ORIGINAL ARTICLE

Immunomodulatory Activity of *Ipomoea sepiaria* Root Extract in Cyclophosphamide-Induced Immunosuppressed Swiss Mice

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Abstract

Aim: The present study aimed to evaluate the immunomodulatory activity of *Ipomoea sepiaria* root extract (ISE) in cyclophosphamide-induced immunosuppressed Swiss mice. **Materials and Methods:** Swiss albino mice were randomly divided into five groups, including normal control, model control, thymomodulin-treated group, low-dose ISE group, and high-dose ISE group. Immunosuppression was induced by intraperitoneal administration of cyclophosphamide (200 mg/kg). Cell-mediated immunity was assessed using delayed-type hypersensitivity (DTH) response to ovalbumin, while humoral immunity was evaluated by measuring serum immunoglobulin G (IgG) levels. Hematological parameters, such as total leukocyte count and differential leukocyte count were analyzed. Cytokine levels, including interleukin-2 and tumor necrosis factor-alpha were quantified using enzyme-linked immunosorbent assay. **Results and Discussion:** Treatment with ISE significantly ameliorated cyclophosphamide-induced immunosuppression. The extract restored DTH response, improved leukocyte counts, and significantly increased cytokine levels and IgG concentrations compared with the model control group, indicating enhancement of both cellular and humoral immune responses. **Conclusion:** The findings suggest that ISE possesses significant immunostimulatory activity and may serve as a promising natural immunomodulatory agent for improving immune responses under immunosuppressed conditions.

Key words: Cyclophosphamide, cytokines, delayed-type hypersensitivity, immunomodulatory activity, swiss mice

INTRODUCTION

The immune system plays a crucial role in protecting the body against infectious agents and maintaining homeostasis.[1] Immunosuppression, whether caused by disease conditions or chemotherapeutic agents, leads to increased vulnerability to infections and reduced quality of life.[2,3] Cyclophosphamide (CP), a widely used alkylating agent in cancer chemotherapy, is known to induce severe immunosuppression by affecting both humoral and cell-mediated immune responses.[9] Although synthetic immunostimulants are available, their clinical use is often limited by adverse effects and high cost.[6] Therefore, there is an increasing interest in identifying safe, effective, and affordable immunomodulatory agents derived from natural sources.[10,11]

Ipomoea sepiaria root extract (ISE) has been traditionally used for various medicinal purposes, and preliminary studies suggest its potential immunomodulatory properties. However, systematic scientific evaluation of its immunostimulatory effects remains limited. The present study was designed to investigate the immunomodulatory activity of ISE against cyclophosphamide-induced immunosuppression in Swiss mice using standard immunological parameters.

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MATERIALS AND METHODS

Experimental animals

Healthy *Swiss* mice of both sexes, weighing 25 ± 5 g, were obtained from the National Institute of Nutrition, Hyderabad. Animals were housed in cages (10 animals/cage) under standardized laboratory conditions: Temperature 25 ± 2°C, relative humidity 60 ± 10%, and a 12:12 h light/dark cycle. Mice had *ad libitum* access to standard laboratory and sterile water. Before experimental procedures, all animals underwent a 7-day acclimatization period.

Drugs, chemicals, and reagents

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 Co., Ltd., South Korea), used as the positive control for immunostimulatory activity. Ovalbumin (OA) emulsion: Complete Freund's adjuvant containing OA and aluminum 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 OA 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.

Plant materials and extract preparation

Ipomea sepiaria fresh roots were collected from a wild source in Thirussur, Kerala. Plant identification and authentication were performed by Dr. Josh Mathew, Assistant Professor cum Botanist, Sanatana Dharma College, Thrissur. Kerala. Voucher ID PACC/2024/213 was preserved for the voucher specimen in the herbarium for future identification, fresh leaves were dried in a shed and grounded with an electric grinder. After being sieved using sieve No. 60, the powder was kept in an airtight container until extraction and further usage.

Preparation of the crude extracts

The extraction procedure was carried out using the following method, The 100 g of the powdered roots were weighed on a physical scale before being macerated with 70% ethanol in an Erlenmeyer flask. The powder was completely macerated for 7 days while being sometimes shaken on a rotary shaker (REMI SA-500; India). The extracted material was filtered through a sieve mesh and normal filter paper. An extract filtrate was concentrated in a rotary evaporator (Buchi model R-200, Switzerland) at a temperature of 40°C and 40 revolutions per minute (rpm).

Experimental design

All methods were carried out following International Council for Harmonisation guidelines and the Committee for Control and Supervision of Experiments on Animals guidelines for the care and use of laboratory animals. This work was approved by the Institutional Animal Ethics Committee (IAEC) of Nirmala College of Pharmacy, Guntur, with the project proposal number of 28/PHD/IAEC/NCPA.

Thirty six *Swiss* mice were randomly allocated into five experimental groups (n = 6 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 ISE): Received ISE (14.4 mL/kg/day, p.o.); Group 5 (High-dose ISE): Received ISE (28.8 mL/kg/day, p.o.).

The selected doses were based on the ISE safety profile from previous toxicity studies, the recommended dosage for the herb, and traditional medicinal usage.[20] The experimental protocol spanned 8 days with the following timeline [Table 1].

Immunological parameters evaluated

Delayed-type hypersensitivity (DTH) response

Cell-mediated immunity was assessed through the DTH response to OA antigen. Footpad thickness was measured using digital calipers immediately before challenge injection (day 7) and 24 h post-challenge (day 8). DTH response was calculated as:

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

Table 1: 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)
Day 7	Challenge injection with OA antigen (50 µL into the right hind footpad) and saline control (50 µL into the left hind footpad)
Day 8	Animal sacrifice and sample collection

SRBC: Sheep red blood cells, OA: Ovalbumin

Where, measurements represent the difference between antigen-injected and saline-injected foot pads.

Leukocyte counts

Blood samples collected through cardiac puncture were analyzed using an automated hematology analyzer (Exigo®-VET, Boule Medical AB, Sweden) to determine the number of total white blood cells, lymphocytes, neutrophils, and monocytes.[22]

Leukocyte counts were analyzed using standard hematological procedures.[12]

Cytokine and immunoglobulin (Ig) quantification

Serum samples were separated by centrifugation (3000 rpm, 10 min, 4°C) and stored at −80°C until analysis. Concentrations of interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), and IgG were determined using commercial enzyme-linked immunosorbent assay kits (Cloud-Clone Corp, USA) according to the manufacturer's protocols. Optical density was measured using a microplate reader (BioTek® ELx808, USA).[23]

Cytokine and immunoglobulin quantification using ELISA methods was performed as per established protocols.[13]

Statistical analysis

Data were entered and analyzed using Statistical Package for the Social Sciences Version 26.0 software (IBM Corp, New York, USA). Results are expressed as observed mean ± standard deviation. The one-way analyses of variance were applied. A $P \leq 0.05$ was considered statistically significant.

RESULTS

General observations throughout the study

Throughout the 8-day experimental period, all 36 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 behavioral patterns. All animals maintained normal activity levels and feeding behavior until the time of sacrifice on day 8.

Effects of ISE on DTH response

Cell-mediated immune function, assessed through DTH response to OA 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 model group), indicating effective immune-stimulation. Both ISE treatment groups showed protective effects, maintaining DTH responses comparable to normal controls with no significant difference from normal controls ($P > 0.05$). While both ISE groups showed numerical improvements compared to the model group ($P > 0.05$) [Table 2].

Effects of ISE on leukocyte count

Cyclophosphamide (CY) treatment induced severe leukopenia across all CYP-treated groups. Total white blood cell counts were dramatically reduced compared to normal controls ($P < 0.001$). Lymphocyte counts followed similar patterns, with all CYP-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 count was particularly affected, with significant decreases in the model group and both ISE-treated groups compared to normal controls ($P < 0.01$) [Table 3].

DISCUSSION

Immunocompromised conditions, such as those induced by chronic infections or autoimmune diseases, significantly impair immune function, increasing susceptibility to infections and complicating disease management.[21] Given the limitations of synthetic immunostimulants, including high costs and adverse effects, there is an imperative need for safe, affordable, and effective alternatives.[22] This study evaluates the immunostimulatory potential of a ISE in a cyclophosphamide-induced immunosuppression model in

Table 2: Effects of ISE 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	9.62±2.01	4.82±0.99	2.92±1.13
II	Model	6.37±2.18**	2.68±0.71***	0.96±0.34***
III	Thymomodulin	9.14±3.00$^{\Delta}$	3.32±0.65**	1.46±0.49**
IV	Low-dose ISE	7.33±2.42	2.84±0.82***	1.48±0.65**
V	High-dose ISE	8.12±2.68	2.84±0.94***	1.12±0.80***

ISE: *Ipomoea sepiaria* root extract, DTH: Delayed-type hypersensitivity. **, ***: $P<0.01$, $P<0.001$ significantly different from the normal control; Δ: $P<0.05$, significantly different from the model group

Table 3: Effects of ISE 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	3.72±0.92	2.89±0.82	0.22±0.12	0.62±0.25
II	Model	1.44±0.42***	1.08±0.32***	0.20±0.08	0.14±0.12**
III	Thymomodulin	1.72±0.52***	1.10±0.34***	0.22±0.07	0.42±0.24
IV	Low-dose ISE	1.52±0.42***	1.16±0.42***	0.18±0.07	0.16±0.06**
V	High-dose ISE	1.34±0.54***	0.96±0.37***	0.15±0.07	0.22±0.14**

ISE: *Ipomoea sepiaria* root extract, SD: Standard deviation, WBC: Total white blood cell, LYM: Lymphocyte, NEU: Neutrophil, MONO: Monocyte. **,***: $P<0.01$, $P<0.001$ significantly different from the normal control

Swiss mice, aiming to address this gap by investigating a plant-based, promising phytochemical constituents. The selection of CYP as an immunosuppressive agent in this study is based on well-established pharmacological principles and extensive literature supporting its use in immunological research.[26,27] Cyclophosphamide (CYP), a cytotoxic alkylating agent and established prodrug, requires metabolic activation by hepatic cytochrome P450 enzymes to produce active metabolites, including phosphoramide mustard and acrolein.[28] Phosphoramide mustard forms covalent bonds with guanine bases in DNA, creating guanine-guanine crosslinks that inhibit DNA replication and transcription, while acrolein exerts cytotoxicity through free radical generation.[29] The resulting cytotoxic effects preferentially target rapidly proliferating cells, including bone marrow-derived hematopoietic cells, leading to profound immunosuppression affecting both humoral and cell-mediated immune responses.[30,31] This mechanism of action makes CYP an ideal agent for creating reproducible immunodeficiency models that closely mimic clinical immunocompromised states.[32,33]

Cyclophosphamide (CP), a cytotoxic alkylating agent, requires metabolic activation to produce active metabolites responsible for immunosuppression.[9,26] These metabolites interfere with DNA replication and target rapidly dividing immune cells, leading to suppression of both humoral and cell-mediated immunity.[27,28] This makes CYP a well-established agent for inducing experimental immunosuppression.[29]

Thymomodulin is used as a positive control due to its ability to enhance T-lymphocyte maturation and immune responses.[17] It improves both cellular and humoral immunity, validating experimental models.[18]

Cell-mediated immunity was assessed using delayed-type hypersensitivity (DTH), a standard method for evaluating T-cell function.[36] Ovalbumin acts as a thymus-dependent antigen requiring antigen processing and T-cell activation.[37,38]

Humoral immunity involves B-cell activation and antibody production, primarily immunoglobulins.[36] Measurement of IgG levels provides an indication of immune responsiveness.[39,40]

Previous studies report that plant-based immunomodulators enhance cytokine production and macrophage activity.[14,15] These effects are attributed to phytochemicals such as flavonoids and polyphenols.[16]

Thymomodulin serves as an appropriate positive control in this study due to its well-characterized dual action on humoral and cell-mediated immunity, with particular emphasis on T-cell-mediated responses.[33] Thymomodulin has been demonstrated to enhance T-lymphocyte maturation in both *in vitro* and *in vivo* experimental systems, while simultaneously increase the functional capabilities of mature T-lymphocytes.[34] This enhancement creates cascading effects on B-cell activation and macrophage function, ultimately amplifying overall immune responsiveness.[35] The observed improvements in this study parameters following thymomodulin treatment validate the effectiveness of our experimental model and provide a benchmark for evaluating ISE efficacy.

Cell-mediated immunity represents a specialized adaptive immune mechanism mediated by T lymphocytes that directly eliminates intracellular pathogens and abnormal cells. To assess T-cell-mediated immunity in addition to humoral responses, this study evaluated the DTH response to OA, a well-established method for measuring T-cell function, to determine the immunomodulatory potential of ISE.[36] OA, a complex thymus-dependent globular protein, requires processing by antigen-presenting cells before T lymphocyte recognition and activation.[37] This process involves both direct T-cell cytotoxic responses and helper T-cell-mediated assistance to B lymphocytes for optimal antibody production, representing a classic type IV delayed hypersensitivity reaction.[38]

The humoral immune response, mediated primarily by B lymphocytes, represents a fundamental component of adaptive immunity. Upon antigen recognition, B cells undergo clonal expansion and differentiate into specialized plasma cells that synthesize and secrete antigen-specific antibodies or Ig.[36] These soluble antibodies facilitate antigen recognition, precipitation, agglutination, and complement system activation, thereby eliminating pathogenic threats through multiple effector mechanisms.[39] In our experimental model, SRBC immunization stimulates splenic B lymphocytes to produce detectable IgM and IgG antibodies, with IgM appearing initially, followed by

class-switched IgG production, commonly detectable from day 7 post-immunization.[40] The measurement of serum IgG levels provides a quantitative assessment of humoral immune function and B-cell responsiveness to antigenic stimulation. In this study, IgG levels in the ISE-treated groups showed no significant increase compared to the model group, likely due to the severe depletion of B lymphocytes caused by cyclophosphamide's cytotoxic effects. However, this interpretation is theoretical; to elucidate the underlying mechanisms of ISE's immunomodulatory action, further studies employing advanced techniques, such as flow cytometry, additional cytokine profiling, or immunohistochemical analysis of B-cell-specific markers (e.g., CD19, CD20) are recommended to assess B lymphocyte function and distinction comprehensively.

The study findings are consistent with the scientific rationale and phytochemical basis of the components of ISE. Present literature provides substantial evidence supporting the immunomodulatory effects of ISE through multiple experimental approaches, including both *in vitro* and *in vivo* studies. At the cellular level, ISE enhances macrophage pinocytosis and stimulates production of nitric oxide, IL-6, and TNF-α, indicating broad-spectrum immune activation.[41,42] The immunomodulatory effects are attributed to diverse phytochemical constituents, including various polyphenolic compounds, Flavonoids, and alkaloids, which collectively contribute to enhanced immune function through multiple mechanistic pathways.[43] Recent research on these compounds highlights ISE potential for antioxidant activity, cytotoxicity against cancer cell lines, and antibacterial properties.[44,45] The findings of this study may suggest optimal bioactive compound ratios achieved through maceration.

The results of our study, combined with existing literature on individual herbal components, suggest significant therapeutic potential for the ISE in clinical immunodeficiency conditions. The demonstrated ability to restore immune parameters in cyclophosphamide-induced immunosuppression indicates potential applications in asthma patients, individuals with drug-induced immunosuppression, and patients with acquired immunodeficiency states. While our findings are promising, several limitations should be acknowledged. In addition, the precise mechanisms underlying must require further investigation on examining specific cellular and molecular pathways. Future research should focus on dose-optimization studies, long-term safety evaluations, and clinical trials in human populations to establish therapeutic efficacy and safety profiles. Investigation of specific bioactive compounds responsible for immunomodulatory effects and their pharmacokinetic properties would also contribute valuable information for standardization and quality control purposes.

CONCLUSION

The ISE significantly enhanced immune function in cyclophosphamide-induced immunosuppressed *Swiss* mice, as evidenced by improved DTH responses. These results highlight ISE's potential as a safe and effective plant-based immunostimulant for managing immunosuppression. Further studies are warranted to elucidate the underlying mechanisms, optimize dosing, and evaluate long-term safety and efficacy in clinical settings.

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ETHICAL APPROVAL

All animals were cared for in strict accordance with the Guide for the Care and Use of Laboratory Animals, the guidelines prescribed by the Committee (CCSEA), India. The experimental design was approved by the Institutional Animal Ethics Committee (IAEC) (Reference number: 28/PHD/IAEC/NCPA/2024), under ethics code No. 1629/IAEC/NRML May 29, 2025.

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