Ind. J. Pharm. Edu. Res., 2026; 60(4):1606-1617.
https://www.ijper.org

Original Article

Effect of Pomiferin against Cyclophosphamide-Induced Mice by Immunomodulatory Effects and Involvement of the Inflammatory Cytokine Pathway

Abdulkarim S. Binshaya[1,*], Khalid Alshaghdali[2]

[1]Department of Medical Laboratory, College of Applied medical sciences, Prince Sattam bin Abdulaziz University, Alkharj, SAUDI ARABIA.
[2]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Hail, SAUDI ARABIA.

ABSTRACT

Background: Cyclophosphamide (Cy) is widely used in oncology and immunology for treating cancers and severe autoimmune diseases. However, its clinical utility is limited by adverse effects such as immunosuppression, hematological alterations, oxidative stress, inflammation, and body weight reduction. Therefore, identifying agents that can mitigate these toxicities is essential. **Objectives:** To evaluate the protective and immunomodulatory effects of Pomiferin against Cy-induced immunosuppression in male Swiss albino mice. **Materials and Methods:** Male Swiss albino mice were divided into four groups: normal control, Cy-treated, and two pomiferin-treated groups (5 mg/kg and 10 mg/kg). Cy was administered to induce immunosuppression. Parameters assessed included spleen and thymus indices, hematological markers (RBC, WBC, platelet counts), cytokine levels, immunoglobulins, oxidative stress markers, and antioxidant enzyme activities. **Results:** Pomiferin treatment significantly restored spleen and thymus indices and normalized hematological parameters. It reduced pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) while enhancing anti-inflammatory cytokine IL-12. Immunoglobulin levels (IgA, IgG, IgM) were markedly improved. Additionally, pomiferin decreased oxidative stress markers (MDA, NO) and enhanced antioxidant defences (CAT, SOD, GSH). **Conclusion:** Pomiferin demonstrates significant protective effects against Cy-induced immunosuppression by modulating immune responses and reducing oxidative stress. These findings suggest its potential as a therapeutic agent for improving immune tolerance and minimizing chemotherapy-induced toxicities.

Keywords: Antioxidant, Cyclophosphamide, Cytokines, Immunomodulatory, Immunosuppression, Pomiferin.

Correspondence:
Abdulkarim S. Binshaya
Department of Medical Laboratory, College of Applied medical sciences, Prince Sattam bin Abdulaziz University, Alkharj-11942, SAUDI ARABIA.
Email: abd.aldosari@psau.edu.sa

Received: 13-04-2026;
Revised: 08-05-2026;
Accepted: 28-07-2026.

INTRODUCTION

The immune system refers to a collection of various cells, chemicals, and processes that act as defense mechanisms to protect the skin, respiratory tract, intestinal tract, and other areas from foreign antigens, such as microbes, viruses, cancer cells, and toxins.[1] To disrupt homeostasis, pathogens encounter harsh environments within the host immune system.[2] Therefore, immunomodulation of immune response could provide a new approach to treating inflammatory and autoimmune diseases associated with immunodeficiency, with ongoing collaboration between clinics and basic research for a better understanding of the complexity among individual immune system components to identify potential new targets for more specific therapeutic interventions.[3] Our immune system is composed of an interrelated framework of lymphoid organs, cells, humoral factors, and cytokines, to achieve immunological surveillance, defense from outside threats, and regulatory functions, as well as to maintain homeostasis in the body, as a protective factor.[4]

Cy is a well-known chemotherapeutic agent for the treatment of numerous forms of cancers and autoimmune diseases due to its potent immunosuppressive ability.[5] Cy-induced immunosuppression raises the risk of infections by thwarting the development of the immune system.[6] Cy is a common cytostatic agent used in malignant lymphoma (WBC become cancerous), multiple myeloma (plasma cells become cancerous), breast cancer, small cell lung cancer, etc., as well as in non-malignant diseases, for example, rheumatoid arthritis and systemic erythema for the treatment of lupus. Cy is commonly used as a chemotherapeutic drug that leads to immunosuppression (bone marrow suppression), leucopenia, and oxidative stress.[7]

There are synthetic drugs used for immunomodulatory effects, but these drugs can harm patients and cause severe allergic reactions, liver damage, and kidney failure. They can also interact

DOI: 10.5530/ijper.20261291

with other medications, leading to serious side effects. Therefore, promising drugs with minimal side effects should be investigated for immunomodulatory effects. The immunomodulatory effects of various natural compounds and plant extracts have been meticulously examined in Cy-treated mice.[8] These agents have demonstrated favourable outcomes to achieve homeostasis by restoring the balance of immune cells and cytokines, thereby optimizing the overall immune function and reducing the side effects of Cy treatment.

Pomiferin is a prenylated isoflavone, a chemical obtained from ethyl acetate extract from fruits of the plant named *Maclura pomifera*, which is a member of the Moraceae family.[9] Pomiferin has been demonstrated to have selective cytotoxicity towards cancer cells.[10] Pomiferin possesses potent antioxidant properties, enabling it to be an effective free radical scavenger. Pomiferin has antioxidant and anti-inflammatory effects[11] and is a potential natural substance for circumventing multi-drug resistance cancers. Such immunomodulating agents act as immunostimulants to enhance the ability to fight against immunosuppressants like Cy to prevent the damage. However, immunomodulators act as either stimulants or suppressants of the immune response.

Regardless of pomiferin's therapeutic action, its effects on Cy-induced immunosuppression have not been thoroughly studied. This study aims to fill this research area by analyzing pomiferin's immunomodulatory and antioxidant effects in a Cy-induced immunosuppressed mouse model. Specifically in the mitigation of the immunosuppressive effects of Cy by restoring immune organ function, enhancing hematopoiesis, modulating cytokine profiles, and boosting antioxidant defenses. However, this investigation suggests new insights into the therapeutic use of pomiferin as a complementary treatment to alleviate the side effects of Cy, thereby improving the possible outcomes for patients undergoing chemotherapy. This design was intentionally selected to mimic a clinically relevant scenario, where treatment is given after the onset of drug-induced immunotoxicity rather than as a preventive measure. Therefore, the observed effects of pomiferin reflect its therapeutic potential in reversing Cy-induced immune and inflammatory disturbances.

MATERIALS AND METHODOS

Chemicals

Cy and pomiferin (≥98%) were procured from MSW Pharma, India. TNF-α (MBS9501927), IL-1β (MBS824958), IL-4 (MBS2504956), IL-6 (MBS730957), and immunoglobulin G (IgG-MBS9135944), IgA (MBS2018930), and IgM (MBS9135874) were quantified using an ELISA kit obtained from MyBioSource, Inc., USA.

Animals

The male Swiss albino mice (20-25 g) were randomly separated into four groups; each kept individually in polypropylene cages. The temperature in the animal facility was maintained at 22±0.5°C. The food and water were provided *ad libitum* to the mice during the entire experiment. The whole process was carried out as per the protocol approved by the Institutional Animal Ethics Committee (IAEC) and the ARRIVE animal research standard.

Acute Toxicity Study

The acute oral toxicity (LD_{50}) of pomiferin was assessed in accordance with OECD Test 423.[12] The compound was administered to rats via oral gavage at doses of 5 and 10 mg/kg body weight, following standard protocols for toxicity studies.[13] The pharmacokinetic properties and ADMET profile of pomiferin were subsequently predicted using the pkCSM computational framework.[14]

Experimental Design

Mice were randomly assigned to 4 groups, with 6 mice per group (i.e., n=6 per group). Group I was assigned as the normal control, receiving oral saline solution daily from days 1 to 10. Group II(Cy-treated) was the immunosuppression model group, receiving intraperitoneal Cy at 80 mg/kg body weight on days 1 to 3 to induce immunosuppression.[15,16] Groups III and IV were the drug-treated groups, receiving co-administration of Cy (80 mg/kg intraperitoneal) (i.e., Cy + Pomiferin 5 mg/kg and Cy + Pomiferin 10 mg/kg, respectively) on days 1 to 3, followed by oral administration of the pomiferin at 5 mg/kg (Group III) or 10 mg/kg (Group IV) daily from days 4 to 10.[13] On the 11th day of the experiment, all mice were sacrificed and euthanized by the cervical dislocation method (Figure 1). Their spleens and thymuses were promptly excised, collected and preserved for further analysis to determine the immunomodulatory potential of the drug against Cy-induced immunosuppression.

Immune Organ Index estimation

Following the established protocol, the spleen and the thymus were dissected, then weighed separately. The immune organs index was determined by using the formula as follows:

Spleen index or Thymus index= weight of spleen or thymus ÷ body weight

Biochemical Estimation

Hematological Analysis

The hematological indices, including total counts of RBC, WBC, Haematocrit (HCT), Haemoglobin (Hb), and Platelets (PLT) as key components of the immune system were investigated using an automated hematology analyzer. Wright's staining technique was employed to facilitate differential cell counting to further determine the WBC population.

Determination of oxidative and antioxidant enzyme parameters

The tissue specimen was homogenized in 50 mL of phosphate-buffered saline (PBS, pH 7.4). The resulting homogenate was centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant collected at this step was used to quantify SOD, CAT, reduced GSH, and MDA levels. All biochemical parameters were determined using assays with procedures conducted in strict accordance with the manufacturer's protocols.[15]

Determination of Nitric Oxide (NO)

Serum NO levels were measured using a colorimetric nitric oxide assay kit according to the manufacturer's instructions. Standards, a sample blank, and 85 µL of each serum sample were added to a microtiter plate. The assay had two steps. First, 5 µL of nitrate reductase and cofactor were added to the sample and standard wells, and the mixture was incubated for 60 min to convert nitrate to nitrite. After adding 5 µL of enhancer, the plate was incubated for 10 min. Second, 50 µL of Griess reagent was added to produce a purple compound from nitrite. Absorbance was read at 450 nm.[17]

Cytokine and immunoglobulin estimation

ELISA was employed to measure the serum levels of TNF-α, IL-1β, IL-6, IL-12, IgA, IgG, and IgM. The Mouse ELISA Kit facilitated the quantitative analysis of these markers in serum samples, following the manufacturer's instructions. Detection involved a solid-phase sandwich immunoassay, with specific antibodies pre-coated on microwells that captured the target proteins for measurement. Results are expressed in pg/mL.[18]

Statistical Analysis

The data acquired from various parameters, such as cytokine levels, hematological indices, as well as immunological organ indices, were examined using an ideal statistical approach. Tukey's *post hoc* test was used to compare the groups, while a one-way ANOVA was used with *post hoc* tests to discover group differences. A p-value <0.05 suggests that the observed differences were not random but rather significant. This verified the immunomodulatory effects of the medication in the setting of Cy-induced immunosuppression.

RESULTS

Acute toxicity study

Pomiferin demonstrates a significant capacity to mitigate Cy-induced organ damage in mice by suppressing oxidative stress and lipid peroxidation. Its high predicted intestinal absorption of 96.6% ensures systemic availability, while its lack of inherent hepatotoxicity makes it a suitable candidate for counteracting chemotherapy-induced injury. Given its low acute toxicity and high LD_{50} value, pomiferin provides a broad therapeutic window for cytoprotection. These properties support its role in preserving tissue architecture and enhancing antioxidant defenses against the toxic side effects of Cy (Table 1).

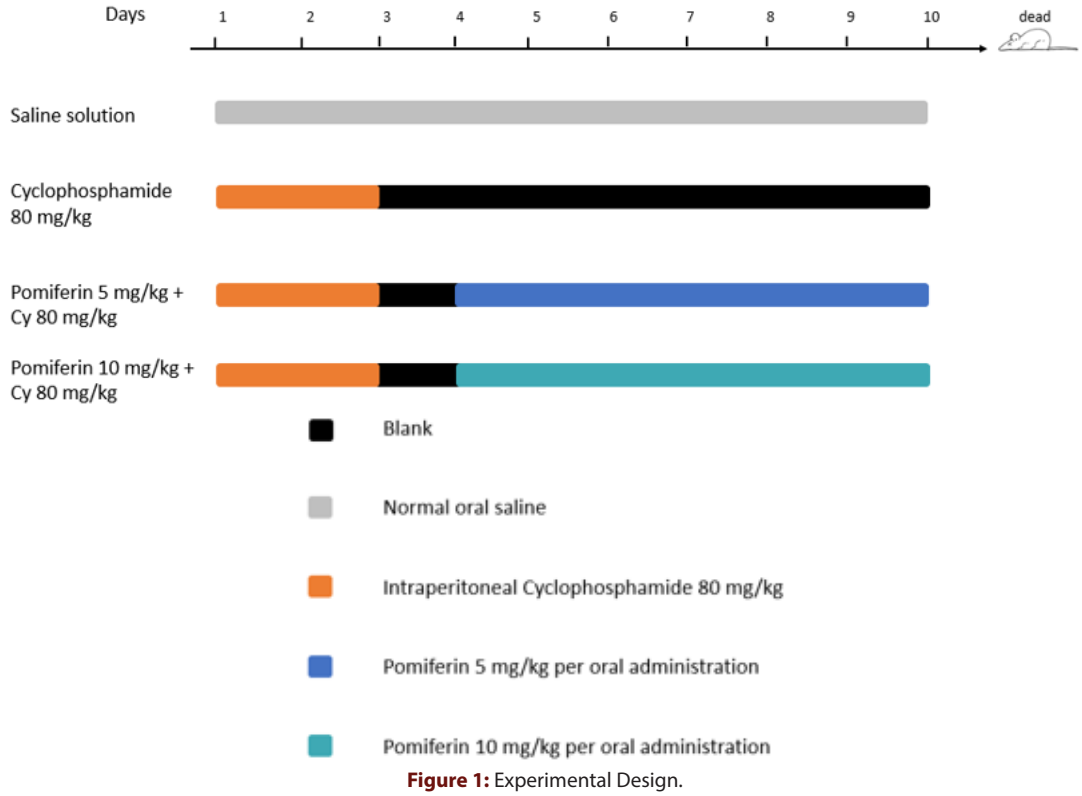

Figure 1: Experimental Design.

Effect of pomiferin on immune organ indices

Cy is a chemotherapeutic drug that exhibits immunosuppressive effects, including reduced body weight and impaired immune organ indices in animal models (Figures 2A, 2B). Compared to the control group, Cy administration significantly reduced both thymus and spleen indices (organ weight/body weight). The body weight of mice in pomiferin-induced therapy was higher than that of Cy-induced mice. Therefore, these findings show a restoration from weight loss in the Cy-induced immunosuppressed mice. Notably, treatment with pomiferin at 5 and 10 mg/kg significantly increased both the spleen [F (3, 20)= 5.318, p=0.0011] and thymus indices [F (3, 20)= 10.71, p<0.0001].

Hematological parameters

Treatment with pomiferin exhibited promising improvements in various hematological parameters, including WBC, RBC, HCT, Hb, and PLT in a mice model of Cy-administered immunosuppression (Figures 3A-3E). In comparison with the normal control group, Cy administration significantly reduced the WBC, RBC, HCT, Hb, and PLT, this reduction caused severe immunosuppression. Notably, pomiferin treatment at both doses

Table 1: Acute oral toxicity study observations after treatment with an oral dose of pomiferin.

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	-3.67	Numeric (log mol/L)
	Caco2 permeability	0.655	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	96.598	Numeric (% Absorbed)
	Skin Permeability	-2.735	Numeric (log Kp)
	P-glycoprotein substrate	Yes	Categorical (Yes/No)
	P-glycoprotein I inhibitor	Yes	Categorical (Yes/No)
	P-glycoprotein II inhibitor	Yes	Categorical (Yes/No)
Distribution	VDss (human)	0.358	Numeric (log L/kg)
	Fraction unbound (human)	0	Numeric (Fu)
	BBB permeability	-1.159	Numeric (log BB)
	CNS permeability	-1.831	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
	CYP3A4 substrate	Yes	Categorical (Yes/No)
	CYP1A2 inhibitor	Yes	Categorical (Yes/No)
	CYP2C19 inhibitor	Yes	Categorical (Yes/No)
	CYP2C9 inhibitor	Yes	Categorical (Yes/No)
	CYP2D6 inhibitor	No	Categorical (Yes/No)
	CYP3A4 inhibitor	No	Categorical (Yes/No)
Excretion	Total Clearance	0.11	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
	Max. tolerated dose (human)	0.232	Numeric (log mg/kg/day)
	hERG I inhibitor	No	Categorical (Yes/No)
	hERG II inhibitor	Yes	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD_{50})	2.452	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	1.72	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	No	Categorical (Yes/No)
	Skin Sensitisation	No	Categorical (Yes/No)
	T. pyriformis toxicity	0.314	Numeric (log ug/L)
	Minnow toxicity	0.974	Numeric (log mM)

ADMET, absorption, distribution, metabolism, excretion, and toxicity; BBB, blood-brain barrier; CNS, central nervous system; CYP, cytochrome; hERG, human ether-à-go-go gene; LD_{50}, lethal dose 50; OCT2, organic cation transporter 2; VDss, volume of distribution.

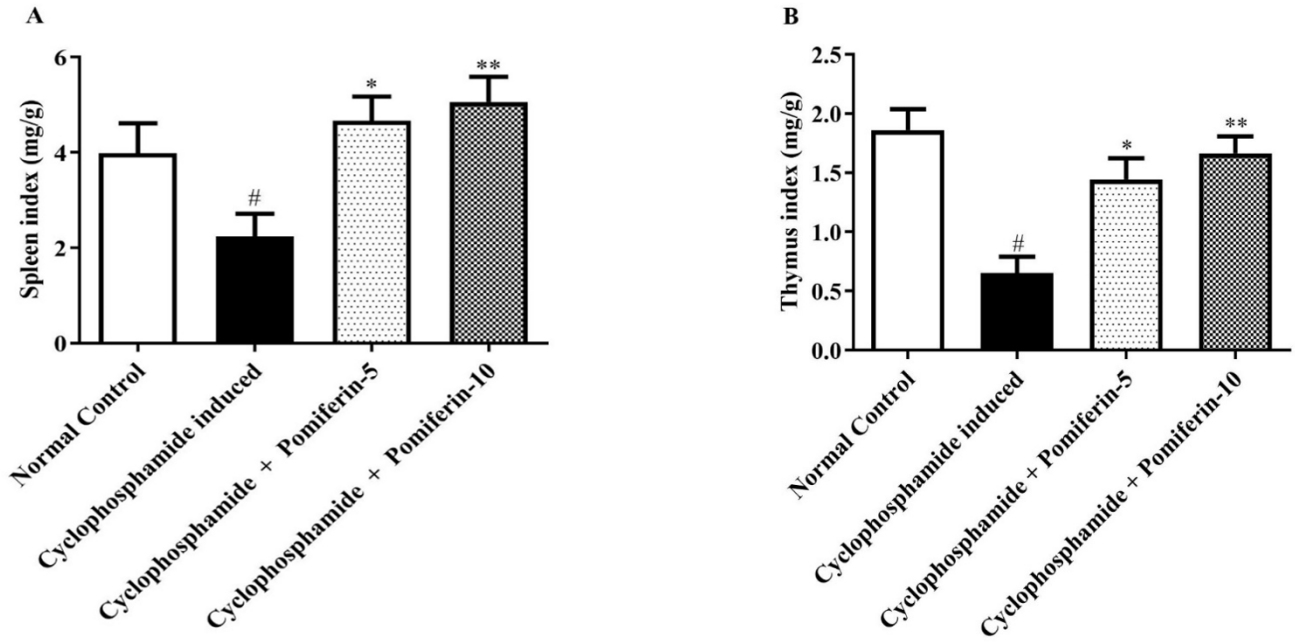

Figure 2: A-B: Effect of pomiferin on bodyweight and immune organs index (A) Spleen index (mg/kg) and (B) Thymus index (mg/kg) in Cy-induced immunosuppressive mice. One ANOVA followed by Tukey's test *post hoc* (n=6). #p<0.001 vs. normal control, * p<0.05, ** p<0.001 Vs 3-Cyclophosphamide group.

significantly reversed these declines, leading to remarkable increases in WBC [F (3, 20)= 10.10, p=0.0003], RBC [F (3, 20)= 9.142, p=0.0005] counts, Hb [F (3, 20)= 6.663, p=0.0027], PLT [F (3, 20)= 8.780, p=0.0006] and HCT [F (3, 20)= 11.69, p=0.0001] than the Cy group.

Estimation of oxidative and antioxidant enzyme parameters

Cy is both a therapeutic agent and a source of oxidative stress leading to cellular damage. Cy administration led to an oxidative injury characterized by a rise in MDA and a subsequent drop in antioxidant enzyme values (CAT, SOD, and GSH) on comparing with the control normal group. The pomiferin treatment at both doses (5 and 10 mg/kg) in the Cy-induced group significantly reduced the MDA level [F (3, 20)= 26.02, p<0.0001], while GSH [F (3, 20)= 6.919, p=0.0022] CAT [F (3, 20)= 5.936, p=0.0046], and SOD [F (3, 20)= 10.05, p=0.0003] were greatly boosted, compared with the Cy-induced group (Figures 4A-4D).

NO estimation

Figure 5 illustrates the impact of pomiferin on NO levels. Spleens from Cy-injected mice exhibited significantly elevated NO levels compared to the control group. Remarkably, pomiferin treatment at both doses (5 and 10 mg/kg) reversed this effect, leading to a substantial reduction in NO levels which effectively counteracted oxidative stress [F (3, 20)= 13.76, p<0.0001].

Effect on cytokines levels

Cy treatment significantly altered the expression of various cytokines, including TNF-α, IL-1β, IL-6, and IL-12, in the animals (Figures 6A-6D). Notably, administration of pomiferin at both doses (5 and 10 mg/kg) significantly inhibited the levels of those cytokines to levels comparable to the control group: TNF-α [F (3, 20)= 14.87, p<0.0001], IL-1β [F (3, 20)=18.24, p<0.0001], IL-6 [F (3, 20)= 16.21, p<0.0001] and IL-12 [F (3, 20)= 30.73, p<0.0001].

Effect on immunoglobulin levels

Figures 7A-7C demonstrates the effect of Cy on immunoglobulin IgA, IgG, and IgM antibody levels in the immunosuppressed animal model. Cy treatment significantly reduced IgA, IgG, and IgM levels in comparison with the control group. However, the administration of pomiferin led to a substantial recovery of those antibodies at both doses (5 and 10 mg/kg), resulting in a marked increase in IgA [F (3, 20)= 13.04, p<0.0001], IgG [F (3, 20)= 9.709, p=0.0004] and IgM levels [F (4, 25)= 6.802, p=0.0008] compared to the Cy group.

DISCUSSION

To study the immune system is of great importance for the development of new drugs.[19] Nevertheless, there is limited research associated with its effectiveness in immunosuppressive conditions. Cy is an immunosuppressive alkylating compound commonly employed as an anticancer drug, shown to have cytotoxic effects resulting in oxidative stress.[20] Our work demonstrated a correlation between therapeutic effect and pomiferin dosage under conditions of Cy-induced immunosuppression in mice. The immunomodulatory effects of pomiferin in Cy-treated mice highlight its ability to mitigate the adverse effects of Cy-induced immunosuppression. In line with existing literature, Cy treatment resulted in a marked reduction

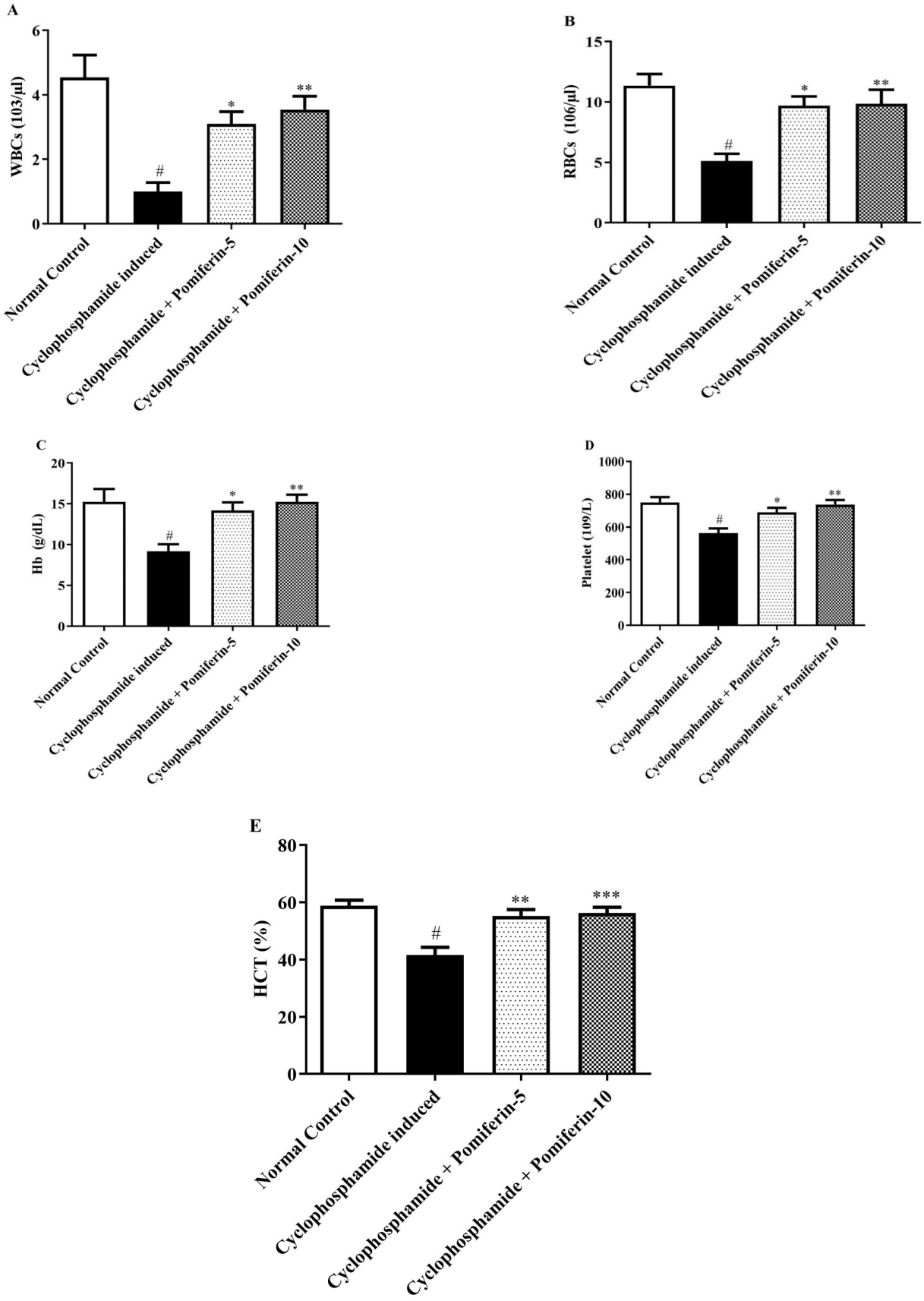

Figure 3: A-E: Effect of pomiferin on hematological parameters (A) WBCs, (B) RBCs, (C) Hb, (D) platelets and (E) HCT, in all experimental groups of Cy-induced mice. One ANOVA followed by Tukey's test *post hoc* (n=6). # p<0.001 vs. normal control, * p<0.05, ** p<0.001 Vs 3-Cyclophosphamide group.

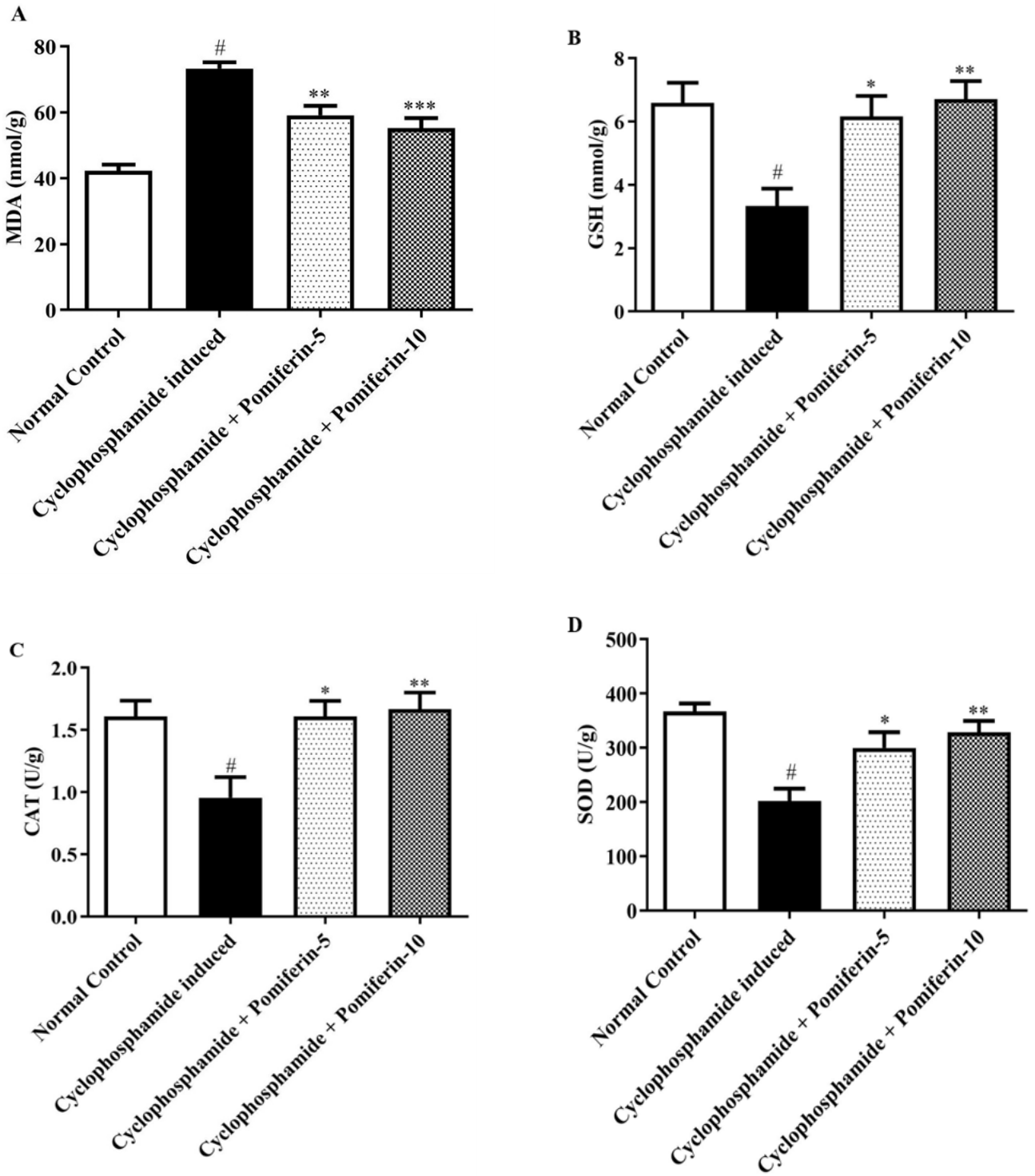

Figure 4: A-D: (A) MDA level, (B) GSH activity (C) CAT level and (D) SOD activity in different experimental groups on treatment with pomiferin in Cy-induced immunosuppressed mice compared with normal. One ANOVA followed by Tukey's test *post hoc* (n=6). # p<0.001 vs. normal control, * p<0.05, ** p<0.001, ** *p<0.0001 Vs 3-Cyclophosphamide group.

in immune organ indices, particularly affecting the spleen and thymus, both of which play crucial roles in immune response and overall health.

Cy can contribute to atrophy, weight loss, and hematopoietic impairment in experimental animals, which, in turn, results in immunosuppression. As a result, the spleen and thymus indices are typically used as precursors of immune health indicators in pursuit of an immune-enhancing medium. The spleen constitutes one of the most significant lymphoid tissues in the body, and it is a complex organ that is responsible for blood purification, removing broken-down or damaged red blood cells,[21] while the thymus provides immune surveillance and protection against

pathogens, tumors, and tissue damage.[22] The existing study revealed that pomiferin treatment successfully restored immune organ indices to near-normal levels, an effect likely attributed to its antioxidant and anti-inflammatory properties,[23] which promotes the structural integrity and functionality of immune tissues. The administration of pomiferin, an immunomodulator, has enough potential to augment the mass of these immune organs.

Cy treatment markedly reduced the count of hematological parameters, RBC, WBC, Hb, and platelets,[24] indicating bone marrow suppression and impaired immune function. Hematological assessments in this study demonstrated that pomiferin improved these parameters, facilitating hematopoietic recovery. This recovery may be attributed to pomiferin's ability to reduce oxidative stress and inflammation. The increase in WBC counts, particularly lymphocytes, underscores pomiferin's role in enhancing the adaptive immune response, while its regulation of neutrophil levels reflects a balanced innate immune response.

Meanwhile, Cy significantly declined the strength of antioxidants (GSH) and enzymes (CAT and SOD), and a rise in MDA compared to the control, indicating lipid peroxidation and oxidative damage.[25] The mice treated with pomiferin showed reduced oxidative stress and restored endogenous antioxidant levels after the introduction of Cy-induced treatment. This pomiferin-induced restoration of antioxidant levels enhances defense mechanisms against Cy-induced oxidative stress. Therefore, pomiferin treatment reduced MDA levels and restored the activities of key antioxidant enzymes such as CAT and SOD, thereby enhancing the body's antioxidant defenses. Additionally, the restoration of GSH levels further supports pomiferin's role in maintaining redox homeostasis and protecting cells from oxidative damage.

The study further explored the role of oxidative stress in Cy-induced immunosuppression. Cy administration elevated Nitric Oxide (NO) levels, signaling increased oxidative stress and inflammation in the spleen. Nitric oxide is a gaseous free radical substance that is formed in living cells during its synthesis, and in higher concentrations, it can become cancerous and promote tumor growth.[26] Treatment with pomiferin reduced NO levels, suggesting that its antioxidant properties mitigated oxidative stress and protected immune organs from Cy-induced damage.

Cytokine molecules are essential regulators of immune responses, released by inflammatory cells and involved in various functions,[27] and were significantly affected by Cy treatment, which elevated pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and reduced the anti-inflammatory cytokine IL-12, disrupting immune regulation.[28] Extreme activation of TNF-α signaling has been linked to chronic inflammation and can eventually lead to clinical repercussions, such as autoimmune diseases.[29] IL-6 has multiple roles in host defense and in human disease, from autoimmunity to cancer.[30] Dysregulation and elevated levels of IL-6 have been found in severe infections, such as sepsis and viral infections.[31] Pomiferin at both doses effectively normalized these cytokine levels by reducing pro-inflammatory markers (TNF-α, IL-1β, and IL-6) and increasing IL-12, demonstrating

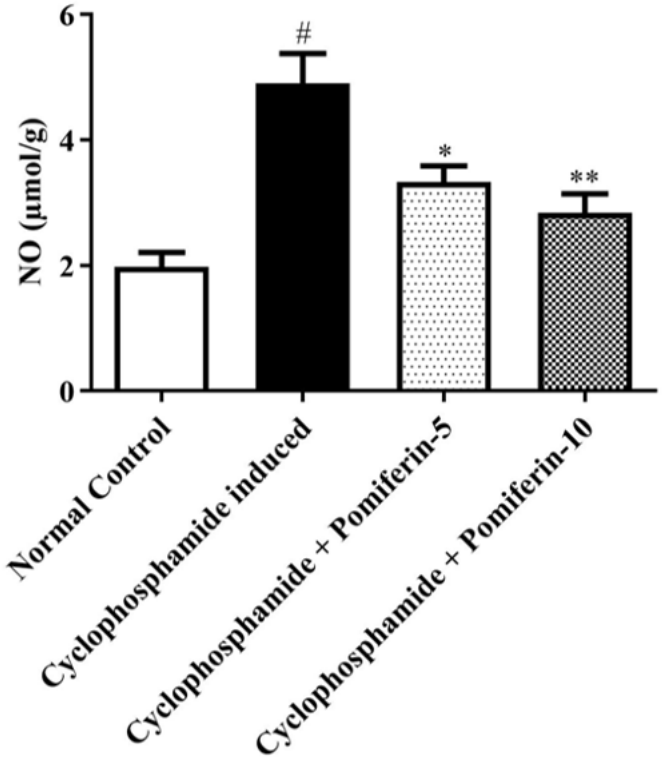

Figure 5: Effect of pomiferin on NO level in Cy-induced immunosuppressed mice compared with normal group. One ANOVA followed by Tukey's test *post hoc* (*n*=6). #*p*<0.001 vs. normal control, * *p*<0.05, ** *p*<0.001 Vs 3-Cyclophosphamide group.

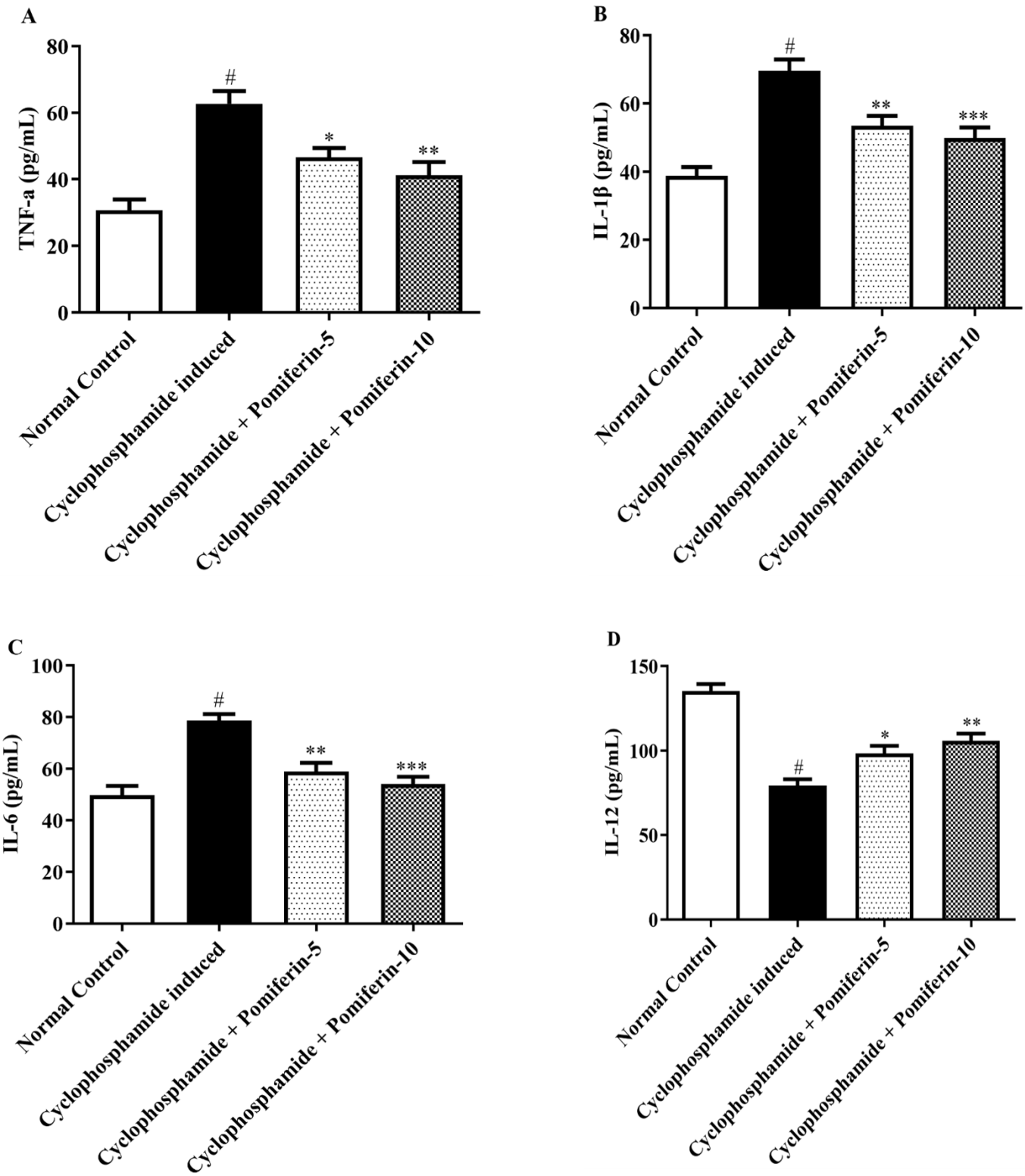

Figure 6: A-D: Effect of pomiferin on (A) TNF-α, (B) IL-1β, (C) IL-6, and (D) IL-12 levels in Cy-induced immunosuppressed mice compared with the normal group. One ANOVA followed by Tukey's test *post hoc* (n=6). #p<0.001 vs. normal control, * p<0.05, ** p<0.001 Vs 3-Cyclophosphamide group.

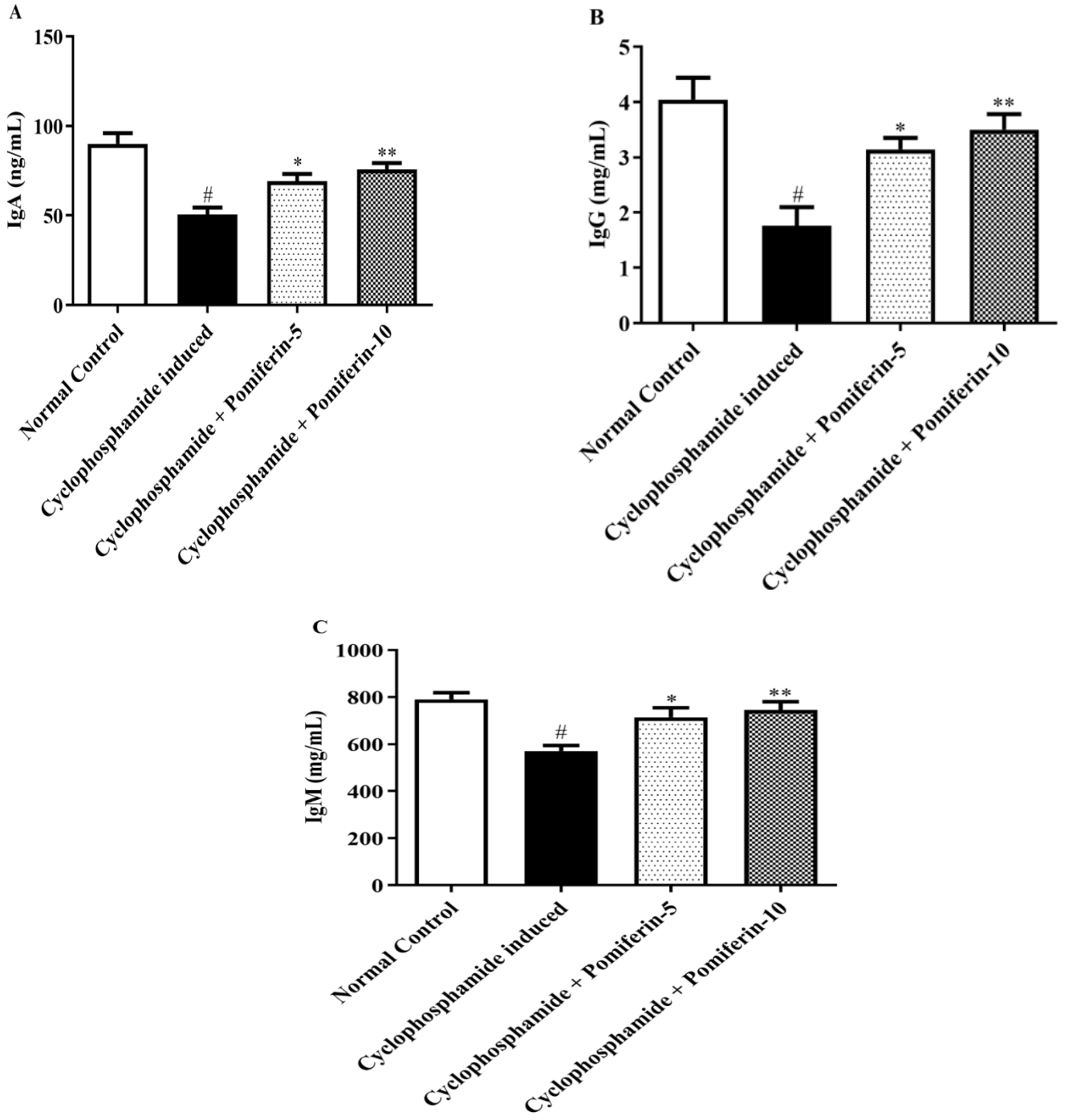

Figure 7: A-C: Effect of pomiferin on (A) IgA, (B) IgG, and (C) IgM levels in Cy-induced immunosuppressed mice compared with the normal group. One ANOVA followed by Tukey's test *post hoc* (n=6). #p<0.001 vs. normal control, * p<0.05, ** p<0.001 Vs 3-Cyclophosphamide group.

its anti-inflammatory properties. This suggests that pomiferin restores immune balance and helps alleviate inflammation associated with Cy-induced immunosuppression.

Immunoglobulins such as IgA, IgG, and IgM perform various functions, determined by their structural variations. IgA is present in many bodily secretions, such as respiratory, tears, saliva, intestinal secretions, and colostrum.[32] IgG is majorly present in blood and tissue fluids; it provides some extent of immunity to the fetus.[33] IgM is particularly effective at attaching to antigenic receptors placed on the outer membrane of bacteria to agglutinate or clump the microorganism.[34] However, pomiferin enhanced or regulated the production of immunoglobulins (IgA, IgG, IgM). As pomiferin possesses strong antioxidant and anti-inflammatory properties, it improves the condition by balancing immune cells, reducing inflammation, and possibly enhancing humoral immunity by producing antibodies such as IgA, IgG, and IgM.

Although Cy is widely used as an immunosuppressive and chemotherapeutic agent, its side effects can be severe. The immunoprotective effects of pomiferin against Cy-induced suppression are mediated through the coordinated modulation of cytokine signaling, antioxidant defense, and hematopoietic recovery. By stabilizing spleen and thymus indices, pomiferin preserves the structural integrity of primary and secondary lymphoid organs, which are essential for immune cell maturation. At the molecular level, pomiferin suppresses the overproduction of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, while simultaneously enhancing IL-12 expression. This shift in the cytokine profile promotes a more balanced immune environment and facilitates the recovery of immunoglobulin levels, specifically IgA, IgG, and IgM, thereby restoring humoral immunity.

Furthermore, pomiferin mitigates the oxidative damage inherent to Cy metabolism. It significantly reduces the accumulation of malondialdehyde and nitric oxide, markers associated with lipid peroxidation and nitrosative stress. This reduction is accompanied by upregulation of endogenous antioxidant enzymes, such as superoxide dismutase and catalase, and by replenishment of reduced glutathione. By neutralizing reactive oxygen species, pomiferin protects hematopoietic precursors, as evidenced by the normalization of red blood cell, white blood cell, and platelet counts. These combined antioxidant and immunomodulatory mechanisms underscore pomiferin's ability to enhance immune tolerance and alleviate systemic toxicity associated with chemotherapy. While these results are promising, the study has certain limitations. To fully understand the signaling pathways involved, future research will need to confirm these mechanisms using different experimental models (e.g., transgenic or knockout mice), genetic analyses (e.g., RT-qPCR for mRNA expression), Western blot analysis, and histopathological examination.

The clinical utility of pomiferin stems from its ability to address the dose-limiting toxicities of Cy, specifically myelosuppression and systemic inflammation. By stabilizing hematological parameters and restoring immunoglobulin production, pomiferin may reduce the risk of opportunistic infections and treatment interruptions in patients undergoing chemotherapy. Ultimately, pomiferin offers a strategic pathway for improving patient outcomes by balancing the potent effects of immunosuppressive therapy with enhanced physiological protection.

CONCLUSION

Pomiferin shows significant potential as a therapeutic agent to mitigate Cy-induced immunosuppression and systemic toxicity. Through its dual capacity to normalize cytokine profiles and bolster endogenous antioxidant defenses, the compound effectively restores lymphoid organ integrity and hematological stability. The marked recovery of immunoglobulin levels and the reduction of oxidative stress markers highlight its efficacy in maintaining immune homeostasis. Given its favorable safety profile and low predicted acute toxicity.

ACKNOWLEDGEMENT

The authors are thankful to the Scientific Research Deanship at the University of Ha'il, Saudi Arabia.

ABBREVIATIONS

Cy: Cyclophosphamide; **RBC:** Red Blood Cells; **WBC:** White Blood Cells; **HCT:** Hematocrit; **Hb:** Hemoglobin; **PLT:** Platelets; **TNF-α:** Tumor Necrosis Factor-alpha; **IL:** Interleukin; **IgA:** Immunoglobulin A; **IgG:** Immunoglobulin G; **IgM:** Immunoglobulin M; **MDA:** Malondialdehyde; **NO:** Nitric Oxide; **CAT:** Catalase; **SOD:** Superoxide Dismutase; **GSH:** Glutathione; **ELISA:** Enzyme-Linked Immunosorbent Assay; **PBS:** Phosphate Buffered Saline; **ANOVA:** Analysis of Variance; **LD_{50}:** Lethal Dose 50; **ADMET:** Absorption, Distribution, Metabolism, Excretion and Toxicity; **CYP:** Cytochrome P450; **BBB:** Blood-Brain Barrier; **CNS:** Central Nervous System; **OCT2:** Organic Cation Transporter 2; **VDss:** Volume of Distribution at Steady State.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy, Asthma and Clinical Immunology. 2018;14(2):49.
2. Yeom J, Shin D, Qiao Y. Editorial: Protein homeostasis in host-pathogen interactions. Frontiers in Microbiology. 2023; 13.
3. Strzelec M, Detka J, Mieszczak P, Sobocińska MK, Majka M. Immunomodulation—a general review of the current state-of-the-art and new therapeutic strategies for targeting the immune system. Frontiers in Immunology. 2023; 14.
4. Sattler S. The Role of the Immune System Beyond the Fight Against Infection. In: Sattler S, Kennedy-Lydon T, editors. The Immunology of Cardiovascular Homeostasis and Pathology. Cham: Springer International Publishing; 2017. p. 3-14.
5. Ahlmann M, Hempel G. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Cancer chemotherapy and pharmacology. 2016;78:661-71.
6. Zong S, Li J, Yang L, Huang Q, Ye Z, Hou G, *et al.* Synergistic antitumor effect of polysaccharide from Lachnum sp. in combination with cyclophosphamide in hepatocellular carcinoma. Carbohydrate Polymers. 2018;196:33-46.
7. Naseri S, Asgarpanah J, Ziai SA. Immunomodulatory and antioxidant effect of liposomal auraptene against cyclophosphamide-induced immunosuppression in BALB/c mice. Experimental Gerontology. 2024;195:112552.
8. Kim H, Hong JY, Lee J, Yeo C, Jeon W-J, Lee YJ, *et al.* Immune-boosting effect of Yookgong-dan against cyclophosphamide-induced immunosuppression in mice. Heliyon. 2024;10(2).
9. Keleş ON, Erol HS, Keleş DV, Karaca M, Çakır A, Koç M, *et al.* Protective effects of pomiferin isolated from *Maclura pomifera* on ischemia-reperfusion injury of rat ovary: biochemical and histopathologic evaluation. International Journal of Research in Medical Sciences. 2023;11(2):1.
10. Alhadrami HA, Sayed AM, Melebari SA, Khogeer AA, Abdulaal WH, Al-Fageeh MB, *et al.* Targeting allosteric sites of human aromatase: a comprehensive *in silico* and *in vitro* workflow to find potential plant-based anti-breast cancer therapeutics. Journal of enzyme inhibition and medicinal chemistry. 2021;36(1):1333-44.
11. Zhao Y, Sang Y, Sun Y, Wu J. Pomiferin Exerts Antineuroinflammatory Effects through Activating Akt/Nrf2 Pathway and Inhibiting NF-κB Pathway. Mediators of Inflammation. 2022;2022(1):5824657.
12. Gothe S, Pawade U, Nikam A, Anjankar M. OECD Guidelines for Acute Oral Toxicity Studies: An Overview. International Journal of Research in Ayurveda and Pharmacy. 2023;14:137-40.
13. Bartošíková L, Nečas J, Bartošík T, Pavlík M, Fráňa P. Effect of pomiferin administration on kidney ischaemia-reperfusion injury in rats. Interdisciplinary toxicology. 2010;3(2):76-81.

14. Alamri A, Al-Abbasi FA, Afzal M, Alqurashi MM, Eid TM, Albishi HM, *et al.* Effect of erucic acid against DMBA-induced breast cancer via the inflammatory/apoptosis pathway. Food Bioscience. 2025;72:107508.
15. Zafar A, Khalid M, Alsaidan OA, Alquraini A, Hazazi A, Abalkhail A, *et al.* Protective effect of erucic acid against cyclophosphamide-induced immunosuppression via IFN-γ/TNF-α/IgG pathways: *In vivo* network pharmacology-based analysis, and molecular docking. Food and Function. 2026.
16. Zhou Y, Chen X, Yi R, Li G, Sun P, Qian Y, *et al.* Immunomodulatory effect of tremella polysaccharides against cyclophosphamide-induced immunosuppression in mice. Molecules. 2018;23(2):239.
17. Omar M, Fathy G, Mohammed S, El-taib A. Investigating the Dichotomous Nature of Nitric Oxide During the Enteral Phase of Trichinella spiralis Infection in Mice: An Experimental Study. Immuno. 2025;5(4):59.
18. Zhou X, Dong Q, Kan X, Peng L, Xu X, Fang Y, *et al.* Immunomodulatory activity of a novel polysaccharide from *Lonicera japonica* in immunosuppressed mice induced by cyclophosphamide. PloS one. 2018;13(10):e0204152.
19. Morsink MAJ, Willemen NGA, Leijten J, Bansal R, Shin SR. Immune Organs and Immune Cells on a Chip: An Overview of Biomedical Applications. Micromachines. 2020;11(9):849.
20. El-Sebaey AM, Abdelhamid FM, Abdalla OA. Protective effects of garlic extract against hematological alterations, immunosuppression, hepatic oxidative stress, and renal damage induced by cyclophosphamide in rats. Environmental Science and Pollution Research. 2019;26(15):15559-72.
21. da Silva RdNO, Santos-Eichler RA, Dias C, Rodrigues SF, Skiba DS, Landgraf RG, *et al.* Immune spleen cells attenuate the inflammatory profile of the mesenteric perivascular adipose tissue in obese mice. Scientific Reports. 2021;11(1):11153.
22. Thapa P, Farber DL. The Role of the Thymus in the Immune Response. Thoracic surgery clinics. 2019;29(2):123-31.
23. Zhao Y, Sang Y, Sun Y, Wu J. Pomiferin Exerts Antineuroinflammatory Effects through Activating Akt/Nrf2 Pathway and Inhibiting NF-κB Pathway. Mediators Inflamm. 2022; 2022:5824657.
24. Wang S, Huang S, Ye Q, Zeng X, Yu H, Qi D, *et al.* Prevention of Cyclophosphamide-Induced Immunosuppression in Mice with the Antimicrobial Peptide Sublancin. Journal of immunology research. 2018; 2018:4353580.
25. Jali AM, Alam MF, Hanbashi A, Mawkili W, Abdlasaed BM, Alshahrani S, *et al.* Sesamin's Therapeutic Actions on Cyclophosphamide-Induced Hepatotoxicity, Molecular Mechanisms, and Histopathological Characteristics. Biomedicines. 2023;11(12):3238.
26. Kumar A, Radzievski R, Xie J. Free Radical: Nitric Oxide in Cancer Therapy. Int J Anal Med Chem. 2018;1:1-4.
27. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, *et al.* Inflammation and tumor progression: signaling pathways and targeted intervention. Signal transduction and targeted therapy. 2021;6(1):263.
28. El-Kholy AA, Elkablawy MA, El-Agamy DS. Lutein mitigates cyclophosphamide induced lung and liver injury via NF-κB/MAPK dependent mechanism. Biomedicine and Pharmacotherapy. 2017;92:519-27.
29. Jang D-i, Lee A-H, Shin H-Y, Song H-R, Park J-H, Kang T-B, *et al.* The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. International journal of molecular sciences. 2021;22(5):2719.
30. Jones BE, Maerz MD, Buckner JH. IL-6: a cytokine at the crossroads of autoimmunity. Current Opinion in Immunology. 2018;55:9-14.
31. Huang L, Zhao X, Qi Y, Li H, Ye G, Liu Y, *et al.* Sepsis-associated severe interleukin-6 storm in critical coronavirus disease 2019. Cellular and molecular immunology. 2020;17(10):1092-4.
32. Li Y, Jin L, Chen T. The Effects of Secretory IgA in the Mucosal Immune System. BioMed research international. 2020; 2020:2032057.
33. Ciobanu AM, Dumitru AE, Gica N, Botezatu R, Peltecu G, Panaitescu AM. Benefits and Risks of IgG Transplacental Transfer. Diagnostics. 2020;10(8):583.
34. Langereis JD, van der Flier M, de Jonge MI. Limited Innovations After More Than 65 Years of Immunoglobulin Replacement Therapy: Potential of IgA- and IgM-Enriched Formulations to Prevent Bacterial Respiratory Tract Infections. Frontiers in Immunology. 2018: 9.

Cite this article: Binshaya AS, Alshaghdali K. Effect of Pomiferin against Cyclophosphamide-Induced Mice by Immunomodulatory Effects and Involvement of the Inflammatory Cytokine Pathway. Indian J of Pharmaceutical Education and Research. 2026;60(4):1606-17.