

Volume 1, Issue 1

Research Article

Date of Submission: 23 May, 2025

Date of Acceptance: 13 June, 2025

Date of Publication: 12 July, 2025

Effect of *Kigelia africana* leaf extract on white blood cell differentials following cyclophosphamide-induced immunocompromised Wistar rats

Onwu Daniel Ocha*, Justina Imere and Dasofunjo Kayode

Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Cross River State (Unicross), Nigeria

*Corresponding Author: Onwu Daniel Ocha, Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Cross River State (Unicross), Nigeria.

Citation: Onwu, D. O., Imere, J., Kayode, D. (2025). Effect of *Kigelia africana* leaf extract on white blood cell differentials following cyclophosphamide-induced immunocompromised Wistar rats. *J Genet Immunol Allergy*, 1(1), 01-06.

Abstract

This research was aimed at assessing the effects of the administration of aqueous extract of *Kigelia africana* on white blood cell differentials in cyclophosphamide-induced immune-compromised Wistar rats. Twenty-five (25) male Wistar rats were allowed to acclimatize for a period of 7 days in a well-ventilated room at room temperature and relative humidity of 29 °C and 70 %, respectively, with a 12-hour natural light-dark cycle. They were allowed food and water ad libitum. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages. The rats were randomly divided into five groups of five rats each. Group A (NC) were fed with normal chow and distilled water only. Group B: immune-compromised rats (induced with cyclophosphamide) without treatment (immune-compromised control). Group C: immune-compromised rats treated with Immunal (Standard control). Group D: immune-compromised rats treated with 100 mg/kg bwt petroleum leaf extract of *K. africana* (PETLEKG1). Group E: immune-compromised rats treated with 200 mg/kg bwt petroleum leaf extract of *K. africana* (PETLEKG2). Immunosuppression in Wistar rats was induced intraperitoneally with 10 mg/kg b.wt. of cyclophosphamide for 7 days in designated rat groups. The dose regimens were administered once daily for an experimental period of 14 days. The result reveals that there was a significant ($P < 0.05$) increase in total white blood cell count, lymphocytes, neutrophils, and basophils when compared to the normal and standard control groups. It can be inferred from this present work that the extract of *K. africana* may fight against infection or foreign bodies through specific and non-specific immune response as well as haematopoietic and erythropoietic properties binding to particular receptors on the phagocyte and lymphocyte cell surfaces, stimulating the production of certain enzymes by the cell that can eliminate infections.

Keywords: Cyclophosphamide, Cytokine-Mediated Immune Modulation, Immunity, Immunosuppression, *Kigelia Africana*, White Blood Cells

Introduction

The immune system is a highly sophisticated network of cells, tissues, and signaling molecules that function collaboratively to maintain immune surveillance, protect against pathogens, and preserve homeostasis. It comprises two primary arms, innate and adaptive immunity, each playing a distinct yet interdependent role in immune defense [1]. The innate immune system serves as the first line of defense, responding rapidly to microbial invasion through pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) that detect pathogen-associated molecular patterns (PAMPs) [2]. This response is mediated by phagocytic cells, including neutrophils and macrophages, which engage in pathogen clearance through oxidative bursts and cytokine secretion. Complement activation further enhances immune defense by promoting opsonization and membrane attack complex (MAC) formation [3]. The adaptive immune system provides specificity and immunological memory, enabling the body to mount a more efficient response upon re-exposure to antigens [4]. T lymphocytes orchestrate cell-mediated immunity through cytotoxic and helper T cell subsets, while B lymphocytes mediate humoral immunity by producing antigen-specific antibodies. The balance between pro-inflammatory and anti-inflammatory cytokines is crucial in regulating immune responses, ensuring the elimination of pathogens while

preventing excessive tissue damage [5]. However, the ability of the immune system to maintain homeostasis depends on a delicate interplay between pro-inflammatory and anti-inflammatory mechanisms, ensuring effective pathogen clearance while preventing immune-mediated tissue damage. Dysregulation in immune function, whether in the form of hyperactivation or suppression, predisposes individuals to various pathological conditions, including autoimmune diseases, chronic infections, and immunodeficiencies [6]. Cyclophosphamide-induced immunosuppression disrupts haematopoietic progenitor cells in the bone marrow, leading to leukopenia and a compromised immune defense [7]. The depletion of white blood cells, particularly lymphocytes, weakens the adaptive immune response, whereas reductions in monocytes and neutrophils impair innate immunity. The resultant immunosuppression increases susceptibility to opportunistic infections, systemic inflammation, and delayed wound healing [8]. Reversing these effects requires interventions that stimulate haematopoiesis, restore immune cell homeostasis, and enhance immune function without eliciting toxicological consequences. Immunosuppressants remain indispensable in managing autoimmune diseases and preventing organ rejection following transplantation [9].

However, prolonged immunosuppression increases the risk of infections and malignancies, underscoring the need for adjunct therapies that selectively restore immune balance. *Kigelia africana*, a medicinal plant known for its pharmacological properties, contains bioactive compounds with potential immunomodulatory effects due to antioxidant such flavonoids, alkaloids, saponins, and terpenoids, which exert anti-inflammatory, antioxidant, and immune-enhancing activities [10]. Flavonoids, in particular, modulate immune cell function by regulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling, a key transcription factor involved in inflammatory responses. By inhibiting NF- κ B activation, flavonoids reduce pro-inflammatory cytokine production while enhancing anti-inflammatory mediators, thereby promoting immune balance. The restoration of white blood cell differentials following immunosuppression (cyclophosphamide) involves the activation of haematopoietic stem cells (HSCs) and the regulation of cytokine networks essential for leukocyte proliferation. Interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), and erythropoietin (EPO) are crucial haematopoietic growth factors that drive the differentiation of progenitor cells into functional leukocytes [10,11]. In addition to haematopoietic recovery, immune regulation involves the modulation of inflammatory pathways that influence leukocyte activity. Monoclonal antibodies targeting inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6), have demonstrated efficacy in managing immune dysregulation [12]. However, the high cost and potential adverse effects of biologic therapies necessitate alternative strategies. The integration of plant-derived immunomodulators into clinical practice requires rigorous scientific validation through preclinical and clinical studies [12]. The immunotherapeutic potential of *K. africana* in reversing cyclophosphamide-induced leukopenia highlights the broader implications of medicinal plants in immune restoration and disease management [13]. Advancements in immunopharmacology continue to explore innovative strategies that harness the therapeutic properties of natural compounds, contributing to the development of targeted immune interventions. The immunomodulatory effects of *K. africana* align with this principle, offering a natural means of restoring immune competence following drug-induced suppression. Further exploration of its molecular targets, immunological pathways, and potential clinical applications will enhance our understanding of plant-derived immunotherapy, reinforcing its role in modern medicine. Therefore, this present research work was designed to evaluate the effect of *Kigelia africana* leaf extract on white blood cell differentials following cyclophosphamide induced immune compromised Wistar rats.

Materials and Methods

Materials

Plant Materials

Fresh leaves of *K. africana* were collected from the University of Cross River State (UNICROSS) environment, Okuku, Cross River State, Nigeria. The leaves were taken to the University of Calabar, Department of Botany for identification and authentication. The voucher number of 205 has been deposited for future reference at the department's herbarium.

Chemicals and Reagents

All chemicals and reagent (Phenyl hydrazine, ethanol, sulphuric acid, ferric chloride, chloroform, hydrochloric acid, Mayer's reagent and Wagner's reagent) used were of analytical grade. Fresh distilled water was used throughout the experimental period. Assay kits used in the analysis in this study were products of Randox Laboratories (England).

Experimental Animals

Twenty-five (25) male Wistar rats were obtained from the animal holding unit of the Department of Medical Biochemistry, University of Cross River State. The animals were allowed to acclimatize for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29°C and 70% respectively with 12 hours natural light-dark cycle. They were allowed food and water ad libitum. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages. All experiments were carried out in compliance with the recommendations of Faculty of Basic Medical Sciences, University of Cross River State, Ethics Committee on guiding principles on care and use of animals.

Method

Preparation of Extract of *K. africana* leaf

The leaves of *K. africana* were collected around UNICROSS and air dried at room temperature for a period of 21 days until constant weight was obtained. The dried leaves were then pulverized to powdered form by a machine blender and sieved. Thereafter, 400g of the pulverized plant material (*K. africana*) was dissolved in 1200ml of 70% petroleum ether

for 72 hours. This was followed with vacuum filtration and extracts was concentrated using an evaporator water bath at 40°C to obtain a solvent free extract, and stored in a refrigerator at 4°C.

Induction of Immunosuppression

Immunosuppression in Wistar rats was induced interperitoneal administration of 10 mg/kg b.w. of Cyclophosphamide for 7 days to induce immunosuppression in designated rat groups.

Experimental Design

The experimental rats were randomly divided into five (5) groups, with five animals per group and treated for a period of fourteen (14) days.

Group A: NC (normal control)

Group C: immune compromised rats treated with immunal (Standard control)

Group B: immune compromised rats (induced with cyclophosphamide) without treatment (immune compromised control)

Group D: immune compromised rats treated with 100mg/kg bwt petroleum leaf extract of *K. africana* extract (PETLEKG1)

Group E: immune compromised rats with 200mg/kg bwt petroleum leaf extract of *K. africana* extract (PETLEKG2)

All administrations were done orally using oropharyngeal cannula once per day for 14days (2 week).

Blood Sample Collection

Blood was collected from all the test rats and control by cardiac puncture using disposable syringe and needle draw blood into plane sterile tubes. The specimens were labeled with the identification alphabets. The samples were kept at room temperature until processing, which occurred within 30 minutes of collection

Determination of Total leucocyte Count

Total leucocytes, also called white blood cells (WBC) were determined using the haemocytometer method (Coles, 1986). The blood sample (0.02 ml) collected with a micropipette was mixed with 0.38 ml of white blood cell diluting fluid held in a test tube.

A drop of the diluted blood sample was used to charge the Neubauer chamber, placed on the microscope stage. White blood cells (leucocytes) were counted in the four corner squares of the Neubauer chamber under x 40 objective using the tally counter. The number of leucocytes counted in the four corner squares was multiple by a factor of 50 to get the total number of leucocytes per microlitre of blood.

Statistical Analysis

The data obtained were analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test at $P < 0.05$. The Graph-pad Prism Software version 8.0.2 was used for the analysis.

Result

The result below reveals the effect of aqueous of *K. africana* on white blood cells cyclophosphamide induced immune compromised rats produced significant ($p < 0.05$).

Increase in WBC, as both 200mg and 400mg b/wt when compared with both the normal and negative control (figure 1).

The extract also significantly ($p < 0.05$) increased the lymphocytes when compared the with the normal and the negative control with exception of the standard control (immunal administration group) (figure 2).

The extract produced similar effect on both neutrophil and basophil when compared with the normal and negative (figure 3-4).

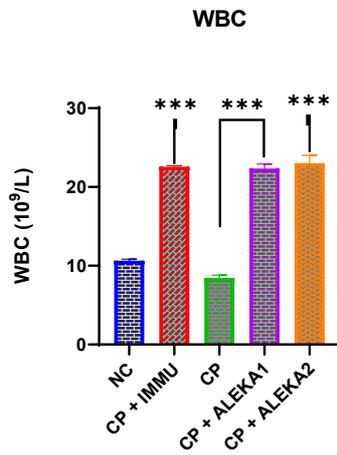


Figure 1: Effect of aqueous extract of *Kigelia africana* leaf on WBC concentration in cyclophosphamide induced immune compromised Wistar rats. Results were expressed as Mean \pm SD (n = 5). *** significant at P<0.05 compared with the control. NC: Normal Control, CP + IMMU: Cyclophosphamide + immunal (20 mg/Kg bwt), CP: Cyclophosphamide control, CP + ALEKA1: Cyclophosphamide + Aqueous extract of *K. africana* (200 mg/Kg bwt), CP + ALEKA2: Cyclophosphamide + Aqueous extract of *K. africana* (400 mg/Kg bwt).

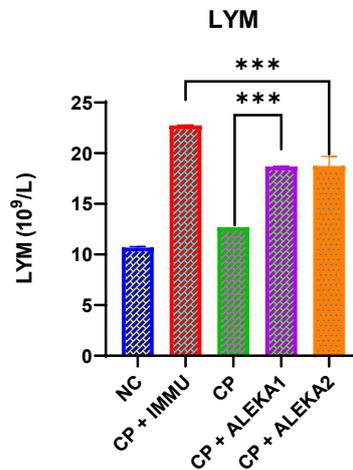


Figure 2: Effect of aqueous extract of *Kigelia africana* leaf on LYM concentration in cyclophosphamide induced immune compromised Wistar rats. Results were expressed as Mean \pm SD (n = 5). *** significant at P<0.05 compared with the control. NC: Normal Control, CP + IMMU: Cyclophosphamide + immunal (20 mg/Kg bwt), CP: Cyclophosphamide control, CP + ALEKA1: Cyclophosphamide + Aqueous extract of *K. africana* (200 mg/Kg bwt), CP + ALEKA2: Cyclophosphamide + Aqueous extract of *K. africana* (400 mg/Kg bwt).

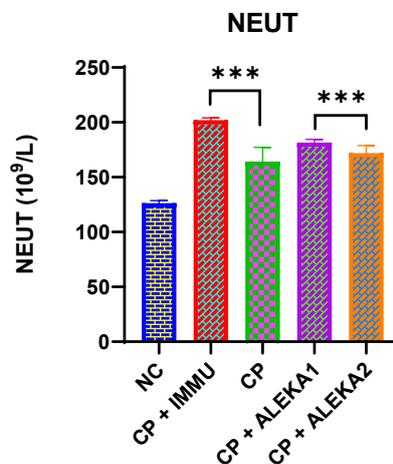


Figure 3: Effect of aqueous extract of *Kigelia africana* leaf on NEUT concentration in cyclophosphamide induced immune compromised Wistar rats. Results were expressed as Mean \pm SD (n = 5). *** significant at P<0.05 compared with the control. NC: Normal Control, CP + IMMU: Cyclophosphamide + immunal (20 mg/Kg bwt), CP: Cyclophosphamide control, CP + ALEKA1: Cyclophosphamide + Aqueous extract of *K. africana* (200 mg/Kg bwt), CP + ALEKA2: Cyclophosphamide + Aqueous extract of *K. africana* (400 mg/Kg bwt).

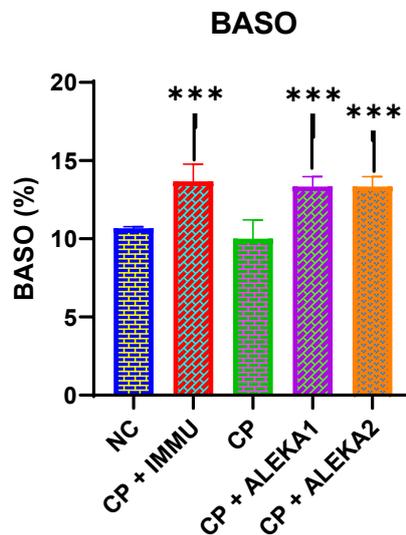


Figure 4: Effect of aqueous extract of *Kigelia africana* leaf on BASO concentration in cyclophosphamide induced immune compromised Wistar rats. Results were expressed as Mean \pm SD (n = 5). *** significant at P<0.05 compared with the control. NC: Normal Control, CP + IMMU: Cyclophosphamide + immunal (20 mg/Kg bwt), CP: Cyclophosphamide control, CP + ALEKA1: Cyclophosphamide + Aqueous extract of *K. africana* (200 mg/Kg bwt), CP + ALEKA2: Cyclophosphamide + Aqueous extract of *K. africana* (400 mg/Kg bwt).

Discussion and Conclusion

Discussion

The immune system is a highly sophisticated network of cells, tissues, and signaling molecules that function collaboratively to maintain immune surveillance, protect against pathogens, and preserve homeostasis. It comprises two primary arms, innate and adaptive immunity, each playing a distinct yet interdependent role in immune defense [1]. Blood is a complicated fluid that carries nutrients to most body tissues and removes waste products from metabolism to prevent tissue buildup and toxicity. It is made up cells, including erythrocytes, leukocytes, and platelets, as well as plasma, an extracellular fluid that is high in proteins [14]. In this study, the significant increase in WBC observed from treatment with *K. africana* suggests that the extract might have enhanced the immune system by increasing WBC production [15]. Granulocytes, macrophage colony-stimulating factor, and interleukins IL-2, IL-4, and IL-5 have also been discovered to control the proliferation, differentiation, and maturation of committed stem cells that produce WBC. Therefore, the significant increase in WBC observed from treatment with *K. africana* in this present study suggests that both the number of lymphocytes and neutrophils in the differential WBC count were raised, thereby boosting the overall WBC count, which agreed with [15]. Additionally, erythropoiesis may be triggered by the antioxidants found in plant extracts [16] when compared with both the normal and negative control. The therapeutic potential of *K. africana* in immune restoration may involve the modulation of EV-mediated signaling, facilitating the transfer of regulatory molecules that may enhance both leukocyte survival and function. This mechanism aligns with [17] and the growing interest in EV-based immunotherapies, which harness cell-derived vesicles for targeted immune modulation. Furthermore, extracellular vesicles (EVs) secreted by immune and non-immune cells play an essential role in intercellular communication, mediating immune responses and tissue regeneration. EVs transport microRNAs (miRNAs) and proteins that regulate immune cell proliferation, apoptosis, and cytokine secretion. Furthermore, the significant increase in LYM observed from treatment with *K. africana* suggests an increase in the production of some chemical cytokines (interferon, interleukins, and complement proteins). These cytokines stimulate other arms of the immune system and increase the activity of natural killer cells as well as T- and B-lymphocytes, which agreed with [18]. Moreover, it enhances natural killer cell and macrophage activity thereby stimulating immune function when compared with both the normal and negative control. In a similar trend, the significant increase in NEUT observed from treatment with *K. africana* suggests that the extract can result in a rise in the synthesis of complement proteins, interleukins, and interferon, among other chemical cytokines. According to [18]. These cytokines activate other immune system branches and boost the activity of T- and B-lymphocytes, natural killer cells, and other immune system components. Additionally, it stimulates the immune system by increasing the activity of macrophages and natural killer cells in experimental rats when compared with both the normal and negative control. Therefore, bioactive constituents of *K. africana* may potentiate the production of these hematopoietic factors, thereby accelerating the recovery of neutrophils, monocytes, and lymphocytes following cyclophosphamide-induced depletion [10]. In like manner, the significant increase in BASO observed from treatment with *K. africana* suggests that the extract has improved leukocyte count, indicating its immunostimulant properties [18]. Immunostimulants attach to specific receptors on the cell surface of phagocytes and lymphocytes, activating these cells to produce enzymes that can destroy pathogens. It can be inferred from this current research, that the extract may stimulate lymphocyte proliferation and, in turn, increase the generation of antibodies by enhancing white blood cells and their differentiation when compared with both the normal and negative control. The ability of *K. africana* to regulate immune homeostasis extends to its antioxidant properties, which mitigate oxidative stress-induced damage to immune

cells [10]. Cyclophosphamide generates reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, and DNA damage in leukocytes. The antioxidant components of *K. africana* scavenge free radicals, preserving cellular integrity and preventing premature immune cell apoptosis [15]. This protective effect contributes to sustained immune function, reducing the severity of immunosuppression and improving host resistance to infections. The immunoregulatory properties of *K. africana* offer a natural approach to modulating inflammatory cytokines, suppressing excessive immune activation while maintaining immune competence [13].

K. Africana, with its diverse pharmacological profile, represents a promising tool for immune modulation, thereby possibly offering a complementary approach to conventional immunotherapeutic strategies. The identification of its active constituents and their mechanistic interactions with immune pathways may also pave the way for novel therapeutic applications in immunocompromised conditions [19].

Conclusion

This study suggests that the extract of *K. africana* may combat infections or foreign bodies through specific and non-specific immune responses, as well as haematopoietic and erythropoietic activities, by binding to receptors on phagocyte and lymphocyte cell surfaces and stimulating the production of enzymes capable of eliminating infections.

References

1. McCully, M. L., Kouzeli, A., & Moser, B. (2018). Peripheral tissue chemokines: homeostatic control of immune surveillance T cells. *Trends in immunology*, 39(9), 734-747.
2. Diamond, M. S., & Kanneganti, T. D. (2022). Innate immunity: the first line of defense against SARS-CoV-2. *Nature immunology*, 23(2), 165-176.
3. Morgan, B. P. (2016). The membrane attack complex as an inflammatory trigger. *Immunobiology*, 221(6), 747-751.
4. Chi, H., Pepper, M., & Thomas, P. G. (2024). Principles and therapeutic applications of adaptive immunity. *Cell*, 187(9), 2052-2078.
5. Iwasaki, A., & Medzhitov, R. (2015). Control of adaptive immunity by the innate immune system. *Nature immunology*, 16(4), 343-353.
6. Costagliola, G., Cappelli, S., & Consolini, R. (2021). Autoimmunity in primary immunodeficiency disorders: An updated review on pathogenic and clinical implications. *Journal of Clinical Medicine*, 10(20), 4729.
7. Sun, C., Yang, J., Pan, L., Guo, N., Li, B., Yao, J., ... & Liu, Z. (2018). Improvement of icaritin on hematopoietic function in cyclophosphamide-induced myelosuppression mice. *Immunopharmacology and Immunotoxicology*, 40(1), 25-34.
8. Kurizky, P. S., dos Santos Neto, L. L., Aires, R. B., da Mota, L. M. H., & Gomes, C. M. (2020). Opportunistic tropical infections in immunosuppressed patients. *Best Practice & Research Clinical Rheumatology*, 34(4), 101509.
9. Terry, L. V., & Oo, Y. H. (2020). The next frontier of regulatory T cells: Promising immunotherapy for autoimmune diseases and organ transplantations. *Frontiers in Immunology*, 11, 565518.
10. Ojediran, T. K., Alagbe, O. J., Victor, D., & Adewale, E. (2024). Analysis of *Kigelia africana* (Lam.) Benth. fruit powder's antioxidant and phytochemical properties. *Brazilian Journal of Science*, 3(7), 38-49.
11. Ahrens, S., Appl, B., Trochimiuk, M., Dücker, C., Serra, G. F., Grau, A. O., & Raluy, L. P. (2022). *Kigelia africana* inhibits proliferation and induces cell death in stage 4 neuroblastoma cell lines. *Biomedicine & Pharmacotherapy*, 154, 113584.
12. Ko, Y. S., Nash, O., Choi, S., & Kim, H. J. (2019). Methanolic extract of *Kigelia africana* exhibits antiatherosclerotic effects in endothelial cells by downregulating RAGE and adhesion molecules.
13. Nagarathna, P. K. M., Reena, K., Reddy, S., & Wesley, J. (2014). Evaluation of Immunomodulatory activity of the flavanoid from *Kigelia africana*. *Indian Journal of Pharmaceutical and Biological Research*, 2(2), 41.
14. Rafeian-Kopaei, M., Baradaran, A., & Rafeian, M. (2014). Plants antioxidants: From laboratory to clinic. *Journal of Nephropathology*, 3(2), 45-46.
15. Dasofunjo, K., Nwodo, O. F. C., Ugwuja, E. I., Onwuka, K., Igwenyi, I. O., & Etim, O. E. (2013). Anti-anemic potentials of *Kigelia africana* in Wistar albino rats. *Journal of Medicinal Plants Research*, 7(32), 2357-2361.
16. Cotoraci, C., Savić, D., Perniu, D., Pinzaru, I., Coricovac, D. E., Copoiu, L., & Dehelean, C. A. (2021). Erythropoiesis and antioxidant effects of plant extracts: Implications for hematopoietic disorders. *Frontiers in Pharmacology*, 12, 746256. <https://doi.org/10.3389/fphar.2021.746256>
17. Li, S., Li, W., Wu, X., Zhang, B., Liu, L., & Yin, L. (2024). Immune cell-derived extracellular vesicles for precision therapy of inflammatory-related diseases. *Journal of Controlled Release*, 368, 533-547.
18. Fazlolahzadeh, R., Rashidi, M., Mahdavi, R., & Ranjbar, F. (2011). Effects of medicinal plants on immune system function and hematological parameters. *Iranian Journal of Basic Medical Sciences*, 14(5), 470-478.
19. Ali, S. A., Singh, G., & Datusalia, A. K. (2021). Potential therapeutic applications of phytoconstituents as immunomodulators: Pre-clinical and clinical evidences. *Phytotherapy Research*, 35(7), 3702-3731.