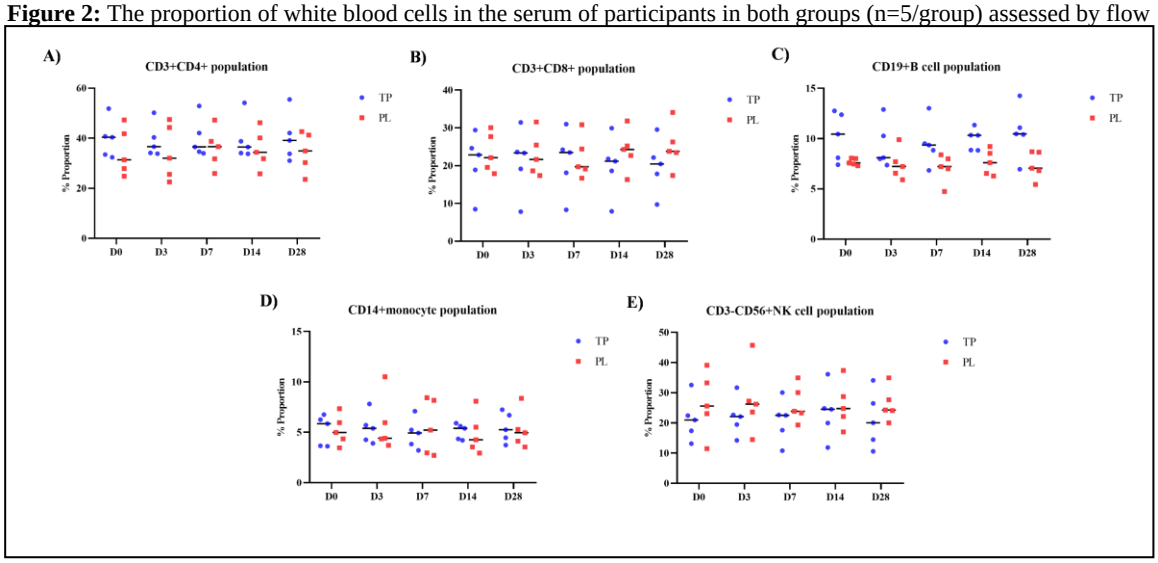
Demographic data	Treatment groups		*p*-value
	TL (n=5)	PL (n=5)	
Gender (M: F)	2:3	2:3	1.000
Age (year)	63 (61-67)	67 (63-69)	0.095
Body weight (kg)	57.60 (50.00-69.80)	54.90 (51.40-56.70)	0.151
Height (m)	1.52 (1.47-1.78)	1.57 (1.50-1.68)	0.841
Body mass index (kg/m^2)	25.13 (19.53-26.66)	21.99 (20.05-24.38)	0.31
Pulse rate (Beats per minute)	78 (57-84)	85 (58-86)	0.151
Systolic blood pressure (mmHg)	127 (119-134)	130 (117-139)	0.841
Diastolic blood pressure (mmHg)	72 (66-81)	72 (64-75)	0.548
Underlying diseases			
- Hypertension	2 (40.0%)	1 (20.0%)	1.000
- Dyslipidemia	3 (60.0%)	3 (60.0%)	1.000
- Benign Prostatic Hyperplasia	1 (20.0%)	0	1.000
- Osteoporosis	1 (20.0%)	0	1.000
- Gastritis	1 (20.0%)	0	1.000
Exercise level			0.261
- No exercise	1 (20.0%)	0	
- 1-2 times/week	0	1 (20.0%)	
- 3-5 times/week	3 (60.0%)	1 (20.0%)	
- >5 times/week	1 (20.0%)	3 (60.0%)	

The data are represented as the median (min-max) and the number of subjects (percentage). Statistical analysis: Independent-samples Mann-Whitney U test, Fisher's exact test, n = 5/group

4.1.3 Immunological measurements for surface marker expression

Flow cytometry was used to detect cell surface markers to differentiate the monocyte, NK cell, T helper cell, cytotoxic T cell, and B cell populations (Beckman Coulter, Inc., USA). Individual white blood cell proportions at baseline and after treatments in both groups (TP and PL) are demonstrated in Figure 2. Neither the TP group nor the PL group showed a significant difference in the within -group or between-group analysis.

Figure 2: The proportion of white blood cells in the serum of participants in both groups (n=5/group) assessed by flow



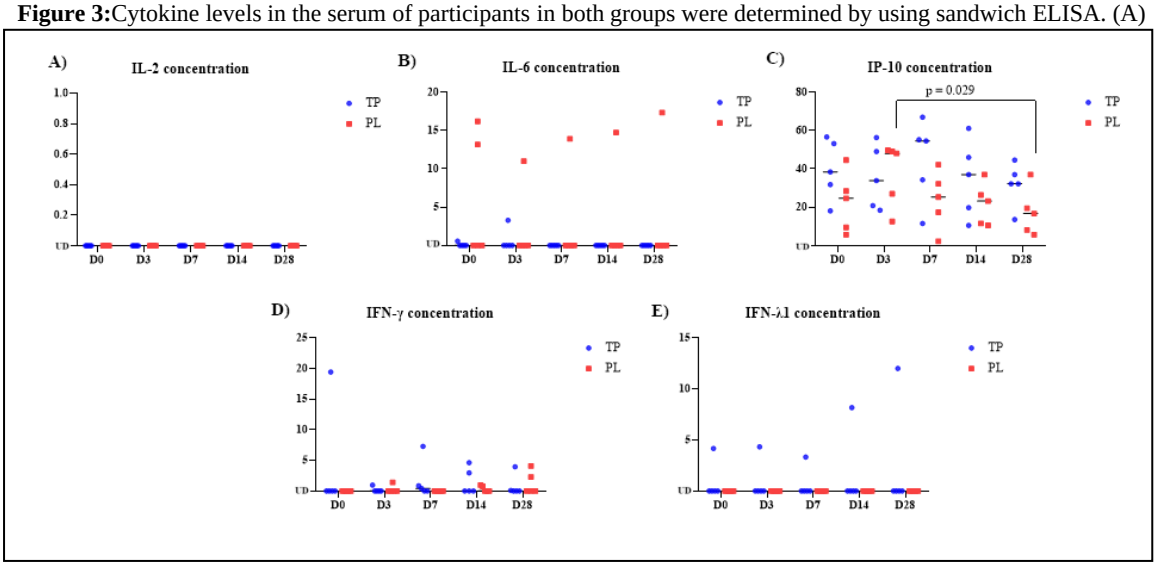
cytometry. (A) CD3+CD4+ T cells proportion; (B) CD3+CD8+ T cells proportion; (C) CD19+ B cells proportion; (D)

CD14+ Monocytes proportion; (E) CD3-CD56+ Natural Killer Cells proportion. The bar represents the median of the cell proportion

4.1.4 Immunological measurement for cytokine concentration

After receiving TL for 28 days, the median levels of IL-6, IFN- γ, and IFN-λ1 did not change from baseline. There was no significant difference in these cytokine levels between the two groups. IL-2 in both groups throughout the study period was undetectable. However, IP-10 in the PL group significantly decreased on day 28 compared to day 3 (median=16.690 (5.655-37.034), *p*-value=0.029). The data are shown in Figure 3.

Figure 3: Cytokine levels in the serum of participants in both groups were determined by using sandwich ELISA. (A)



IL-2 concentration; (B) IL-6 concentration; (C) IP-10 concentration; (D) IFN- γ concentration (E) IFN-λ1 concentration, UD. Undetectable

4.1.5 EQ-5D-5L Health State

The result of the quality-of-life evaluation was presented as the median (min-max) of the EQ-5D-5L index score and VAS score. Throughout the study, the median value showed that the majority of participants reported 'no problems' in all five health dimensions, which was interpreted as 1.000 for index sore. Consequently, there was no difference in health state conditions between the two groups. The median values of the EQ-VAS scores during the study of both groups were not less than 85 scores, which indicates that they had good health conditions. The data for the EQ-5D-5L health state are shown in Table 2.

Table 2: Median (min-max) of EQ-5D-5L index score and EQ-5D-5L VAS score in Triphala (TP) and Placebo (PL) groups in each visit

Groups (n=5/group)	Day 0	Day 7	Day 14	Day 28	*p*-value
EQ-5D-5L index score					
TP	0.9436 (0.8194-1.000)	1.000 (0.8855-1.000)	1.000 (1.000-1.000)	1.000 (0.8855-1.000)	0.096
PL	1.000 (0.8775-1.000)	1.000 (1.000-1.000)	1.000 (0.8855-1.000)	0.9436 (0.9419-1.000)	0.348
VAS score					

TP	85 (80-90)	85 (80-90)	90 (85-90)	90 (80-90)	0.284
PL	90 (75-100)	90 (85-95)	90 (85-100)	90 (80-95)	0.822

4.1.6 Safety assessment

In both groups, after 28 days of the study period, there was no significant difference in hemoglobin, hematocrit, white blood cells, and platelet count when compared to baseline levels. On day 3, the hemoglobin, hematocrit, and platelet count of the PL group significantly increased from baseline when compared to those of the TP group. However, these data were within the normal range.

There was no significant difference in liver and kidney function, fasting blood glucose levels, and lipid profiles in both the within-group and between-group studies.

During the study, adverse events occurred in 9 out of 10 of the participants (90%), including bloating, dyspepsia, difficulty defecation, diarrhea, polyuria, myalgia, dizziness, vertigo, and blurred vision. Bloating was the most common adverse event observed in the placebo group (80%) and treatment group (40%), with a *p*-value of 0.524. Diarrhea affected 20% of participants in both groups (p=1.000). Polyuria was observed only in the PL group at a rate of 40% (p=0.444). Dyspepsia and difficulty defecation were reported in the PL group at a rate of 20% for each event (p=0.1000). In the TP group, myalgia, dizziness, vertigo, and blurred vision were reported by 20% of participants, with a *p*-value of 0.1000 for each occurrence. There was no statistically significant difference in the proportion of adverse events occurring in either group. The adverse events in this study were mild to moderate in severity. Thus, administering TP at a dosage of 3,232 mg per day for 4 weeks appears to be safe.

4.2 Discussion

Aging is a complex process that directly impacts the immune system. The aging process reduces the organism's functions, and the ability to adaptively react to preserve homeostasis, which finally leads to an increased susceptibility to infectious illness and eventually results in mortality and morbidity. Aging-related changes in both innate and adaptive immune responses are generally defined as immunosenescence. The alteration of multiple immune cells and cytokines, which include T- and B-cell compartments, phagocytic cells, and cytokine-associated levels, affected by age is constantly concerning.

Although Triphala has been found to significantly increase the percentage of NK cells, cytotoxic T lymphocytes, and B cells in healthy adults aged 20-45 (Phetkate et al., 2012), in this preliminary study, the administration of Triphala 3,232 mg per day for 28 days in old age participants did not significantly show effects on the proportion of leukocyte subsets and cytokine levels.

1) The subjects in this study were old-age participants whose physiological changes typically occur in all organ systems in the body. For this reason, the response to Triphala may be different from that of younger, healthier volunteers. It has been reported that lactic acid bacteria in the intestine can alter Triphala into active compounds that are beneficial to the body (Peterson, Denniston, & Chopra, 2017). For example, chebulinic acid is transformed by the human gut microbiota into metabolites such as urolithin, which has antioxidant properties (Olennikov, Kashchenko, & Chirikova, 2015). In the elderly, the gut microbiota is different from that of young people in that the bacterial diversity is reduced, the amount of gut commensal bacteria, including bacteroides, bifidobacteria, and lactobacilli, is decreased, whereas the number of pathogenic bacteria, such as enterobacteria, *C. difficile*, and *C. perfringens*, is increased (Nagpal et al., 2018). Because the intestines of old people contain a low number of lactic acid bacteria, such as lactobacilli, their ability to convert Triphala into active compounds is decreased. Therefore, the effect of Triphala on the immune system of old people is quite low. Hence, administration of prebiotics and probiotics in combination with Triphala may possibly enhance the immunological effect of Triphala in old people.

2) Administration frequency in the form of twice a day may have an impact on the pharmacological action of Triphala when compared to that in the form of three times a day, especially if the half-life of the active compounds is short. A previous study focusing on the pharmacokinetics of gallic acid in healthy

volunteers who were administered a single dose of the aqueous extract Triphala (Jumpa-ngern et al., 2022) revealed that the median terminal elimination half-life of gallic acid was approximately 1 hour. In accordance with this study, another team studying the pharmacokinetics of active compounds in *T. chebula* ethanolic extract after a single dose oral administration in rats reported that the half-life of gallic acid was around 1.5 hours, whereas the half-life of other active compounds, including chebulic acid, protocatechuic acid, corilagin, chebulagic acid, chebulinic acid, 1,2,3,4,6-O-pentagalloylglucose, ellagic acid, and ethyl gallate, was 6.60, 2.06, 26.39, 19.98, 43.30, 6.52, 8.23, and 2.08 h, respectively. Noticeably, even though the half-life of gallic acid is very short (just 1.5 hours), other important compounds, including chebulagic acid and chebulinic acid, used to be reported to have the highest antioxidant value compared to other compounds found in Triphala (Wang, Li, & Hu, 2018), have a half-life of more than 12 hours. Therefore, administering Triphala twice a day appears to provide sufficient important active compounds for the body to accumulate and demonstrate their efficacy. Moreover, during the first three days in our study, the dosing pattern for the volunteers was four times a day, which was frequent enough to contribute to the accumulation of important active compounds in the body. Nevertheless, there was no change in the proportion of leukocyte subpopulation and level of cytokines in the blood samples of the volunteers who received Triphala during this early period.

3) This report provided preliminary data obtained from only 10 volunteers with an age equal to or above 60 years. This small sample size created data with a high degree of deviation and a low power of test. Therefore, to obtain a definitive conclusion about the effect of Triphala on the immune system, it requires a larger sample size.

4) This study determined the proportion of immune cells and cytokines in participants with good health conditions without any acute illness or infection, and it may be reasonable to not observe a significant alteration in the immune response. To verify the immunological effect of Triphala, additional *in vitro* studies should be performed by assaying the function of isolated white blood cells exposed to bacterial components such as lipopolysaccharide (LPS) or viral particles to see whether there will be a change in the production and secretion of distinct cytokines.

However, in terms of safety, oral administration of Triphala at a dose of 3,232 mg per day for 28 days showed no severe adverse events or affected liver or kidney function. All participants could be tolerable for Triphala.

The reason for the significant decrease of IP-10 in the PL group on day 28 compared to day 3 may be due to two subjects in the placebo group having IP-10 values on day 3 significantly higher than the baseline. After that, IP-10 levels gradually decreased. The increased level of IP-10 on day 3 in these participants may be a result of inflammation in their gastrointestinal tract, as they reported experiencing abdominal distension and loose bowel movements. As a result, IP-10 levels on day 3 were significantly higher than on day 28.

5. Conclusion

In this preliminary study, taking Triphala at a dose of 3,232 mg per day for 28 days in elderly participants was found to be safe. However, this dose regimen does not cause any change in the proportion of white blood cells and cytokine levels in the blood. Notably, these results do not indicate that Triphala has any effect on the function of immune cells. To clearly verify whether Triphala enhances the activity of immune cells, additional functional assays pertaining to the effects of Triphala on the physiological functions of individual types of immune cells (including cytotoxic T cells, natural killer cells, and monocytes/macrophages) should be performed. Additionally, further study about the co-administration of Triphala and pre/probiotics should be done to prove whether the production of Triphala's active compounds is enhanced.

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