IMMUNOSTIMULATORY EFFECT OF *JASMINUM SUBTRIPLINERVE* BLUME LEAVES EXTRACT ON CYCLOPHOSPHAMIDE-INDUCED IMMUNOSUPPRESSION IN MICE

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ABSTRACT

The objective of this study was to evaluate the immunostimulatory activity of Jasminum subtriplinerve Blume. extract (JM). We induced the immunosuppression in Swiss mice by cyclophosphamide (CP). Jasminum subtriplinerve Blume. extract 14.4 g/kg body weight was administered orally 7 consecutive days to animals and CP (200 mg/kg i.p.) was injected on 4th day. The result showed that Jasminum subtriplinerve Blume. leaves extract 14.4 g/kg ameliorated the effects of CP on delayed type hypersensitivity (DTH) response, leukocyte quantity, relative organ weight, IgM concentration and micro-histological images. This suggested the potential of Jasminum subtriplinerve Blume leaves extract to treat immunosuppression diseases in clinical practice.

Keywords: *Immunostimulatory activity, Immunosuppression, Swiss mice, Cyclophosphamide, Jasminum subtriplinerve Blume. leaves.*

I. INTRODUCTION

Immunosuppression is a state of the immunity responding to pathogens below the required level [9]. Immunostimulators are often used in conjunction with chemotherapy drugs to activate or induce the mediators or components of the immune system that have been suppressed by therapy [8]. Studies of new immunostimulatory agents are important with regard to the prevention and treatment of the diseases that occur in patients who are immunosuppressed.

Most prescription immunostimulants (i.e. levamisole, isoprinosine) are synthetic substances which possess serious side effects. There is a growing interest to find naturally occurring immunomodulators to modulate the complex immune system which may possess fewer side effects. A large number of natural materials have been suggested to have immunomodulatory effects [10].

Jasminum subtriplinerve Blume., a species of Oleaceae, is widely distributed worldwide, growing naturally in the mountains and midlands of countries such as India, Myanmar, Cambodia, Laos, and the southern provinces of China [11]. In Vietnam, *J. subtriplinerve* can be found in numerous provinces across mountains, midlands, and plains, including Lao Cai, Hoa Binh, Hanoi, Nghe An, Ha Tinh, Quang Tri, and Thua Thien Hue [1]. Traditional medicine employs J. subtriplinerve leaves, which are primarily utilized to manage irregular menstruation in postpartum women with high fever, lymphadenitis, mastitis, breast abscess, leukorrhea, rheumatism-induced bone and joint pain, scabies,

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impetigo, and pruritic skin conditions [5]. The plant serves various medicinal purposes, such as supporting liver function, promoting bile secretion, aiding digestion, improving appetite, facilitating sleep, and demonstrating antibacterial and anti- inflammatory properties [6].

In pre-clinical research, *Jasminum subtriplinerve* Blume extract was shown to possess a significant immunostimulatory effect on both the cell-mediated and humoral immune systems. In this study, our aim was to validate the immunostimulatory potency of *Jasminum subtriplinerve* Blume extract in subjects which underwent immunosupression induced by cyclophosphamide.

II. MATERIALS AND METHODS

2.2. Preparation of Jasminum subtriplinerve Blume leaves extract

Fresh tea leaves bought in Hanoi are cleaned and dehydrated, then extracted 2 times by pot with a ratio of 1: 4 (1kg of leaves, 4 liters of water) at 100 degrees Celsius, under normal pressure for 10 minutes. -12 hours will get 1g of extract in a plastic form.

2.2. Drugs and chemicals

Cyclophosphamide (Endoxan 200 mg, Baxter Oncology GmbH, Germany) was a standard immunosuppressive agent. Levamisole was obtained from Sigma (Aldrich) and used as positive control in this experiment. Sheep red blood cells (SRBC) and OA solution (ovalbumin + Al(OH)3) were used as the antigenic materials.

2.3. Subjects

Swiss albino mice of either sex, weighing 20 ± 2 g were purchased from National Institute of Hygiene and Epidemiology. The mice were kept in cages (10 animals per cage) and provided with water and standard food ad libitum. Before the experiment was carried out, mice were adapted to their laboratory condition for 7 days.

2.4. Experimental design

The animals were randomly selected into 4 groups of ten mice per cage.

Group I (control) was given an i.p. injection of physiological saline. Group II (CP) was injected CP with a single dose (200 mg/kg) on the 4th day of the experiment. Group III (levamisole 100 mg/kg + CP) and group IV (JM leaves extract 14.4 g/kg + CP) were orally administered levamisole and JM leaves extract for 7 consecutive days with a single injection of CP on the 4th day. On the 8th day, mice were sacrificed to collect blood samples, spleen and thymus to evaluate immune parameters.

Delayed type hypersensitivity (DTH) response

On the 2nd day of the experiment, animals were given an i.p. injection of sheep's red blood cells and injected OVA 0,1 mL into spinal cord. DTH reaction was elicited 5 days later by the injection of OVA 50 µL into right hind paw and physiological saline into left one after measuring thickness of footpad. After 24 hours, the paw volume was measured again to assess the degree of swelling of the footpad.

Counting leukocyte quantity

On the day of sacrifice, blood samples were collected to determine the number of total WBC, lymphocytes, neutrophils and monocytes by using an automatic blood analyzer.

Relative organ weight

The related weight of the spleen and thymus was calculated using the following formula:

Relative organ weight = Organ weight (mg) / Body weight (g)

Assay for serum IgM, IL-2 and TNF- α

On the 8th day of the experiment, blood samples were assayed to measure the concentration of serum IgM, IL-2 and TNF-α by using an ELISA kit (Cloud-Clone Corp., USA).

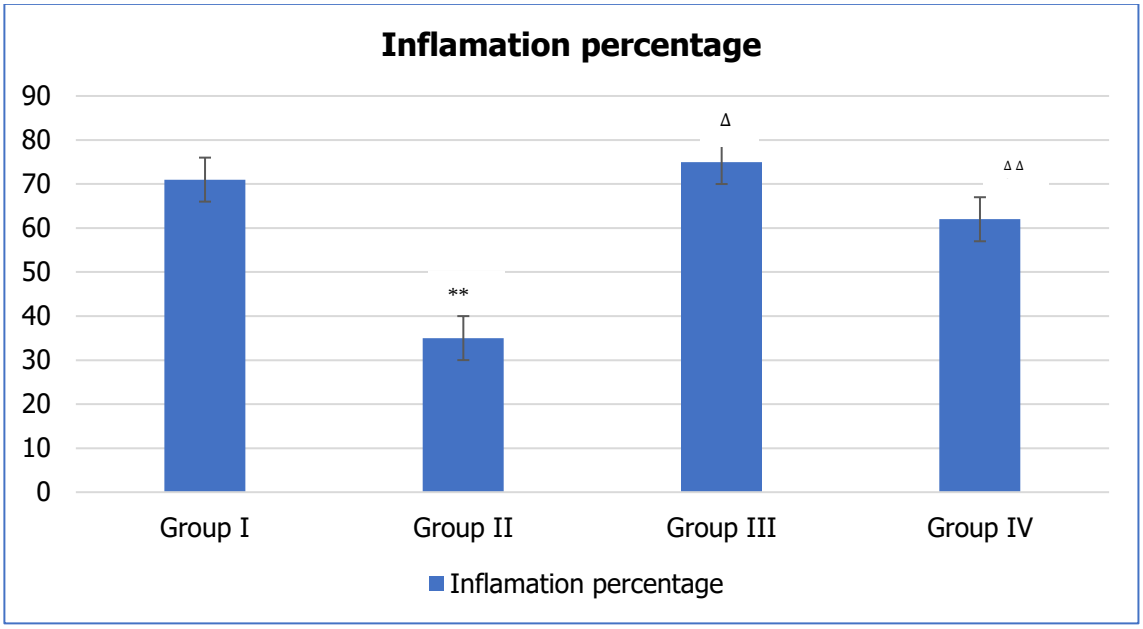
2.5. Statistical analysis

The data were expressed as the Mean ± SD and statistical analysis was carried out employing a student's t-test.

III. RESULTS

1. Effect of *Jasminum subtriplinerve* Blume leaves extract on delayed type hypersensitivity (DTH) response

Administration of CP (200 mg/kg, i.p) showed a significant decrease in the DTH response as compared with the control group (group I) ($p < 0.01$). There was a significant increase in the paw volume at groups treated with levamisole ($p < 0.001$) and the extract of JM leaves 14.4 g/ kg ($p < 0.05$) as compared with group II (Figure 1).

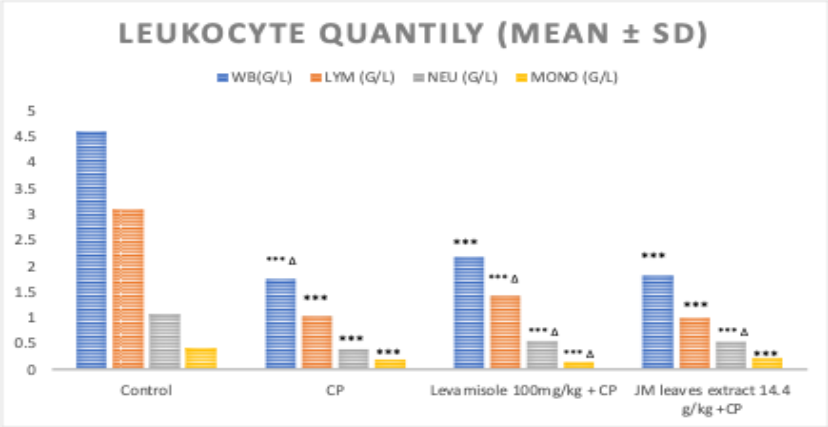


*** compared with group I ($p < 0.01$; .Δ, ΔΔ Groups III, IV were compared with group II ($p < 0.05$, $p < 0.01$).*

Figure 1. ***Effect of Jasminum subtriplinerve Blume leaves extract on delayed type hypersensitivity (DTH) response***

2. Effect of *Jasminum subtriplinerve* Blume leaves extract on leukocyte quantity

Figure 2 showed that mice treated with CP showed a significant reduction in leukocyte quantity as compared with the control group. Levamisole increased the white blood cells (WBC), lymphocytes (LYM) and the neutrophil (NEU), but had no the improvement in monocytes (MONO) as compared with group II. There was an increase in the NEU quantity at group treated JM leaves extract 14.4 g/kg ($p < 0.05$) as compared with group II; however, JM had no clear effect on WBC, LYM and MONO quantities.

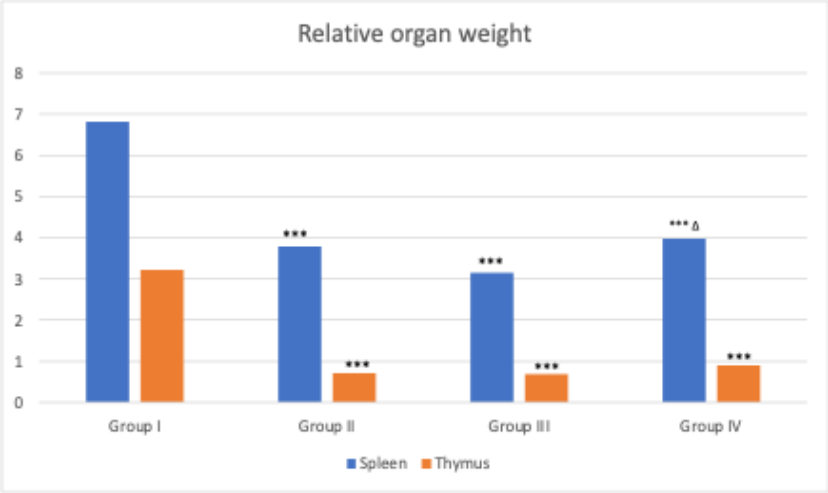


**** compared with group I (p < 0.001); Δ Groups III, IV were compared with group II (p < 0.05).*

Figure 2. ***Effect of Jasminum subtriplinerve Blume leaves extract on leukocyte quantity***

3. Effect of *Jasminum subtriplinerve* Blume leaves extract on relative organ weight

Figure 3 showed that the thymus relative weight of the animals treated with JM leaves extract at dose of 14.4 g/kg increased as compared with group II, but no significant action was observed (p > 0.05). However, there was a marked increase in spleen relative weight at mice treated with JM leaves extract 14.4 g/kg as compared with group II (p < 0.05).



**** compared with group I (p < 0.001); Δ Groups III, IV were compared with group II (p < 0.05).*

Figure 3. ***Effect of Jasminum subtriplinerve Blume leaves extract on relative organ weight***

4. Effect of *Jasminum subtriplinerve* Blume leaves extract on serum antibody, IL-2 and TNF-α concentration

The below table (Table 1) illustrated that levamisole increased IgM and TNF-α concentration as compared to group II with p < 0.05 and p < 0.001 respectively. The IgM concentration at JM leaves extract 14.4 g/kg group was higher than that at group II, but no significant action was observed (p > 0.05). There was no substantial difference between the JM extract 14.4 g/kg and group II in terms of IL-2 and TNF-α concentration.

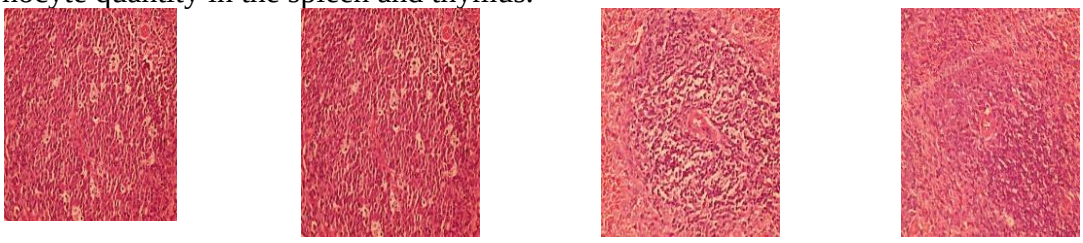
Table 2. ***Effect of Jasminum subtriplinerve Blume leaves extract on serum antibody, IL-2 and TNF-α concentration***

Group	Treatment	IgM concentration Mean ± SD (mg/mL)	IL-2 concentration Mean ± SD (pg/mL)	TNF-α concentration Mean ± SD (pg/mL)
I	**Control**	25.09 ± 7.19	58.27 ± 10.75	676.39 ± 114.24
II	**CP**	21.83 ± 4.14*	52.38 ± 9.98	616.46 ± 108.7*
III	**Levamisole 100 mg/ kg + CP**	22.01 ± 5.09 Δ	46.51 ± 8.10	806.53 ± 111.14 ΔΔ
IV	**JM leaves extract 14.4 g/kg + CP**	24.92 ± 4.68	43.29 ± 9.18	614.09 ± 114.17

** compared with group I ($p < 0.05$); Δ, ΔΔ Groups III, IV were compared with group II ($p < 0.05$, $p < 0.01$).*

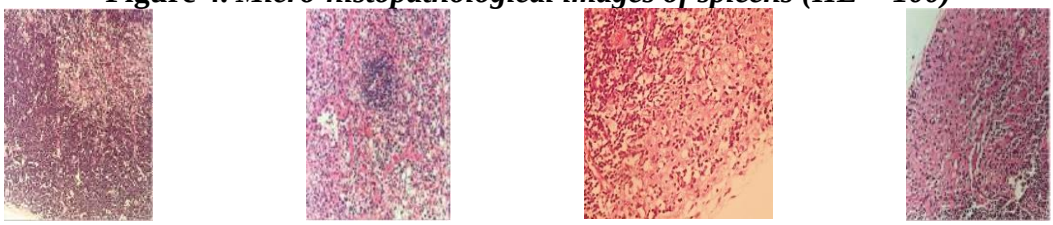
5. Histopathological study of spleen and thymus

The microhistological images (Figure 4 and 5) demonstrated that in the spleen and thymus of animals treated with CP, the number of lymphocytes decreased dramatically as compared with the control mice. Levamisole and JM extract 14.4 g/kg significantly restored the lymphocyte quantity in the spleen and thymus.



Control **CP** **Levamisole** **JM leaves extract**

Figure 4. ***Micro-histopathological images of spleens (HE × 100)***



Control **CP** **Levamisole** **JM leaves extract**

Figure 5. ***Micro-histopathological images of thymuses (HE × 100)***

IV. DISCUSSION

Cyclophosphamide is one of the most commonly used alkylating agents which produces toxic side effects including immunotoxicity, hematotoxicity and mutagenicity. It has a pronounced effect on lymphocytes and is usually used as an immunosuppressant [4]. In this study, CP at the dose of 200 mg/kg i.p caused a significant decrease in some parameters including: delayed type hypersensitivity (DTH) response; leukocyte quantity; relative organ weight; serum antibody, IL-2 and TNF-α concentrations.

In the DTH test, the DTH response that directly correlates with the cell mediated immunity was found to be considerable in animals treated with levamisole and JM extract. After being challenged by an antigen, the sensitized T-lymphocytes were converted to lymphoblasts secreting a variety of molecules including pro-inflammatory cytokines, thereby attracting scavenger cells to the site of reaction [2]. An increased footpad thickness indicated the stimulatory effect of JM leaves extract on the lymphocytes and accessory cell types required for the expression of this reaction.

Cytokines secreted by a range of cells, including lymphocytes, have an important role in the inflammatory response of the body, especially IL-2 and TNF-α. IL-2 has a wide spectrum of immunostimulatory activities, such as inducing the differentiation of lymphocytes, promoting the function of NK cells, and releasing interferons [3]. In this study, there was no significant improvement in cytokine concentrations in the JM extract group compared with CP group.

Antibody production induced by thymus dependent antigen (sheep red blood cells) requires a co-operation of T- and B-lymphocytes and macrophages. The high value of the IgM titer in mice treated JM extract demonstrated the immunostimulation of JM extract through the humoral immunity.

JM extract at dose of 14.4 g/ kg increased the weight of lymphoid organs (spleen and thymus). The increase in thymus and spleen weight was accompanied by the increase in its cell numbers, improving the stimulatory effect of this extract on the lymphocytes and bone marrow haematopoietic cells. This result was consistent with the histopathological changes in thymus and spleen in experimental groups (Figure 4 and 5).

Bone marrow is a site of continued proliferation and turnover of blood cells, and is a source of cells involved in immune reactivity. T-lymphocytes and other key cells of immune system are known to activate the production of antibody and polymorphonuclear granulocytes to destroy the antigen. Loss of stem cells and inability of bone marrow to regenerate new blood cells will result in leucopenia. In this study, JM extract improved leucocyte quantity as compared with the CP group, suggesting the improvement of JM leaves extract on the bone marrow activity suppression induced by CP.

These results indicated that JM extract potentiates the humoral as well as the cellular immunity. Currently, there are not many studies on evaluating the immune stimulation of plants in the world. Protocatechuic acid (PCA) has been isolated from the leaves of *Jasminum subtriplinerve* and has numerous pharmacologic effects, including anti-inflammatory and antitumoral activities [7]. Following the sudy of Solomon E Owumi (2015), PCA suppressed MTX-mediated increases in interleukin-1β, tumour necrosis factor alpha and caspase-3 activity in treated animals [12].

V. CONCLUSION

Jasminum subtriplinerve Blume. had stimulatory effects on the immune systems suppressed by CP. Oral administration of *Jasminum subtriplinerve* Blume. leaves extract at dose of 14.4 g/kg for 7 consecutive days ameliorated the effects of CP on delayed type hypersensitivity (DTH) response, leukocyte quantities, relative organ

weight, IgM concentration and micro-histological images.

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