© 2024 Journal of Pharmacy & Pharmacognosy Research, 12 (5), 892-899, 2024
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres23.1751_12.5.892

Original Article

Potential effects of *Linh Loc Son* hard capsule – a Vietnamese herbal combination in immunodeficiency induced by cyclophosphamide on mice

[Efectos potenciales de la cápsula dura *Linh Loc Son*, una combinación de hierbas vietnamitas en la inmunodeficiencia inducida por ciclofosfamida en ratones]

Binh Quoc Pham[1], Ngan Kim Thi Nguyen[1], Su Quoc Pham[1], Cuong Duy Nguyen[2], Lam Vu Trinh[1], Hang Thu Thi Dinh[3], Quang Vinh Trinh[3], Van Anh Thi Pham[3]*

[1]Vietnam University of Traditional Medicine, Hanoi, Vietnam.
[2]Department of Health, Vietnam Ministry of Public Security, Hanoi, Vietnam.
[3]Hanoi Medical University, Hanoi, Vietnam.
*E-mail: phamvananh@hmu.edu.vn

Abstract

Context: Traditional medicine and herbal extracts have long been recognized for their immunomodulatory effects, enhancing patient immune responses. The use of traditional medicine to target specific pathogens and support the immune system is increasingly studied. *Linh Loc Son* (*LLS)* hard capsule, a product derived from nature, consists of the main four natural ingredients: *Curculigo orchioides* Gaertn., *Dioscorea persimilis* Prain et Burk., *Morinda officinalis* F.C.How. and *Fallopia multiflora* (Thunb.) *Haraldson*.

Aims: To evaluate the potential effects of LLS hard capsules in complementary treatment of immunodeficiency state in mice.

Methods: An experiment was conducted to assess the impact of *LLS* hard capsules on an immunosuppressed model of cyclophosphamide-induced Swiss mice of either sex. Two doses of *LLS* hard capsules (0.69 and 1.38 g/kg body weight) and levamisole (100 mg/kg body weight), serving as a positive control, were administered orally for seven consecutive days; cyclophosphamide (200 mg/kg i.p.) was administered on the fourth day.

Results: Both doses of *LLS* hard capsule did insignificantly ameliorate the immunosuppressive effects of cyclophosphamide on the delayed-type hypersensitivity response but maintained leukocyte counts, relative organ weight, and cytokines as compared to the levamisole group and showed a significant improvement in micro-histological images.

Conclusions: This study has demonstrated *in vivo* the immunostimulatory effect of *LLS* hard capsules, highlighting their potential to boost and regulate the immune response in mice models of immunodeficiency.

Keywords: experimental; herbal medicine; immunologic; immunosuppression.

Resumen

Contexto: La medicina tradicional y los extractos de hierbas son reconocidos desde hace mucho tiempo por sus efectos inmunomoduladores, que mejoran las respuestas inmunitarias de los pacientes. Se estudia cada vez más el uso de la medicina tradicional para atacar patógenos específicos y apoyar el sistema inmunológico. La cápsula dura *Linh Loc Son* (*LLS*), un producto derivado de la naturaleza, consta de los cuatro ingredientes naturales principales: *Curculigo orchioides* Gaertn., *Dioscorea persimilis* Prain et Burk., *Morinda officinalis* F.C.How. y *Fallopia multiflora* (Thunb.) *Haraldson*.

Objetivos: Evaluar los efectos potenciales de la cápsula dura de *LLS* en el tratamiento complementario del estado de inmunodeficiencia en ratones.

Métodos: Se llevó a cabo un experimento para evaluar el impacto de las cápsulas duras de *LLS* en un modelo inmunosuprimido de ratones suizos de ambos sexos inducidos por ciclofosfamida. Se administraron por vía oral dos dosis de cápsulas duras de *LLS* (0,69 y 1,38 g/kg de peso corporal) y levamisol (100 mg/kg de peso corporal), que sirvieron como control positivo, por vía oral durante siete días consecutivos; Se administró ciclofosfamida (200 mg/kg i.p.) el cuarto día.

Resultados: Ambas dosis de la cápsula dura de *LLS* mejoraron de manera insignificante los efectos inmunosupresores de la ciclofosfamida en la respuesta de hipersensibilidad de tipo retardado, pero mantuvieron los recuentos de leucocitos, el peso relativo de los órganos y las citocinas en comparación con el grupo de levamisol y mostraron una mejora significativa en las imágenes microhistológicas.

Conclusiones: Este estudio ha demostrado *in vivo* el efecto inmunoestimulador de la cápsula dura de *LLS*, destacando su potencial para estimular y regular la respuesta inmune en un modelo de inmunodeficiencia en ratones.

Palabras Clave: experimental; medicina herbaria; inmunológico; inmunosupresión.

ARTICLE INFO
Received: July 19, 2024.
Accepted: March 26, 2024.
Available Online: April 17, 2024.

AUTHOR INFO
ORCID: 0000-0001-8837-3829 (HTTD)
0000-0003-4548-104X (QVT)
0000-0002-8977-9377 (VATP)

Abbreviations: BW: Body weight; CP: Cyclophosphamide; DTH: Delayed type hypersensitivity; IgM: Immunoglobulin M; IL-1β: Interleukin-1β; IL-2: Interleukin-2; iNOS: Inducible nitric oxide synthase; *LLS*: *Linh Loc Son* - a nature-derived hard capsule of Bach Thao Duoc Co. Ltd.; MERS-CoV: Middle East respiratory syndrome coronavirus; OVA: Ovalbumin; SARS-CoV: Severe acute respiratory syndrome coronavirus; SRBC: Sheep red blood cells; TNF-α: Tumor necrosis factor alpha; WBC: White blood cell.

INTRODUCTION

The recent surge in viral outbreaks, including potential pandemics, highlights the critical role of the immune system in defense against viruses. Immunocompromised individuals with a weakened response to viral invasion face a higher risk of severe infections. There are lots of innovative drug inventions to stimulate this protection mechanism, including drugs derived from herbal medicine. Most immunostimulants prescribed worldwide are chemical substances with several adverse effects and can be contraindicated in some circumstances. On the other hand, natural materials have been investigated to have immunomodulatory effects and influence the human body's immune system with lower costs and fewer side effects (Jantan et al., 2015). Therefore, the approach of innovative agents has a promising potential concerning the prevention of the state of immunodeficiency and the enhancement of the human immune system in general.

The respiratory viral disease outbreaks to date have the same routes and means of transmission; the recent viral COVID-19 pandemic tends to spread more quickly, with large numbers of people infected and dying. The respiratory viral pandemics (i.e., SARS-CoV, SAR-CoV-2, MERS-CoV) become a significant public health problem due to the rise of new subtypes is more frequent and the ability to weaken and escape the host immunity (Noor and Maniha, 2020). Non-immunosuppressed individuals infected by respiratory viruses are generally asymptomatic or have mild manifestations. In contrast to the above, patients with an immunodeficiency state are more susceptible to viral infection because of genetic causes, drug therapy, or organ transplantation, and the viral infection may result in a life-threatening scenario (Dropulic and Cohen, 2011). Besides vaccination therapy, immunomodulator herbs have been investigated in the management of severe viral infection by boosting the host's immune capacity (Burns et al., 2010; Liu et al., 2016). *Linh Loc Son (LLS)* hard capsule is a combination of herbal ingredients, with *Curculigo orchioides* Gaertn., *Dioscorea persimilis* Prain et Burk., *Morinda officinalis* F.C.How, *Fallopia multiflora* (Thunb.) Haraldson. as the main components. These are based on a traditional formula developed by the founding headmaster of Vietnam University of Traditional Medicine. The product aims to improve human immune capacity. These herbs are known to be safe, cost-effective, and popular, exhibiting immunostimulatory, anti-inflammatory, and hepatoprotective effects. Thus, their combination could offer a more efficient complementary therapy soon (Bafna and Mishra, 2006; Lakshmi et al., 2003; Liu et al., 2018; Zhang et al., 2017; 2023). Unlike costly synthetic immunostimulants like interferons and interleukins, *LLS* offers a natural, orally administered alternative. This study evaluated the efficacy of *LLS* hard capsules as a viable replacement for injectable immunostimulants. The hard capsule formulation has been adopted for its convenience, reliability, and ease of manufacturing and administration. Specifically, this study aims to validate the immune-stimulatory effects of the four major herbs in *LLS* hard capsules using an immunosuppressed mouse model induced by cyclophosphamide.

MATERIAL AND METHODS

Drugs and chemicals

Cyclophosphamide (CP) (Endoxan 200 mg, Baxter Oncology GmbH, Germany) was used as the immunosuppressive agent. Levamisole was obtained from Sigma-Aldrich Chemicals Pvt. Ltd. USA and used as a reference compound in this experiment. Sheep red blood cells (SRBC) obtained from sheep veins in sterile condition (Nam Khoa Co. Ltd., Viet Nam) and OVA solution (ovalbumin + $Al(OH)_3$) (Sigma-Aldrich Chemicals Pvt. Ltd. USA) were used as the delayed-type hypersensitivity inducer. SRBC was preserved in Alsever's solution (Sigma-Aldrich Chemicals Pvt. Ltd., USA) and distilled water to form 1200 mL solution (pH 6.1), then was diluted in NaCl 0.9% (Otsuka, Japan) to create the 5% solution to use in the experiment. 100 mg $Al(OH)_3$ was dissolved in 50 mL NaCl 0.9% (Otsuka, Japan), the ratio of ovalbumin to $Al(OH)_3$ was 1:4.

Plant material

The preparation *LLS* hard capsule was conducted by the Bach Thao Duoc Duoc Company Limited, Hanoi, Vietnam. Fresh plant materials were cultivated from the company farm and identified based on **the** Vietnamese Pharmacopeia 5th edition (Vietnamese Ministry of Health, 2017).

Preparation of *LLS* hard capsule

The fresh plant materials were first boiled two times at 100°C for 3 hours, each with distilled water, to collect the extracted liquid. Then, the total amount of extracted liquid was concentrated at 100°C until the

humidity had reached under 25%. The extract was dried entirely in the next step at 80°C for under 3% humidity extract. Then, the dried extract product was ground to a fine powder and preserved in vacuum bags (storage conditions: temperature (25°C), humidity (under 75%).

A 480 mg fine powder was mainly produced from 833 mg *Curculigo orchioides* Gaertn., 1666 mg *Dioscorea persimilis* Prain et Burk., 1666 mg *Morinda officinalis* F.C.How and 1666 mg *Fallopia multiflora* (Thunb.) Haraldson then it was formulated in a 600 mg hard capsule. After the preparation process, the investigational products were subjected to a quality examination confirmed by Bach Thao Duoc, Legal Director (Registration number: TCKT.TP.03). The quality examination conformed to the guidelines of Vietnamese Pharmacopeia 5th edition (Vietnamese Ministry of Health, 2017).

Three *LLS* hard capsules (equal to 1.44 g fine powder) were dissolved in distilled water to form an investigational product (20.8 mL) before oral administration by gastric tube (1 mL/100 g body weight). The investigational product was freshly prepared every day before the administration procedure.

Animals

Swiss albino mice (Mus musculus) of either sex, weighing 20 ± 2 g, were purchased from the National Institute of Hygiene and Epidemiology. The mice were kept in cages (10 animals per cage) under standard conditions of humidity (50 ± 5%), temperature (25 ± 2°C), and light (12 h light/12 h dark cycle) and fed with standard food (National Institute of Hygiene and Epidemiology) and water *ad libitum*. Before the experiment, mice were adapted to their laboratory condition within seven days. This study was approved by the Scientific Board Committee of Hanoi Medical University (ref number: IRB00003121).

Animal study conduct

Fifty mice were included in this experiment and were randomly assigned into five groups of ten mice per cage: control group (Group I), CP-induced group (Group II), positive control group (Group III), *LLS* pretreated at 0.69 g/kg BW group (Group IV), *LLS* pretreated at 1.38 g/kg BW group (Group V). On the 4th day of the experiment, the control group was given an i.p. injection of physiological saline (10 mL/kg), and four other groups (Group II-V) were injected CP with a single dose (200 mg/kg). Positive control group (Group III): levamisole 100 mg/kg, *LLS* pretreated group (Group IV and V): *LLS* hard capsules pretreated at two doses (equal to 0.69 g fine powder/kg and 1.38 g fine powder/kg, respectively), were administered orally levamisole and *LLS* hard capsules for seven consecutive days. The dose of 0.69 g/kg was selected based on findings from studies of acute toxicity, sub-chronic toxicity, and other pharmacological effects (Chu, 2023). On the eighth day, mice were anesthetized with ketamine (100 mg/kg, i.p.) and then sacrificed to collect blood samples (using cardiac puncture with thoracotomy method), spleen, and thymus (using abdominal surgery) to evaluate immune parameters.

Delayed type hypersensitivity (DTH) response estimation

On the second day of the experiment, animals were given an i.p. injection of sheep red blood cells 5% (500 µL) and injected subcutaneously OVA 100 µL into the nape of the neck site. DTH reaction was elicited five days later by the injection of OVA 50 µL into the right hind paw and physiological saline into the left one after measuring the thickness of the footpad in the center of the walking pads using an INSIZE® digital external caliper (Code: 2312–20). After 24 hours, the paw volume was measured again to assess the swelling degree through differential percentage between the two footpads.

Relative organ weight

The related weight of the spleen and thymus was calculated using the following formula [1].

$$\text{Relative organ weight} = \frac{\text{Organ weight (mg)}}{\text{Body weight (g)}} \quad [1]$$

Leukocyte counts

Blood samples were collected on the day of sacrifice to determine the number of total WBCs, lymphocytes, neutrophils, and monocytes using a HORIBA® ABX MircoES60 blood analyzer.

Estimation of IgM, IL-2, and TNF-α concentration in serum

On the eighth day of the experiment, blood samples were assayed to measure the concentration of serum IgM, IL-2, and TNF-α using an ELISA kit (Cloud-Clone Corp., USA) and a BioTek® ELx808 Reader.

Histopathological examination

A histopathological examination was performed in 15 spleens/thymi: 3 in each group. After removal, the organs were weighed using a digital scale (Precisa®, Swiss, Model 321LX Type 2200C) and then preserved in 10% formalin for fixation. Sections stained with hematoxylin and eosin (HE) were examined under a

light microscope (Olympus BX10, Japan). The images were snapped by an attached digital camera (Olympus DP12 camera, Japan) with a magnification of 20×.

Statistical analysis

The data were expressed as the mean ± standard deviation (SD), and statistical analysis was carried out employing the T-student test using SPSS 20. The $p<0.05$ was considered to be statistically significant.

RESULTS

General results after the study period

Fifty mice, ten in each group, were included in this experiment. After the experiment period, all mice were still alive. All mice were healthy at the time of sacrifice (Day 8). No adverse effect was observed, and there was no notable change in vital signs, skin, fur, or daily behavior.

Effect of *LLS* hard capsules on delayed-type hypersensitivity (DTH) response

Administration of CP (200 mg/kg, i.p) significantly decreased footpad swelling compared to the control group ($p<0.001$). Levamisole treatment showed no significant difference in swelling degree compared to the CP-induced group ($p>0.05$). Both *LLS* doses slightly increased the DTH response, but no significant improvement was observed ($p>0.05$) (Table 1).

Effect of *LLS* hard capsules on relative organ weight

CP administration significantly decreased the relative weight of all organs ($p<0.001$) in all four CP-treated groups. However, the *LLS* 0.69 g/kg pretreatment group displayed a significant increase in spleen weight compared to the CP-only group ($p<0.05$). No significant differences were observed in the relative weight of the thymus or spleen in the remaining CP-treated groups (Table 1).

Effect of *LLS* hard capsules on leukocyte counts

There was a notable reduction in leukocyte counts compared to the normal control group. Both the levamisole group and the groups pretreated with *LLS* at both doses exhibited no significant difference in leukocyte counts in comparison to the CP-induced group ($p>0.05$) (Table 2).

Effect of *LLS* hard capsules on serum IgM, IL-2, and TNF-α concentrations

The levamisole group exhibited a significant improvement in IL-2 concentration compared to the normal control group ($p<0.05$), while no significant differences in IL-2 concentration were observed among the other four groups. Fig. 1 demonstrates notable decreases in TNF-α concentration in both the CP-induced group and the pretreated *LLS* 0.69 g/kg group compared to the control group ($p<0.05$). Additionally, treatment with levamisole and *LLS* at both doses significantly enhanced TNF-α concentration compared to the CP-induced group, with $p<0.001$, <0.05, and <0.001, respectively. A significant decrease in serum IgM level was noted in all four groups administered CP, relative to the normal control group ($p<0.001$). Among these, the most significant improvement was observed in the pretreated *LLS* 0.69 g/kg group compared to the CP-induced group ($p<0.05$), as illustrated in Fig. 1.

Histopathological examination of spleens and thymus

The number of lymphocytes decreased significantly in all four groups administered with CP. Severe damage to organ structures was observed in the white pulp of spleens and the cortex area of the thymus. Levamisole and LLS hard capsules restored the spleen and thymus lymphocyte counts at different levels. Significant improvements were observed in pretreated *LLS* groups of both doses (Figs. 2 and 3).

Table 1. Effect of *LLS* hard capsules on swelling degree of footpads and relative organ weight of the spleen and thymus.

Group	Treatment (n = 10)	Swelling degree of footpads (%) (Mean ± SD)	Relative weight of spleen (mg/g) (Mean ± SD)	Relative weight of thymus (mg/g) (Mean ± SD)
I	Control	20.24 ± 6.72	7.10 ± 1.63	6.50 ± 1.56
II	CP	9.62 ± 3.01***	3.12 ± 0.62***	1.16 ± 0.31***
III	Levamisole + CP	9.20 ± 2.90***	2.67 ± 0.48***	1.17 ± 0.42***
IV	*LLS 0.69 g/kg* + CP	10.44 ± 3.23***	3.21 ± 0.42***	1.11 ± 0.16***
V	*LLS 1.38 g/kg* + CP	11.77 ± 2.45**	4.00 ± 0.84***$^{\Delta}$	0.95 ± 0.21***

**, ***$p<0.01$, $p<0.001$ compared with group I. $^{\Delta}$ Groups III, IV, and V were compared with group II ($p<0.05$). CP: Cyclophosphamide.

Table 2. Effect of *LLS* hard capsules on leukocyte counts.

Group	Treatment (n = 10)	TLC count (Mean ± SD)		
		WBC (g/L)	LYM (g/L)	NEU (g/L)
I	Control	6.11 ± 1.84	4.17 ± 1.38	0.55 ± 0.29
II	CP	1.63 ± 0.58***	1.01 ± 0.34***	0.18 ± 0.10**
III	Levamisole 100 mg/kg + CP	1.41 ± 0.26***	0.96 ± 0.20***	0.22 ± 0.13**
IV	*LLS 0.69 g/kg* + CP	1.57 ± 0.55***	0.96 ± 0.35***	0.21 ± 0.08**
V	*LLS 1.38 g/kg* + CP	1.73 ± 0.59***	1.05 ± 0.34***	0.23 ± 0.09**

, * p0.01, p0.001 compared with group I. TLC: Total leukocyte count; WBC: White blood cells; LYM: Lymphocytes; NEU: Neutrophils; CP: Cyclophosphamide.µg

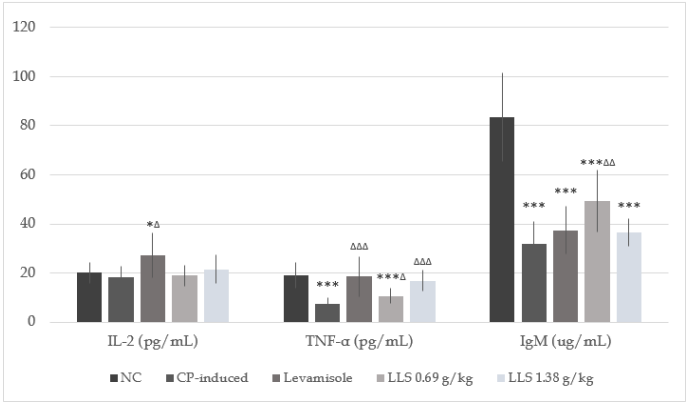

Figure 1. Effect of *LLS* hard capsule on serum IgM, IL-2, and TNF-α concentration.

Data are expressed as mean ± SD, n = 10. *, *** p<0.05, p<0.001 compared with group I. Δ, ΔΔ, ΔΔΔ Groups III, IV, V were compared with group II (p<0.05, p<0.01, p<0.001).

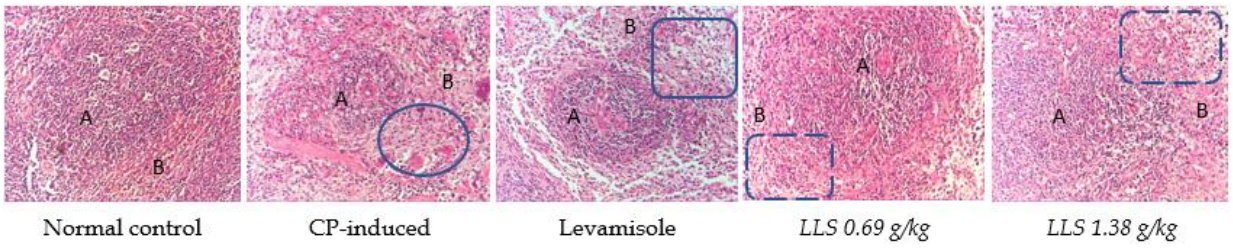

Figure 2. Histopathology of the spleens in cyclophosphamide (CP)-induced immunosuppressed mice.

A. Red pulp, **B**. White pulp. The oval area indicates that the white pulp lacks lymphocytes compared to the normal control group, while the **Rectangle area** shows a mild improvement. The **dashed rectangle area** indicates a manifest improvement with a significant increase in lymphocytes. HE, 20×.

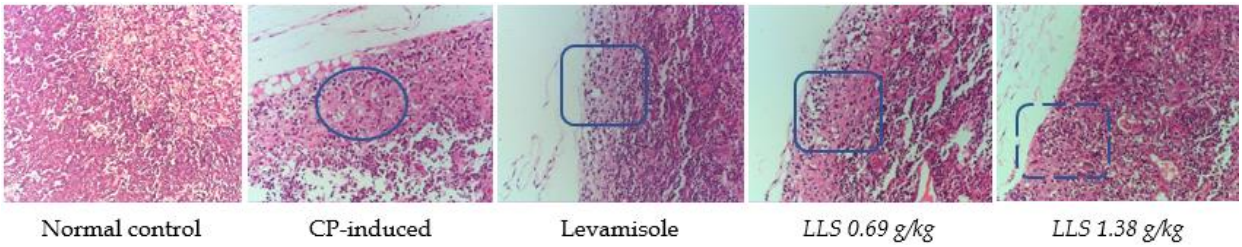

Figure 3. Histopathology of the thymuses in cyclophosphamide (CP)-induced immunosuppressed mice.

The oval area indicates that the cortex lacks lymphocytes compared to the normal control group**. The rectangle area** shows that Levamisole and LLS 0.69 g/kg show a slight improvement, but the thymus structure still has been significantly damaged. **The dashed rectangle area** indicates an evident restoration of *LLS 1.38* g/kg in the lymphocyte count. HE, 20×.

DISCUSSION

Herbal remedies have long been utilized for daily consumption and in traditional treatment for specific diseases. All four major herbs in the *LLS* hard capsule have a history of traditional medicinal use for years. Extensive research, both *in vitro* and *in vivo*, has investigated their potential health effects (Byeon et al., 2018; Lin et al., 2015; Nie et al., 2013; Oh and Lim 2008; Zhang et al., 2017). Each herb ingredient in the *LLS* hard capsule has been previously studied for its immunomodulatory properties through various mechanisms.

Curculigo orchioides Gaertn., for instance, not only enhances the phagocytic activity of macrophages but also exhibits immunostimulatory effects on hemagglutination, plaque-forming cell, and delayed-type hypersensitivity, which are the integral processes of both humoral and cell-mediated-immune responses (Bafna and Mishra, 2006; Lakshmi et al., 2003). The polysaccharide isolated from *Dioscorea batatas* Decne. has demonstrated pharmacological effects in ulcerative colitis mice through modulation of microbiota composition (Zhang et al., 2023). *Dioscorea* spp. also possesses high nutritional value, particularly in micronutrients, and its phytochemicals exhibit antioxidant properties related mainly to radical-scavenging capacity in both in vitro and in vivo assays (Adomėnienė and Venskutonis, 2022; Jingying et al., 2023). Major constituents of *Morinda officinalis* F.C.How include polysaccharides, oligosaccharides, anthraquinones and iridoid glycosides, which have been associated with anti-oxidative, immune-regulatory, and anti-inflammatory activities (Zhang et al., 2017). *Fallopia multiflora* (Thunb.) Haraldson. is known for its immunomodulating effect, primarily attributed to its polysaccharides and anthraquinone glycosides, which can accelerate lymphocyte proliferation and mix lymphocyte reaction, improve macrophage phagocytosis, and increase TNF secretion and the activity of NK cells, antagonize the restraining effect of lymphocyte helper/suppressor ratio induce by mitomycin (Guibo, 2006; Lin et al., 2015). Given the popularity and efficacy of these herbal ingredients, the primary objective of this study is to evaluate the immunostimulatory effects of *LLS* hard capsule across various parameters. The vital signs and daily behavior of the mice were monitored throughout the experiment to assess safety. In the study's conclusion, the absence of mortality or adverse events indicated the safety of the LLS hard capsule at the experimental doses administered to mice.

In recent decades, respiratory viral pandemics, notably the SARS-CoV-2 pandemic, have posed significant challenges. Severe cases of viral infections often present with a lymphopenia manifestation (Yang et al., 2020). The viruses typically weaken the immune system upon invasion, with SARS-CoV-2 no exception (Jacques and Apedaile, 2020). In immunosuppressed states, abnormalities of lymphocyte counts, immunoglobulins, and cytokines are common. Cyclophosphamide effectively mimics the immunosuppressed state observed in viral infection, leading to immune organ injuries with decreased leukocyte counts, lymphocyte cytokine (IL-2), immunoglobulin (IgM), TNF-α. Undoubtedly, a SARS-CoV-2-infected case has two stages of disease: the first is immunosuppression, and the second is hyperinflammation characterized by cytokine storm and leukocyte infiltration (Jacques and Apedaile, 2020). This study elucidates the initial stage of viral infection and explores the DTH response.

In the DTH test, cyclophosphamide significantly damaged the short-lived suppressor T-cells in the immune regulatory system in all four groups except the control one. No notable change was observed in the DTH response of the levamisole group and two pretreated *LLS* groups. Bafna and Mishra (2006) demonstrated that pretreatment of methanol extract of *Curculigo orchioides* Gaertn. increased the footpad thickness, which indicated *Curculigo orchioides* Gaertn. possesses a stimulatory effect on the lymphocytes and accessory cell types required for the expression of this reaction. The DTH response directly correlates with cell-mediated immunity, in which sensitized T-lymphocytes are converted to lymphoblasts and start the biological pathways of the reaction (Jacysyn et al., 2001). The lack of notable changes in the DTH response among the levamisole group and two pretreated *LLS* groups can be attributed to a dramatic increase in lymphocyte count, including T-lymphocyte, resulting from cyclophosphamide-induced damage, which may be irrecoverable. The cyclophosphamide dose used in this study (200 mg/kg, i.p.) was substantially higher than that used in Bafna and Mishra's study (30 mg/kg, i.p.)

IL-2 exhibits a broad spectrum of immune-promoting activities, such as inducing lymphocyte differentiation, promoting NK cells' function, and releasing interferons (Ross and Cantrell, 2018). There was only levamisole, which showed an improvement in serum IL-2 levels. This is equivalent to the result of Yan's study published in 2021 (Yan et al., 2021). *LLS* hard capsule had a mild influence on IL-2 level but was not significant compared to the CP-induced group. TNF-α is a pleiotropic cytokine, considered a pro-inflammatory molecule, but it also mediates an anti-inflammatory and immunomodulatory effect in various chronic inflammatory and autoimmune diseases (Salomon et al., 2018). The immunostimulatory effect of *Dioscorea persimilis* Prain et Burk. and *Fallopia*

multiflora (Thunb.) Haraldson., two major constituents of cytokine secretion, particularly TNF-α, may explain the noticeable improvement in serum TNF-α level observed in a dose-dependent manner with *LLS* hard capsule treatment (Adomėnienė and Venskutonis, 2022; Guibo, 2006). Humoral immunity, characterized by immunoglobulins, was also evaluated in this study through the IgM level. The dose expected to be used in future clinical trials of *LLS* hard capsule (0.69 g/kg) effectively restored IgM titer, indicating that the immunostimulatory effect of *LLS* hard capsule may depend on the humoral immune pathway.

The protective effect of *the LLS hard capsule is consistent with the histopathological changes in the* thymus and spleen in experimental groups (Figs. 2 and 3). Serving as a critical source of cells for immune reactivity, the bone marrow, targeted primarily by the inducer cyclophosphamide, sustains damage leading to leukopenia—a hallmark of immunosuppressive conditions. Significant pathological improvements were noted in the spleen and thymus groups pretreated with *LLS*. An increase in leukocyte presence was evident in the spleen's white pulp and the thymus's cortex area compared to the CP-treated group. This indicates the *LLS* hard capsule's potential to mitigate the suppression of bone marrow activity and stimulate the function of immune organs.

These findings suggest the potential of *LLS* hard capsules as an adjunctive therapy for immunodeficiency or for enhancing immune function in patients with viral infections or undergoing chemotherapy. This naturally derived treatment offers ease of production and a favorable safety profile. However, further research is required to fully understand the immunomodulatory effects of LLS hard capsules. Additional studies should investigate its specific anti-viral properties (particularly against SARS-CoV-2), its potential for reducing inflammation and cytokine storms, and its impact on lung injury models.

Future clinical trials should evaluate the safety and efficacy of *LLS* hard capsule on volunteers, providing further evidence of its potential applications in immunosuppressive diseases, viral infections, and complementary therapies.

CONCLUSION

The fine powder of *LLS* hard capsules, administered at both doses, significantly enhanced the immune system enhancement of immunosuppressed mice. Treatment with *LLS* hard capsules effectively prevented the decline in serum TNF- α and IgM levels and ameliorated the organ injuries in histopathological examinations of the spleen and thymus. These findings suggest that *LLS* may hold promise as an effective traditional medicine for use as adjunctive therapy in managing immunosuppressed states and bolstering the human immune system.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This study was supported by the Department of Physiology and the Department of Pathophysiology and Immunology at Hanoi Medical University, Hanoi, Vietnam. The authors, therefore, acknowledge and thank the HMU staff for their helpful advice and technical support. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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AUTHOR CONTRIBUTION:

Contribution	Binh QP	Ngan NTK	Su QP	Cuong ND	Lam TV	Hang DTT	Quang TV	Van Anh PT
Concepts or ideas	x							x
Design	x							x
Definition of intellectual content	x							x
Literature search		x	x	x	x	x	x	x
Experimental studies		x	x	x	x	x	x	x
Data acquisition		x	x	x	x	x		
Data analysis						x		
Statistical analysis						x		
Manuscript preparation							x	x
Manuscript editing	x					x	x	x
Manuscript review	x	x	x	x	x	x	x	x

Citation Format: Binh QP, Ngan NTK, Su QP, Cuong ND, Lam TV, Hang DTT, Quang TV, Van Anh PT (2024) Potential effects of *Linh Loc Son* hard capsule – a Vietnamese herbal combination in immunodeficiency induced by cyclophosphamide on mice. J Pharm Pharmacogn Res 12(5): 892–899. https://doi.org/10.56499/jppres23.1751_12.5.892