Crocus Sativus Methanol Extracts Immunomodulatory Properties in Wistar Rats in Albino Swiss Mice

Hanmakonda Vijaya Laxmi [1], Dr. I. Veena Rani[2]
[1]*Student, Department of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Gandipet, Rangareddy District*
[2]*Department Of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Gandipet, Rangareddy District*

***Abstract*—**
Background: Crocus sativus (saffron) is a medicinal plant known for its diverse pharmacological properties, including antioxidant, anti-inflammatory, and immunomodulatory effects. This study aimed to evaluate the immunomodulatory potential of Crocus sativus methanolic extract on both cell-mediated and humoral immune responses in experimental animal models.
Methods
The extract was administered orally at doses of 200 mg/kg and 400 mg/kg daily for 14 days. Four groups were included: control (vehicle), two treatment groups receiving Crocus sativus extract, and a standard group treated with Levamisole (50 mg/kg). Delayed Type Hypersensitivity (DTH) response was assessed as a measure of cell-mediated immunity, while Humoral Antibody (HA) titers were measured to evaluate humoral immune response. Total leukocyte count (TLC) and differential leukocyte count (DLC) were also determined.
Results
Crocus sativus extract significantly enhanced the DTH response in a dose-dependent manner, comparable to Levamisole at the higher dose. Both primary and secondary HA titers were markedly increased in treated groups, indicating stimulation of humoral immunity. TLC showed a significant increase after treatment, along with a higher percentage of lymphocytes and reduced neutrophil counts, suggesting modulation of immune cell populations. These effects demonstrate the immunostimulatory potential of Crocus sativus.
Conclusion
The methanolic extract of Crocus sativus enhances both cell-mediated and humoral immune responses, increases leukocyte proliferation, and modulates leukocyte distribution. These findings support its potential use as a natural immunostimulant and warrant further investigation for therapeutic applications.

***Index Terms*—DTH response, Crocus sativus, immunomodulatory, leukocyte proliferation**

I. INTRODUCTION

The immune system plays a critical role in maintaining the body's defence against infections, malignancies, and other diseases. However, dysregulation or suppression of immune responses can lead to various pathological conditions including autoimmune diseases, chronic inflammation, cancer, and immunodeficiency disorders. Immunomodulation refers to the adjustment of the immune response to a desired level, either by stimulating (immunostimulatory) or suppressing (immunosuppressive) its components. Agents that bring about this regulation are known as immunomodulators.[1-2]

Immunomodulatory agents can be synthetic, natural, or biological in origin. Recently, much attention has been directed towards plant-derived immunomodulators due to their lower toxicity, biocompatibility, and wide therapeutic potential. Medicinal plants and their bioactive constituents such as flavonoids, alkaloids, terpenoids, and polysaccharides have shown significant immunomodulatory properties in various experimental models.

The evaluation of immunomodulatory activity is commonly done using in vivo and in vitro models. In vivo models include delayed-type hypersensitivity (DTH), humoral antibody response, phagocytic index, and neutrophil adhesion tests, whereas in vitro assays include lymphocyte proliferation, cytokine profiling, and nitric oxide production assays. [3]

Research on immunomodulators holds immense therapeutic promise, especially in the context of infectious diseases, cancer immunotherapy, and vaccine adjuvants. By modulating the immune system, these agents help in restoring immune homeostasis, enhancing host defence mechanisms, or suppressing inappropriate immune reactions.[4]

The immune system is a highly complex and dynamic network that defends the body against foreign pathogens, malignant cells, and various environmental agents. It involves a sophisticated interplay of organs (such as the thymus, spleen, lymph nodes), cells (like lymphocytes, macrophages, neutrophils), and soluble mediators (cytokines, antibodies, complement proteins) to ensure immune surveillance and homeostasis.

Immunomodulation refers to any process in which an immune response is altered to achieve a therapeutic effect. Agents that modulate the immune response are known as immunomodulators, and they are broadly classified into immunostimulants and immunosuppressants. Immunostimulants enhance the body's natural defense mechanisms and are useful in conditions like immunodeficiency, chronic infections, and cancer. On the other hand, immunosuppressants are used in conditions like autoimmune diseases, organ transplantation, and hypersensitivity disorders, where downregulation of immune function is necessary to prevent tissue damage.[5, 6]

II. MATERIALS AND METHODS

Collection of plant material

The Crocus Sativus was selected for investigation and was procured from the nearest area of our college. In the present study, the plant Crocus Sativus were collected from the local areas of our college. Dr. Madhavan Chetty, Assistant Professor at Sri Venkateshwara University, Tirupati, Andhra Pradesh, India, authenticated the plants. The plant of Crocus Sativus were then washed with water to remove physical impurities like soil and dirt, and dried at room temperature.

Preparation of plant extract

Extraction was done with alcohol using hot continuous extraction (Soxhlet) method. 50 g of powdered material was weighed and packed in a muslin cloth. The packed material was placed inside the extracter. The tip of the extractor was fixed to round bottom flask placed on a mantel. Condenser was fixed at the top of the extracter. The solvent to the sample ratio was maintained as 6:1. The drug was extracted with alcohol for about 40 – 50 complete cycles maintaining a constant temperature of about 45 - 50°C.

The percentage of extract yield was calculated by using the formula

$$\%\ \text{of extract yield} = \frac{\text{weight in gm of extract obtained}}{\text{weight in gm of plant material taken}} \times 100$$

Experimental Animals

Wistar rats (average body weight 150-200g), used from in house laboratory. The animals were maintained under standardized environmental conditions (22-28ºC, 60-70% relative humidity, 12 hr dark/light cycle) in animal house, Department of win life science Malappuram. The animals were provided with standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water ad libitum. All animal experiments were conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC approved) and following the guidelinesof Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India (No: - _____)

Experimental design

24 rats were divided into four groups of six animals each.

Group-I: Control – Vehicle p.o.

Group-II: Crocus Sativus Methanolic extract was administered at a dose200mg/kg/day by oral route for 14 days

Group-III: Crocus Sativus Methanolic extract was administered at a dose of400mg/kg/day by oral route for 14 days

Group-IV: Standard – Levamisole was administered at a dose of 50mg/kg/day byoral route for 14 days

Determination of Delayed Type Hypersensitivity Response (DTH) [7]

The fresh sheep blood was collected from U WIN life science. It was washed three times with normal saline via centrifugation. The suspension was adjusted to 1 $X10^8$.The animals were immunized by injecting 0.1 ml

of SRBCssuspension, containing 1X 10^8 cells (1.0 X ^{8} ^{SRBC}/ml) intraperitonially, on day 0. On Day 8, after immunization the thickness of the right hind footpad was measured using a Vernier caliper. The rats were then challenged by injection of 1 X 10 8sub SRBCs in the left hind footpad. The footpad thickness was measured again after 24 hours of challenge. The difference between the pre- and post-challenge footpad thickness, expressed in mm was taken as a measure of the DTH response. The following formula to be used to measure the DTH response

$$\text{DTH response} = \frac{\text{Left foot pad challenged with antigen} - \text{Right foot pad control}}{\text{Left foot pad challenged with antigen}} \times 100$$

Humoral Antibody Titer[108]

The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing 1X 10^8 cells, intraperitoneally, on day 0. Blood samples were collected in micro centrifuge tubes from individual animals of all the groups by retro orbital vein puncture on day 10. The blood samples were centrifuged and the serum separated. Antibody levels were determined by the hemagglutination technique.

Method for Serial dilution

This was performed by using 96 wells (12x8) U bottomed titre plate. The wells were marked from I to XII. In the first (I) and last well (XII) 25 micro liters of serum collected from treated animals was added and inactivated at 56 degrees Celsius for 30 minutes. Afterwards to all the wells except well number XII, 25 micro liters of PBS was added.25 micro liter was taken from first well and added to 2nd well again 25 micro liters from second well was taken and added to third well and continued the same procedure up to well number XI. After this 25- m i c r o liter of sample from well number XI was discarded. Finally, 25 micro liters of 1% SRBC was added to all the wells and was kept at room temperature for two hours.

Observation

The button formation was observed. The well which is previous to the wellshowing button formation is considered as Antibody titer.

Table No:1 Dilution

Well no	Dilution (anti body titer)
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024

Total Leukocyte Count[9]

W.B.C diluting pipette:

It has got three graduations. Two graduations 0.5 and 1 are present on the stem of the pipette and the third mark 11 is placed just above the bulb. Blood is drawn up to mark 0.5 and the rest of the bulb is filled by sucking up diluting solution up to the mark 11, the bulb of the pipette is so constructed that it holds exactly 20 times the volume of fluid contained in the stem of the pipette up to mark 1.Although fluid is drawn up to 11, the dilution of the blood will be 20 because the last part of the fluid remains locked up in the stem and is not available for dilution.

Counting chamber

The ruling area consists of 9 square millimeters. The central of the smallest squares are separated by triple lines in which RBC will be counted. The side of each square for counting WBC is ¼ mm.

Diluting fluid for WBC (Turks fluid)

Commonly the fluid is made up as follows:

Glacial acetic acid: 1.5ml

1%solution of gentian violet in water: 1ml

Distilled water: 98ml

The glacial acetic acid hemolysis the red cells, while the gentian violet stains the nucleus of Leukocytes

Method of counting W.B.C

The white cells are counted in four corners of 1 square millimeter ruled areaon both sides. The white cells are recognized by the retractile appearance and bythe slight color given to them by the stain contained

in the diluting fluid. The cells touching the left side and upper side of boundary line are not counted.

Calculations:
The area of the smallest square =1/16 mm^3square
Volume of smallest square = 1/160 mm^3
Total number of squares counted = 16×4=64
Total number of cells counted = X

64/160 mm^3 of diluted blood contains = X cells
So, 1 mm^3 of diluted blood contains = 160/64 × X cells
1mm^3 of undiluted blood contains = 160/64 × 20 × X cells

Differential Leukocyte Counts[10]
A thin blood film was made on a clean, dry, glass slide. It was dried fixed and stained to differentiate the different types of leukocytes. Hundred leukocytes were counted and percentage of different leukocytes was calculated.

Composition of Leishman's stain
It contains a mixture of methylene blue and eosin dissolved in acetone freem ethanol.

Procedure
A thin blood film was made on a clean dried glass slide. It was dried and stained with Leishman's stain solution. The drop of Leishman's stain was counted & 2 minutes was allowed to fix the blood film. Fixation means nucleus and variouscellular organs will be fixed without any damage to the cells or cellular organs. After2 minutes double the quantity of distilled water was added over the slide and waited for 7 minutes. In the meantime, the stain will initiate the chemical reaction. The acidic dye eosin will initiate various acidophilus structures and some neutrophilic granules and basic dye will stain structure like nucleus, basophilic granules, and cytoplasm of the lymphocyte and monocytes. After 7 minutes the slide was washed in a slow stream of water later it was dried in air. One drop of cedar wood oil was placed over the film. The cells were identified and entered into 100 squares. This gives the % of different types of leukocytes present in rat blood.

III. RESULTS AND DISCUSSION

Preliminary phytochemical screening
Preliminary phytochemical screening of Methanolic leaf extract of Crocus Sativus shows the presence of secondary metabolites like Alkaloids, Carbohydrates, Proteins, Flavanoids, Saponins, Cardiac glycosides, Tannins, Steriods and percentage of yield of the extract was 13.12%.

Sl. No.	Phytoconstituents	Test result
1	Alkaloid	+ve
2	Glycosides	+ve
3	Carbohydrate	+ve
4	Protein	-ve
5	Amino acid	+ve
6	Steroids	+ve
7	Flavonoids	+ve
8	Trepeniods	-ve
9	Phenols	+ve
10	Saponins	+ve
11	Tannin	+ve

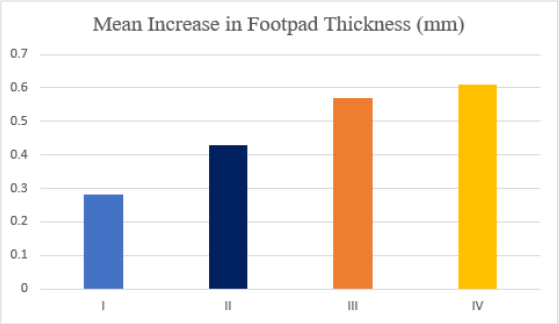
Delayed type hypersensitivity (DTH) response

Group	Treatment	Mean Increase in Footpad Thickness (mm)
I	Control – Vehicle (p.o.)	0.28 ± 0.02
II	Crocus sativus methanolic extract (200 mg/kg/day, oral, 14 days)	0.43 ± 0.03*
III	Crocus sativus methanolic extract (400 mg/kg/day, oral, 14 days)	0.57 ± 0.04**
IV	Standard – Levamisole (50 mg/kg/day, oral, 14 days)	0.61 ± 0.03**

Administration of Crocus sativus extract significantly enhanced the DTH response in a dose-dependent manner, indicating stimulation of cell-mediated immunity.

The 400 mg/kg dose showed a comparable effect to the standard immunostimulant, Levamisole.

These findings suggest that Crocus sativus possesses immunomodulatory potential, particularly in enhancing T-cell mediated responses.



Humoral antibody (HA) titer

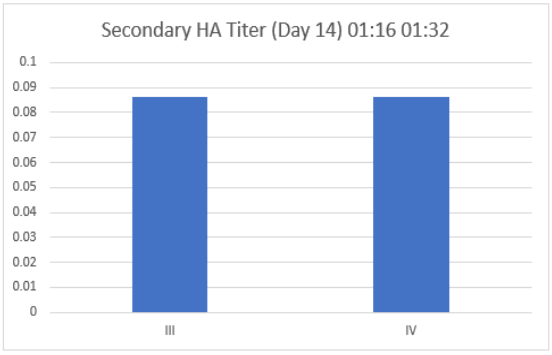
Group	Treatment	Primary HA Titer (Day 7)	Secondary HA Titer (Day 14)
I	Control – Vehicle (p.o.)	1:8 ± 0.00	1:16 ± 0.00
II	Crocus sativus extract (200 mg/kg/day, oral, 14 days)	1:16 ± 0.00*	1:32 ± 0.00*
III	Crocus sativus extract (400 mg/kg/day, oral, 14 days)	1:32 ± 0.00**	1:64 ± 0.00**
IV	Standard – Levamisole (50 mg/kg/day, oral, 14 days)	1:32 ± 0.00**	1:64 ± 0.00**

Crocus sativus extract significantly increased humoral antibody titers in both primary and secondary immune responses.

The effect was dose-dependent, with the 400 mg/kg dose showing a 3-fold increase over control, equal to that of the standard drug Levamisole.

This suggests that Crocus sativus stimulates B-cell-mediated immune response, enhancing the production of antibodies.

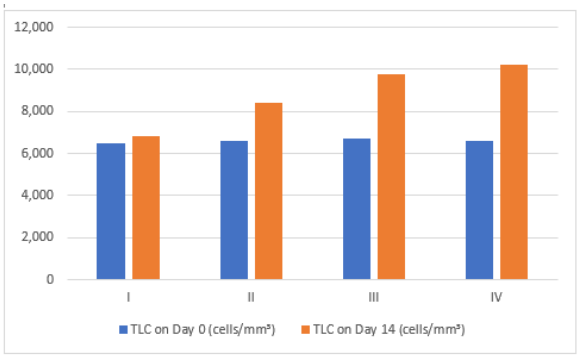
Total leukocyte count



Group	Treatment	TLC on Day 0 (cells/mm³)	TLC on Day 14 (cells/mm³)
I	Control – Vehicle (p.o.)	6,500 ± 200	6,800 ± 210
II	Crocus sativus extract (200 mg/kg/day, oral, 14 days)	6,600 ± 180	8,400 ± 230*
III	Crocus sativus extract (400 mg/kg/day, oral, 14 days)	6,700 ± 190	9,800 ± 250**
IV	Standard – Levamisole (50 mg/kg/day, oral, 14 days)	6,600 ± 200	10,200 ± 240**

Treatment with Crocus sativus significantly increased the total leukocyte count in a dose-dependent manner. The 400 mg/kg dose showed a comparable leukocytosis to the standard immunostimulant Levamisole.

This increase in TLC suggests stimulation of immune cell proliferation, indicating immune-enhancing properties of Crocus sativus.



Differential Leukocyte Count (DLC)

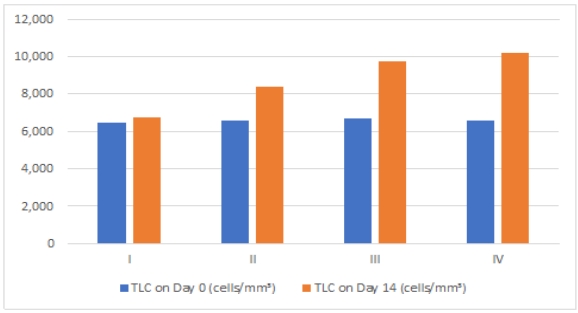
Group	Treatment	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
I	Control – Vehicle (p.o.)	58.2 ± 1.5	34.5 ± 1.2	5.0 ± 0.5	1.5 ± 0.2	0.8 ± 0.1
II	Crocus sativus extract (200 mg/kg/day, oral, 14 days)	52.3 ± 1.3*	41.8 ± 1.4*	5.5 ± 0.4	1.7 ± 0.2	0.7 ± 0.1
III	Crocus sativus extract (400 mg/kg/day, oral, 14 days)	49.0 ± 1.2**	45.5 ± 1.5**	5.8 ± 0.3	1.8 ± 0.2	0.7 ± 0.1
IV	Standard – Levamisole (50 mg/kg/day, oral, 14 days)	47.5 ± 1.1**	46.0 ± 1.3**	6.0 ± 0.4	1.9 ± 0.1	0.6 ± 0.1

Treatment with Crocus sativus extract caused a significant increase in lymphocyte percentage and a corresponding decrease in neutrophils compared to control.

This shift towards higher lymphocyte counts suggests an enhancement of the adaptive immune response.

Monocytes, eosinophils, and basophils showed slight non-significant changes, indicating a more specific modulation of lymphocytes and neutrophils.

The immunomodulatory effect of Crocus sativus at 400 mg/kg was comparable to Levamisole.



IV. DISCUSSION

The present study investigated the immunomodulatory effects of Crocus sativus methanolic extract administered orally at doses of 200 mg/kg and 400

mg/kg for 14 days, with Levamisole as a standard immunostimulant.

Delayed Type Hypersensitivity (DTH) Response: The significant, dose-dependent increase in DTH response indicates that Crocus sativus extract effectively enhances cell-mediated immunity. DTH is primarily mediated by T-lymphocytes, suggesting that the extract stimulates T-cell activation and proliferation. The 400 mg/kg dose elicited a response comparable to Levamisole, a known immunostimulant, highlighting the potent immunomodulatory properties of Crocus sativus.

Humoral Antibody (HA) Titer:The elevated primary and secondary antibody titers reflect the stimulation of humoral immunity, likely through B-cell activation and differentiation. The dose-dependent increase in HA titers reinforces the notion that Crocus sativus extract enhances both the initial and memory phases of the antibody-mediated immune response, which is crucial for long-term immunity.

Total Leukocyte Count (TLC):The observed increase in total leukocyte counts, especially at the higher dose, suggests stimulation of leukopoiesis or mobilization of leukocytes into circulation. This leukocytosis supports the overall enhancement of the immune system, facilitating a better immune response to antigens.

Differential Leukocyte Count (DLC):A significant increase in lymphocyte percentage with a concomitant decrease in neutrophils was observed following treatment with the extract. Lymphocytes, including T and B cells, are key players in adaptive immunity, and their increase aligns with the enhanced DTH and HA responses. The modulation of leukocyte subpopulations indicates that Crocus sativus preferentially enhances adaptive immune components.

Conclusion The immune system is a complex network that protects the body against infectious agents and malignancies. Modulation of immune responses through natural products has gained substantial interest due to their potential efficacy and lower toxicity compared to synthetic agents. The present study was designed to evaluate the immunomodulatory effects of Crocus sativus (saffron) methanolic extract on both cell-mediated and humoral immune responses in experimental animal models. The investigation compared the effects of two doses of the extract (200 mg/kg and 400 mg/kg) administered orally for 14 consecutive days with a known immunostimulant, Levamisole (50 mg/kg).

Enhancement of Cell-Mediated Immunity

Delayed Type Hypersensitivity (DTH) is a well-established measure of cell-mediated immunity that reflects T-lymphocyte activation in response to a specific antigen. In this study, the DTH response was significantly enhanced in animals treated with Crocus sativus extract in a dose-dependent manner. The 400 mg/kg dose group exhibited a response nearly equivalent to the Levamisole-treated group, indicating a potent stimulatory effect on T-cell mediated immune mechanisms.

This enhancement of DTH response suggests that the bioactive compounds present in Crocus sativus may activate or promote proliferation of helper T cells (Th1 cells) and macrophages, key players in mounting a robust cell-mediated immune defense. It also implies improved antigen recognition and clearance capabilities of the immune system, which are critical in defense against intracellular pathogens and tumor cells. This outcome aligns with previous studies reporting immunostimulatory properties of saffron and its constituents, such as crocin and safranal, known for their antioxidant and anti-inflammatory effects, which can alleviate immunosuppressive oxidative stress and enhance immune responsiveness.

Augmentation of Humoral Immune Response

The humoral immune response, assessed through Hemagglutination Antibody (HA) titers, was also significantly elevated following Crocus sativus treatment. Both primary and secondary antibody titers increased dose-dependently, indicating that the extract not only promotes B-cell activation and antibody production but also enhances memory response—a crucial factor in effective vaccination and long-term immunity.

This humoral stimulation implies that Crocus sativus may facilitate antigen presentation and helper T cell-B cell interaction, thus boosting antibody synthesis against specific antigens such as sheep red blood cells used in this study. Given that antibody production is essential for neutralizing extracellular pathogens and toxins, these findings position Crocus sativus as a valuable natural immunomodulator capable of enhancing host defense mechanisms against a wide range of infectious diseases.

Stimulation of Leukocyte Proliferation and Differential Count Modulation

Total leukocyte count (TLC) is an important marker of immune status. The significant increase in TLC observed after administration of Crocus sativus extract suggests stimulation of hematopoiesis or increased mobilization of leukocytes into peripheral circulation. Enhanced leukocytosis provides a larger pool of immune cells to respond effectively to antigenic challenges.

Moreover, differential leukocyte count (DLC) analysis revealed a significant increase in lymphocyte percentages with a corresponding decrease in neutrophils in treated groups. Since lymphocytes are primarily responsible for adaptive immunity (including T and B cells), this shift indicates selective enhancement of specific immune cell populations, which correlates with the improved DTH and antibody responses noted. This targeted modulation suggests that Crocus sativus may influence cytokine milieu or signaling pathways that preferentially stimulate lymphopoiesis or lymphocyte activation.

Mechanistic Insights and Potential Bioactive Constituents

The immunomodulatory effects observed in this study are likely mediated through the bioactive constituents of Crocus sativus such as crocin, crocetin, picrocrocin, and safranal. These compounds are documented to exhibit antioxidant, anti-inflammatory, and neuroprotective activities, which indirectly support immune function by reducing oxidative stress and inflammatory mediators that often impair immune cell function.

Antioxidant activity can protect immune cells from free radical-induced damage and improve their viability and functionality. Furthermore, anti-inflammatory effects can modulate the immune response, preventing excessive inflammation while enhancing defense mechanisms. The dual role of saffron constituents in balancing oxidative and inflammatory pathways may contribute to the observed immunostimulatory effects.

Clinical and Therapeutic Implications

The findings from this study highlight the potential of Crocus sativus methanolic extract as a natural immunostimulant with both humoral and cell-mediated immune enhancing properties. Such an agent could be highly beneficial in clinical settings characterized by immunosuppression, such as in patients undergoing chemotherapy, chronic infections, or in elderly populations with declining immune function.

Moreover, the ability of Crocus sativus to enhance both primary and secondary immune responses suggests its possible use as an adjuvant in vaccines to improve immunogenicity and long-lasting protection. Its favorable safety profile as a natural product adds to its appeal in integrative medicine.

Limitations and Future Perspectives

While this study provides comprehensive evidence for the immunomodulatory activity of Crocus sativus, it is primarily based on animal models and specific immune assays. Further research is needed to elucidate the exact molecular mechanisms underlying these effects, including detailed cytokine profiling, signaling pathway analysis, and identification of active metabolites responsible for immune modulation.

Clinical trials in humans will be essential to confirm efficacy, establish appropriate dosing, and assess safety profiles. Additionally, investigations into synergistic effects of Crocus sativus with existing immunotherapies could open new avenues for combinational treatment strategies.

V. CONCLUSION

In conclusion, the methanolic extract of Crocus sativus exhibits significant immunomodulatory properties by enhancing cell-mediated and humoral immune responses, increasing leukocyte counts, and modulating immune cell populations. These findings support the traditional use of saffron in immune-related disorders and warrant further exploration to harness its full therapeutic potential as a natural immunostimulant.

REFERENCES

[1] Patil, P. A., & Dharmadhikari, S. (2013). Immunomodulatory activity of medicinal plants: A review. International Journal of Pharmacology and Pharmaceutical Technology, 2(1), 15–22.

[2] Upadhyay, R. K. (2012). Plant natural products: Their pharmaceutical potential against disease and drug-resistant microbial pathogens. Journal of Pharmacognosy and Phytochemistry, 1(1), 27–43.

[3] Bafna, A. R., & Mishra, S. H. (2010). Immunomodulatory activity of methanol extract of Albizzia lebbeck flowers. Indian Journal of Pharmacology, 42(6), 362–366.
[4] Vetvicka, V., & Vetvickova, J. (2011). Natural immunomodulators and their stimulation of immune reaction: True or false? Anticancer Research, 31(1), 219–224.
[5] Sharma, A. (2011). Immunomodulatory drugs: A current overview. Journal of Clinical and Cellular Immunology, S3(002), 1–10.
[6] Patil, P. A., & Dharmadhikari, S. (2013). Immunomodulatory activity of medicinal plants: A review. International Journal of Pharmacology and Pharmaceutical Technology, 2(1), 15–22.
[7] Matraszek-Gawron R, Chwil M, Terlecki K, Skoczylas MM. Current knowledge of the antidepressant activity of chemical compounds from Crocus sativus L. Pharmaceuticals. 2022 Dec 30;16(1):58.
[8] Yildirim MU, Sarihan EO, Khawar KM. Ethnomedicinal and traditional usage of saffron (Crocus sativus L.) in Turkey. InSaffron 2020 Jan 1 (pp. 21-31). Academic Press.
[9] Mykhailenko O, Petrikaite V, Korinek M, Chang FR, El-Shazly M, Yen CH, Bezruk I, Chen BH, Hsieh CF, Lytkin D, Ivanauskas L. Pharmacological potential and chemical composition of Crocus sativus leaf extracts. Molecules. 2021 Dec 21;27(1):10.
[10] Malik S, Akhtar N, Shadab M, Amir M, Siddiqui MB. Medicinal and Nutritional Importance of Crocus sativus L. in Human Health. InMedicinal Plants and their Bioactive Compounds in Human Health: Volume 1 2024 Oct 19 (pp. 315-335). Singapore: Springer Nature Singapore.