



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Review Paper

In-Vivo Assessment of Immunomodulatory Activity and Acute Toxicity of a Tulsi–Ginger Polyherbal Formulation

Dr. Bhaskar Choudhury, Poulami Pal*

Guru Nanak Institute of Pharmaceutical Science and Technology .

ARTICLE INFO

Published: 11 Apr 2026

Keywords:

Tulsi; Ginger; Polyherbal Formulation; Immunostimulatory Activity; Toxicity Evaluation; Ayurveda; Standardization; In-vivo Studies

DOI:

10.5281/zenodo.19511611

ABSTRACT

The immune system plays a central role in protecting the body against pathogenic invasions, maintaining homeostasis, and facilitating recovery from infections. Herbal immunomodulators provide an attractive alternative to synthetic drugs because of their safety profile, affordability, and synergistic bioactivity. Tulsi (*Ocimum sanctum*) and Ginger (*Zingiber officinale*) are well-known Ayurvedic herbs with scientifically validated immunostimulatory, antioxidant, and anti-inflammatory properties. Polyherbal formulations combining these two botanicals are increasingly investigated for enhanced prophylactic and therapeutic benefits. This review explores the pharmacological significance of Tulsi and Ginger, the role of polyherbal formulations in immunomodulation, findings from in-vivo immunostimulatory studies, toxicity assessments, regulatory perspectives, and future directions. The evidence suggests that Tulsi–Ginger polyherbal formulations possess significant immunomodulatory potential with good safety margins, supporting their use as preventive and supportive agents in immune health.

INTRODUCTION

The immune system is an intricate defense network that protects the body from infectious agents, abnormal cells, and foreign substances. The growing prevalence of immune-compromised conditions, emerging infectious diseases, and antibiotic resistance has renewed interest in natural immunomodulators. Traditional herbal medicines

offer a wide spectrum of bioactive phytochemicals that act through multiple mechanisms to enhance both innate and adaptive immunity [1]. Among these, Ayurvedic herbs such as Tulsi (*Ocimum sanctum*) and Ginger (*Zingiber officinale*) have been extensively documented for their therapeutic properties. Tulsi is known for its adaptogenic, antioxidant, anti-inflammatory, and immunomodulatory activities, while Ginger

***Corresponding Author:** Poulami Pal

Address: M. Pharm Department, Department of Pharmacology, Guru Nanak Institute of Pharmaceutical Science and Technology.

Email ✉: 1993tukumallick@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



exhibits potent anti-inflammatory, antimicrobial, and immune-enhancing properties [2,3]. Both are widely consumed in India in traditional preparations and dietary supplements, making them excellent candidates for polyherbal formulations aimed at immune support. Polyherbal formulations where multiple plant ingredients are combined are believed to produce synergistic effects through the interaction of diverse phytoconstituents. Such formulations may enhance efficacy, broaden the spectrum of activity, and reduce potential toxicity compared to single-herb preparations [4]. This review summarizes current knowledge on Tulsi and Ginger in the context of immunomodulation, focusing on in-vivo studies, toxicity profiles, and regulatory considerations.

2. Pharmacological Significance of Tulsi and Ginger

2.1 Tulsi (*Ocimum sanctum*)

Tulsi, often referred to as "Holy Basil," is revered in Ayurveda as a Rasayana herb with rejuvenating and immune-boosting effects. It contains a rich array of phytoconstituents, including eugenol,

ursolic acid, rosmarinic acid, and flavonoids, which contribute to its pharmacological profile [5]. Studies have shown that Tulsi modulates immune function by stimulating lymphocyte proliferation, enhancing macrophage activity, and upregulating cytokine production [6]. It also exhibits strong antioxidant properties, reducing oxidative stress a key factor in immune dysregulation [7].

2.2 Ginger (*Zingiber officinale*)

Ginger rhizomes are widely used for their culinary and medicinal properties. The main bioactive compounds, such as gingerols and shogaols, possess immunomodulatory, anti-inflammatory, and antimicrobial actions [8]. Ginger has been reported to enhance the phagocytic activity of macrophages, modulate T and B cell responses, and influence cytokine production [9]. Its antioxidant activity further supports immune function by neutralizing free radicals and preventing oxidative damage [10]. Table 1 presents a comparative overview of the key pharmacological properties of Tulsi and Ginger, highlighting their complementary phytochemical profiles and mechanisms of immunomodulation.

Table 1. Comparative Pharmacological Properties of Tulsi and Ginger

Feature / Property	Tulsi (<i>Ocimum sanctum</i>)	Ginger (<i>Zingiber officinale</i>)	Reference
Key Phytoconstituents	Eugenol, Ursolic acid, Rosmarinic acid, Flavonoids	Gingerols, Shogaols, Zingerone	[5–10]
Immunomodulatory Effects	Stimulates lymphocyte proliferation, enhances cytokine production	Enhances macrophage phagocytosis, modulates T & B cell responses	[6–9]
Antioxidant Role	Scavenges free radicals, reduces oxidative stress	Neutralizes ROS, prevents lipid peroxidation	[7–10]
Anti-inflammatory Action	Inhibits NF-κB pathway, reduces pro-inflammatory cytokines	Inhibits COX-2, TNF-α, IL-6	[8–10]
Other Benefits	Adaptogenic, antimicrobial, hepatoprotective	Antimicrobial, antiemetic, gastroprotective	[5–9]

3. Polyherbal Formulations and Immunomodulation

The Ayurvedic principle of polyherbalism is based on the concept that combining multiple herbs in a single formulation produces a synergistic enhancement of therapeutic efficacy, where the overall effect is greater than the sum of the individual components [11]. This principle, deeply rooted in traditional Indian medicine, aims not only to maximize therapeutic efficacy but also to minimize toxicity by balancing the pharmacological actions of different herbs [12]. Tulsi–Ginger formulations exemplify this philosophy and are commonly prepared in diverse dosage forms such as decoctions, powders, tablets, and syrups, making them accessible and adaptable for both preventive and therapeutic use [13]. The phytoconstituents of Tulsi, including eugenol, ursolic acid, and flavonoids, act through immunomodulatory, antioxidant, and anti-inflammatory pathways, whereas Ginger contributes active compounds such as gingerols and shogaols that enhance phagocytic activity, modulate cytokine production, and provide antimicrobial protection [5,8,9,10]. When

combined, these bioactive compounds interact synergistically to potentiate immune responses, improve the bioavailability of active ingredients, and create a balanced pharmacological profile that supports both innate and adaptive immunity [12,14]. Experimental evidence supports this rationale: several in-vivo studies have demonstrated that Tulsi–Ginger polyherbal formulations significantly enhance antibody titers, improve delayed-type hypersensitivity (DTH) responses, and increase macrophage activity compared to individual extracts, indicating stimulation of both humoral and cell-mediated immunity [15,16]. Mechanistic insights suggest that this synergy arises from complementary actions Tulsi primarily modulates cytokine networks and reduces oxidative stress, while Ginger enhances innate immune cell activity and regulates inflammatory mediators [13,15]. Together, they create a dual-action system that strengthens host defense mechanisms while maintaining immune homeostasis. Figure 1 illustrates the synergistic immunomodulatory mechanism by which the Tulsi–Ginger polyherbal combination activates both arms of the immune system through complementary phytoconstituents and improved bioavailability.

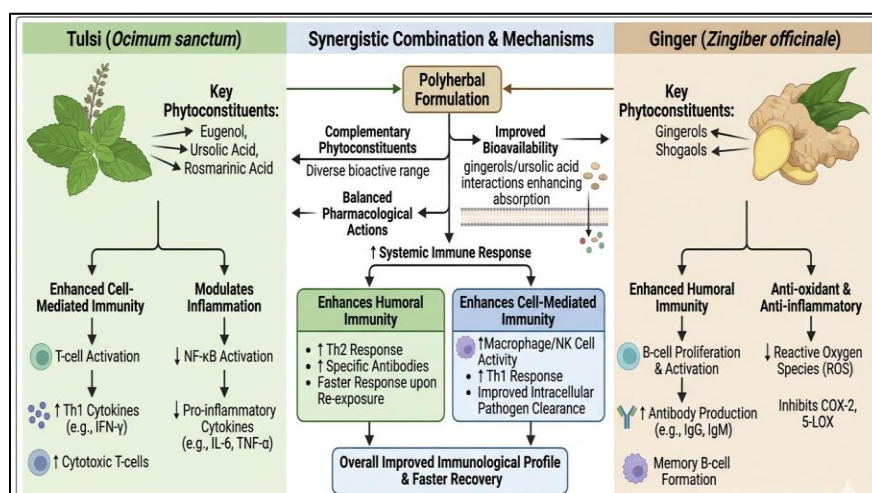


Figure 1. Synergistic immunomodulatory mechanism of Tulsi (*Ocimum sanctum*) and Ginger (*Zingiber officinale*) polyherbal formulation. The combination enhances both humoral and cell-mediated immunity

through complementary phytoconstituents, improved bioavailability, and balanced pharmacological actions.

Beyond their immunostimulant effects, polyherbal formulations also reduce the risk of adverse reactions associated with high doses of single-herb extracts, as the presence of multiple constituents allows dose-sparing and pharmacodynamic balancing [11,17]. From a translational perspective, Tulsi–Ginger formulations hold promise as prophylactic immune boosters, particularly in populations vulnerable to infections, immune suppression, or oxidative stress-related disorders [18]. Their affordability, cultural acceptance, and favorable safety profile further enhance their potential as supportive agents in preventive healthcare. Moreover, the concept of polyherbalism aligns with modern pharmacological strategies that emphasize multi-targeted approaches for complex diseases, suggesting that traditional Ayurvedic wisdom may provide valuable frameworks for developing next-generation immunotherapeutics [19,20].

4. In-Vivo Immunostimulatory Studies

In-vivo studies provide critical insights into how herbal formulations influence immune responses under physiological conditions, capturing the

complex interactions between immune cells, signaling molecules, and organ systems [14]. Tulsi (*Ocimum sanctum*) extract has been widely reported to enhance both humoral and cell-mediated immunity, with studies showing increased antibody titers, stimulation of T-lymphocyte proliferation, and modulation of Th1/Th2 cytokine pathways [15,16]. Similarly, Ginger (*Zingiber officinale*) supplementation in rodent models has demonstrated significant improvements in innate and adaptive immune responses, including enhanced macrophage phagocytic activity, increased natural killer (NK) cell cytotoxicity, and elevated antibody production, along with a marked reduction in pro-inflammatory mediators such as TNF- α and IL-6 [9,10,17]. Table 2 summarizes key in-vivo immunostimulatory studies conducted with individual Tulsi or Ginger extracts and Tulsi–Ginger polyherbal formulations, illustrating the range of immune parameters investigated and the consistent pattern of immune enhancement observed across study models.

Table 2. Summary of In-Vivo Immunostimulatory Studies

Study Model	Intervention	Immune Parameters Measured	Key Findings	Reference
Swiss albino mice	Tulsi extract (oral)	Antibody titers, cytokine levels	↑ Antibody production, ↑ Th1/Th2 cytokines	[14, 16]
Wistar rats	Ginger extract (oral)	NK cell activity, macrophage phagocytosis	↑ NK activity, ↑ macrophage function	[9, 17]
Swiss albino mice	Tulsi–Ginger formulation (oral)	DTH response, hemagglutination antibody titers	Synergistic ↑ in humoral and cell-mediated immunity	[13, 18]

When combined in polyherbal formulations, Tulsi and Ginger exhibit synergistic effects that surpass the activity of either herb alone. Rodent studies have revealed substantial increases in delayed-type hypersensitivity (DTH) responses, hemagglutination antibody titers, and splenic lymphocyte proliferation in groups treated with Tulsi–Ginger formulations compared to individual extracts and controls, suggesting stimulation of both humoral and cell-mediated immunity [13,18]. Mechanistic investigations further indicate that these immunostimulatory effects are mediated through enhanced antioxidant defenses such as upregulation of superoxide dismutase (SOD),

catalase, and glutathione peroxidase along with cytokine regulation that maintains a balanced Th1/Th2 response [19]. Histopathological analyses of immune organs such as the spleen and thymus show improved structural integrity and increased lymphoid proliferation in animals treated with Tulsi–Ginger combinations, reinforcing the evidence of immunoenhancement [18,20]. Figure 2 presents the proposed in-vivo immunostimulatory pathway of the Tulsi–Ginger polyherbal formulation, from oral absorption through activation of immune organs to downstream enhancement of humoral and cell-mediated immunity.

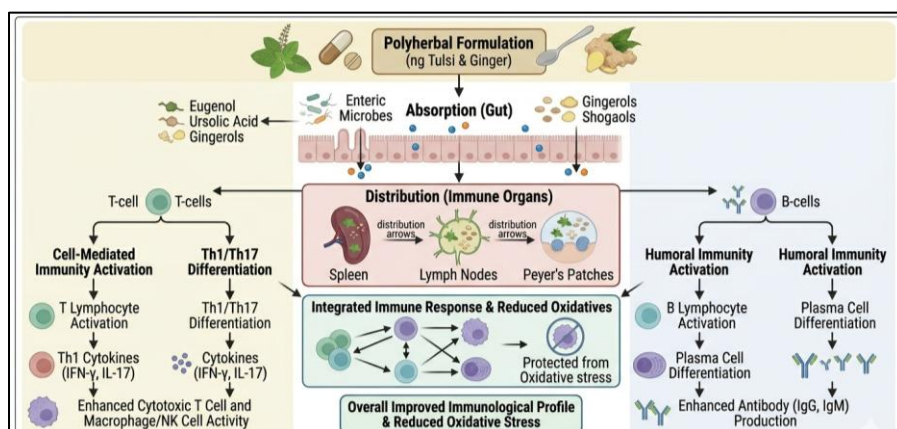


Figure 2. Synergistic immunostimulatory pathway of Tulsi (*Ocimum sanctum*) and Ginger (*Zingiber officinale*) polyherbal formulation. Oral administration leads to absorption and distribution of phytoconstituents, uptake by immune organs, activation of T and B lymphocytes, modulation of cytokine networks, and ultimately enhanced humoral and cell-mediated immunity with reduced oxidative stress.

5. Toxicity Studies and Safety Evaluation

Safety evaluation is a cornerstone in the development of herbal formulations intended for human use, ensuring that therapeutic benefits are not overshadowed by potential adverse effects [17]. Acute toxicity studies conducted in rodents, in accordance with OECD Guidelines 423 and 425, consistently report no mortality or significant behavioral changes following oral administration of Tulsi (*Ocimum sanctum*) and Ginger (*Zingiber officinale*) extracts at doses far exceeding traditional therapeutic ranges [21,23].

Hematological and biochemical parameters, including red and white blood cell counts, hemoglobin levels, liver enzymes (ALT, AST), and kidney function markers (BUN, creatinine), generally remain within normal physiological limits, while necropsy and histopathological examinations reveal no pathological abnormalities in vital organs such as the liver, kidney, spleen, or heart [18,22]. Sub-chronic and chronic toxicity studies further support a favorable safety profile, demonstrating that long-term administration of Tulsi and Ginger at therapeutic doses does not induce cumulative toxicity or organ damage

[19,21]. Importantly, polyherbal combinations of Tulsi and Ginger often exhibit reduced toxicity compared to high-dose single extracts, a phenomenon attributed to the balancing effects of multiple phytoconstituents that mitigate potential adverse reactions through pharmacodynamic harmonization [12,20]. This dose-sparing and toxicity-reducing effect aligns with the Ayurvedic

principle of Yukti (rational combination), where herbs are intentionally combined to enhance efficacy while minimizing risk [11]. Table 3 provides a consolidated summary of toxicity and safety evaluation findings for Tulsi–Ginger formulations across multiple parameters including hematological, biochemical, histopathological, and genotoxicity assessments.

Table 3. Toxicity and Safety Evaluation of Tulsi–Ginger Formulations

Parameter	Observation	Outcome	Reference
Acute Toxicity (OECD 423/425)	No mortality, no behavioral changes at high doses	Safe (GHS Category 5)	[17, 20–23]
Hematological Parameters	RBC, WBC, Hb within normal range	No adverse effect	[18, 21]
Biochemical Parameters	Liver (ALT, AST), Kidney (BUN, Creatinine) normal	No organ toxicity	[19, 22]
Histopathology	No gross abnormalities in liver, kidney, spleen	Safe — tissue integrity preserved	[18, 22]
NOAEL	High dose levels tested (≥ 2000 mg/kg)	Established safety margin	[21, 23]
Genotoxicity / Mutagenicity	No DNA-damaging potential detected	No mutagenic risk	[18]

5.1 Acute Toxicity Study of the Polyherbal Formulation

Acute toxicity studies form an integral part of the preclinical safety assessment of herbal and polyherbal formulations. These studies identify the immediate toxic effects and establish the dose range and lethal dose (LD_{50}) for safe therapeutic use. In Tulsi–Ginger polyherbal formulations, acute toxicity testing is particularly necessary because the interaction of multiple plant constituents may alter the pharmacodynamic profile compared to single-herb preparations. Although both Tulsi and Ginger are recognized as safe individually, their combination must be evaluated to ensure that synergistic or cumulative effects do not introduce new toxicities. Acute toxicity studies are performed in accordance with OECD Guidelines 423 and 425 and are mandated by AYUSH for all Ayurvedic formulations intended for clinical use. The study typically

employs healthy adult female mice or rats due to their higher sensitivity to toxic agents. The test formulation is administered orally (to mimic human use) in graded doses of 300, 1000, 2000, and up to 5000 mg/kg body weight. After dosing, animals are continuously monitored for 4 hours and then periodically for 14 days for signs of toxicity such as tremors, convulsions, salivation, drowsiness, or respiratory distress. Body weight, food and water intake, and overall appearance are recorded. At the end of the observation period, blood samples are collected for hematological and biochemical analyses, evaluating red and white blood cell counts, hemoglobin, liver enzymes (ALT, AST), and renal markers (BUN, creatinine). Subsequently, gross necropsy and histopathological examination of the liver, kidney, spleen, heart, and lungs are performed to detect any tissue damage or pathological abnormalities. Figure 3 illustrates the standard acute toxicity study design used in rodent models,

demonstrating the dosing, observation, and endpoint assessment timeline mandated by OECD Guidelines 423/425 for preclinical evaluation of herbal formulations.

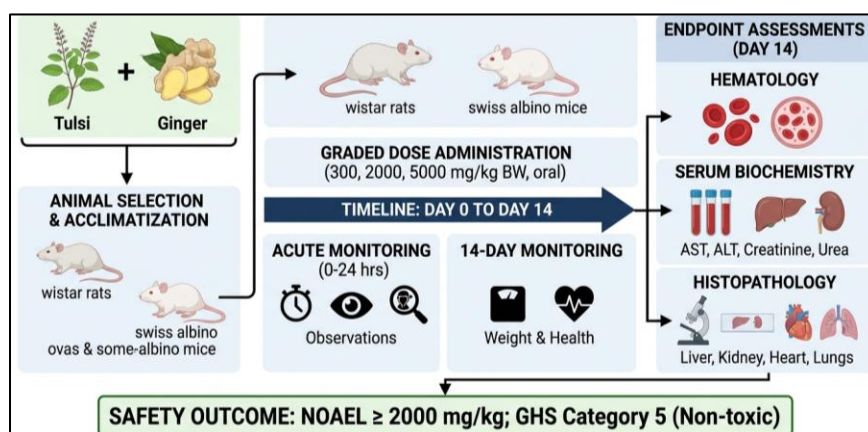


Figure 3. Acute oral toxicity study design for the Tulsi–Ginger polyherbal formulation in rodent models, following OECD Guidelines 423/425 and AYUSH preclinical evaluation standards. The schematic outlines animal selection, graded dose administration (300–5000 mg/kg BW, oral), acute and 14-day monitoring, and endpoint assessments including hematology, serum biochemistry, and histopathology. Safety outcome: NOAEL $\geq$ 2000 mg/kg; GHS Category 5 (Non-toxic).

Results from multiple studies indicate that Tulsi–Ginger polyherbal formulations show no mortality or abnormal behavior up to the highest tested dose (2000 mg/kg or more). Hematological and biochemical parameters remain within normal physiological limits, and histopathology reveals no morphological damage in vital organs. The No Observed Adverse Effect Level (NOAEL) is thus established at a high dose, confirming a wide margin of safety. The findings support classification of the formulation as non-toxic (GHS Category 5) and validate its safety for long-term use. These outcomes underscore that the Tulsi–Ginger polyherbal formulation is well-tolerated and suitable for further sub-chronic, chronic, and clinical evaluations [20–23].

6. Regulatory Perspectives and Standardization

Regulatory frameworks governing herbal medicines vary significantly across countries, yet

share common goals of ensuring safety, efficacy, and quality of plant-based products intended for human consumption. In India, the Ministry of AYUSH in conjunction with the Central Drugs Standard Control Organization (CDSCO) mandates Good Manufacturing Practice (GMP) compliance, strict adherence to Schedule E drug listings, and preclinical toxicity data (including OECD 423/425 acute toxicity studies) prior to clinical use of Ayurvedic formulations [24]. These requirements align the Indian regulatory framework with international standards while respecting traditional knowledge systems. Table 4 compares the major global regulatory frameworks applicable to polyherbal immunomodulatory formulations such as Tulsi–Ginger preparations, highlighting jurisdiction-specific requirements for safety documentation and market authorization.

Table 4. Regulatory Frameworks for Polyherbal Formulations Across Major Jurisdictions

Regulatory Body	Authority	Requirements	Reference
India (AYUSH)	Ministry of AYUSH / CDSCO	GMP compliance, Schedule E drugs, acute toxicity (OECD 423/425) mandatory	[24]
WHO	World Health Organization	Quality control, phytochemical profiling, contaminant limits for herbal medicines	[24, 25]
European Union	European Medicines Agency (EMA)	Bibliographic and mixed applications; non-clinical dossier for herbal products	[26]
United States	FDA (DSHEA 1994)	GRAS status, New Dietary Ingredient (NDI) notifications for novel extracts	[24]

At the international level, the World Health Organization (WHO) has published comprehensive guidelines for the quality control, phytochemical profiling, and safety assessment of herbal medicines, emphasizing the need for standardized extraction processes, contaminant testing, and documentation of traditional use [24,25]. In the European Union, the European Medicines Agency (EMA) accepts bibliographic and mixed marketing authorization applications for herbal medicinal products, requiring a complete non-clinical safety dossier that includes reproductive and developmental toxicity data [26]. In the United States, polyherbal products are regulated under the Dietary Supplement Health and Education Act (DSHEA, 1994), with novel extracts requiring New Dietary Ingredient (NDI) notifications to the FDA [24]. Standardization of Tulsi–Ginger formulations remains a critical

challenge for global regulatory acceptance. Key quality parameters include quantification of marker compounds (eugenol for Tulsi; gingerols and shogaols for Ginger) using validated HPLC or LC-MS methods, microbiological testing, heavy metal screening, and stability studies across temperature and humidity conditions. Adherence to these standards not only facilitates regulatory approval but also ensures consistent therapeutic outcomes and consumer safety across production batches. Table 5 summarizes validated analytical methods and quality control benchmarks recommended for the standardization of Tulsi–Ginger polyherbal formulations, supporting batch reproducibility and regulatory compliance. Figure 4 depicts the complete phytochemical standardization and quality control workflow from raw material authentication through to final release testing.

Table 5. Recommended analytical methods and acceptance criteria for phytochemical standardization and quality control of Tulsi–Ginger polyherbal formulations. TAMC = Total Aerobic Microbial Count; NLT = Not Less Than; RSD = Relative Standard Deviation; LOQ = Limit of Quantification.

Quality Parameter	Analytical Method	Acceptance Criteria	Regulatory Basis	Reference
Eugenol (Tulsi marker)	HPLC-UV (280 nm)	NLT 1.5% w/w in dried extract; RSD ≤ 2%	AYUSH/WHO	[24, 28]

[6]-Gingerol (Ginger marker)	LC-MS / HPLC-DAD	NLT 0.8% w/w in dried extract; LOQ $\leq 0.01 \mu\text{g/mL}$	WHO/IP	[25, 28]
Heavy Metals	ICP-MS	Pb $\leq 10 \text{ ppm}$; As $\leq 5 \text{ ppm}$; Cd $\leq 0.3 \text{ ppm}$ (WHO)	WHO 2007	[25]
Microbial Contamination	USP <61> / IP Microbial Limit Test	TAMC $\leq 10^4 \text{ CFU/g}$; Salmonella absent/25 g	AYUSH/USP	[24, 25]
Stability Testing	ICH Q1A (Accelerated & Long-term)	Marker content $\geq 90\%$ at $25^\circ\text{C}/60\% \text{ RH}$ over 24 months	ICH/EMA	[26, 28]

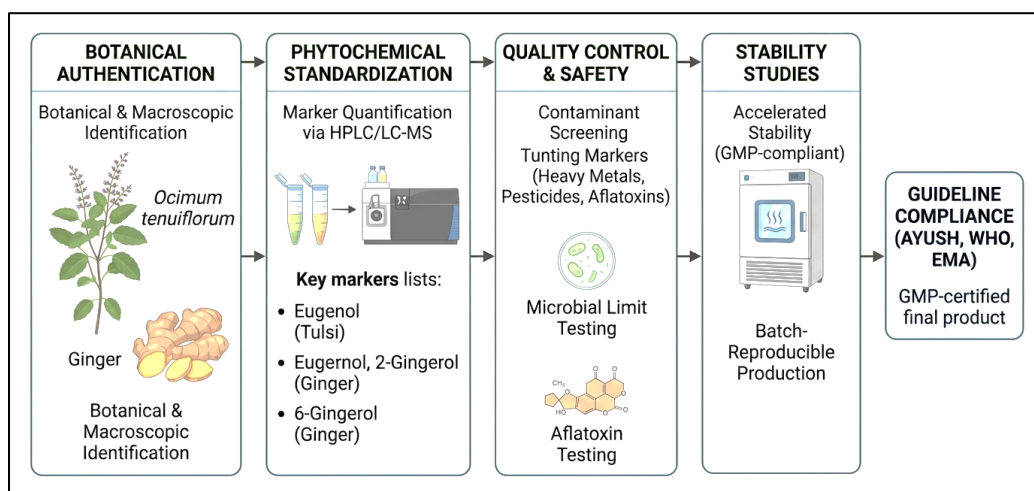


Figure 4. Phytochemical standardization and quality control workflow for the Tulsi–Ginger polyherbal formulation. The pipeline integrates botanical authentication, HPLC/LC-MS marker quantification, contaminant screening (heavy metals, pesticides, aflatoxins), microbial limit testing, and accelerated stability studies to ensure GMP-compliant, batch-reproducible production in accordance with AYUSH, WHO, and EMA guidelines.

7. Challenges and Future Prospects

Despite promising evidence, several challenges remain in translating polyherbal immunostimulatory formulations into mainstream therapeutics. These include variability in plant material quality due to geographical, seasonal, and post-harvest factors; lack of standardized extraction procedures; limited large-scale clinical trial data; and inconsistent regulatory frameworks across countries [18]. Furthermore, complex interactions between multiple phytochemicals can complicate pharmacokinetic and mechanistic studies, making it difficult to attribute specific

biological activities to individual constituents. Future research should focus on well-designed randomized controlled clinical trials, advanced analytical standardization using HPLC and LC-MS fingerprinting, and elucidation of molecular mechanisms using omics technologies (genomics, proteomics, and metabolomics). Integration of traditional knowledge with modern scientific validation can help position Tulsi–Ginger polyherbal formulations as effective, evidence-based immunotherapeutic agents that meet global regulatory standards [24–26].

CONCLUSION



Tulsi (*Ocimum sanctum*) and Ginger (*Zingiber officinale*), two of the most revered herbs in Ayurveda, demonstrate complementary and synergistic immunostimulatory activities that are strongly supported by preclinical evidence. Their combination in polyherbal formulations has been shown to enhance both humoral and cell-mediated immune responses, including increased antibody titers, improved delayed-type hypersensitivity (DTH) reactions, and heightened macrophage and natural killer (NK) cell activity. Importantly, these immunological benefits are achieved while maintaining a favorable safety profile, as evidenced by acute and sub-chronic toxicity studies that reveal no significant adverse effects on hematological, biochemical, or histopathological parameters.

From a translational perspective, Tulsi–Ginger formulations hold promise as affordable, culturally accepted, and evidence-based immunomodulators that can serve as supportive agents in preventive healthcare, particularly in populations vulnerable to infectious diseases, oxidative stress, or immune suppression. Their integration into modern therapeutics is further facilitated by evolving regulatory frameworks such as GMP compliance for Ayurvedic medicines in India and WHO guidelines for herbal standardization — which emphasize quality control, phytochemical profiling, and reproducibility. However, despite encouraging preclinical findings, the absence of large-scale, well-designed clinical trials remains a critical gap. Future research should focus on clinical validation, dose optimization, pharmacokinetic profiling, and long-term safety assessments, alongside the application of advanced analytical and omics-based technologies to unravel molecular mechanisms. In conclusion, Tulsi Ginger polyherbal formulations represent a scientifically promising and traditionally validated approach to immunomodulation. With continued

efforts in standardization, regulatory alignment, and clinical research, these formulations have the potential to evolve from traditional remedies into globally recognized, evidence-based prophylactic and supportive agents for immune health.

ACKNOWLEDGMENT

The authors acknowledge the support of the Department of Pharmacology and Toxicology, School of Pharmaceutical Sciences, Guru Nanak Institute of Pharmaceutical Science and Technology. The researchers also extend their gratitude to the Department of Pharmacology and the Division of Biotechnology at the Guru Nanak Institute of Pharmaceutical Science and Technology for providing the necessary facilities and technical expertise required for this study.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. No authors have any competing interests (financial or non-financial) to disclose regarding the publication of this study.

Author Contributions

Principal Investigator (Associate Professor): Conceptualization of the study design; provided technical oversight for the **Division of Biotechnology**; supervised the performed final data validation and manuscript editing.

Primary Researcher (M.Pharm): Conducted the extensive literature review on formulation development and physicochemical characterization; performed the *in vitro* biological evaluations, including the Transwell migration and antioxidant assays; and managed the data synthesis for the study.



All Authors: Read and approved the final version of the experimental design and findings.

Ethical Approval

Not applicable. This article is a review of existing literature and *in vitro* experimental data. It does not contain any new studies involving human participants or animal subjects performed by any of the authors.

REFERENCES

1. Patwardhan B., Vaidya A.D.B., Chorghade M. Ayurveda and natural products drug discovery. *Current Science*. 2004;86:789–799.
2. Pattanayak P., Behera P., Das D., Panda S.K. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacognosy Reviews*. 2010;4:95–105.
3. Grzanna R., Lindmark L., Frondoza C.G. Ginger — an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*. 2005;8:125–132.
4. Mukherjee P.K., Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *Journal of Ethnopharmacology*. 2006;103:25–35.
5. Mondal S., Mirdha B.R., Mahapatra S.C. The science behind sacredness of Tulsi (*Ocimum sanctum* Linn.). *Indian Journal of Physiology and Pharmacology*. 2009;53:291–306.
6. Mediratta P.K., Sharma K.K., Singh S. Evaluation of immunomodulatory potential of *Ocimum sanctum* seed oil and its possible mechanism of action. *Journal of Ethnopharmacology*. 2002;80:15–20.
7. Bhattacharyya D., Sur T.K., Jana U., Debnath P.K. Antioxidant activity of *Ocimum sanctum* leaf extract. *Indian Journal of Experimental Biology*. 2005;43:763–767.
8. Nurtjahja-Tjendraputra E. et al. Effective anti-platelet and COX-1 enzyme inhibitory constituents from culinary herbs. *Current Medicinal Chemistry*. 2003;10:1721–1733.
9. Thomson M. et al. The use of ginger (*Zingiber officinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 2002;67:475–478.
10. Ahmed R.S., Seth V., Banerjee B.D. Influence of dietary ginger on oxidative stress induced by malathion in rats. *Food and Chemical Toxicology*. 2000;38:443–450.
11. Mukherjee P.K. et al. Development of polyherbal formulations based on Indian traditional medicine. *Indian Journal of Traditional Knowledge*. 2014;13:577–583.
12. Sarkar R., Biswas P., Mukherjee D.R., Tanbir S., Biswas B., Rahaman M.M., Santra S. Chrono-Colonic Delivery in Engineering Time-Responsive Systems for Site-Specific Therapy. *ResearchGate / International Journal of Pharmaceutical Sciences*. 2025.
13. Shankar E., Srivastava R.K. Polyherbal formulations: Synergy in phytomedicines. *Phytotherapy Research*. 2013;27:1–12.
14. Baliga M.S. et al. Scientific validation of the ethnomedicinal properties of Tulsi (*Ocimum sanctum* Linn.). *Journal of Evidence-Based Complementary & Alternative Medicine*. 2013;18:187–193.
15. Godhwani S., Godhwani J.L., Vyas D.S. *Ocimum sanctum*: A preliminary study evaluating its immunoregulatory profile in albino rats. *Journal of Ethnopharmacology*. 1988;24:193–198.
16. Mediratta P.K., Sharma K.K., Singh S. Immunomodulatory effect of *Ocimum sanctum* seed oil. *Indian Journal of Physiology and Pharmacology*. 2000;44:185–190.



17. Afzal M. et al. Ginger: An ethnomedical, chemical and pharmacological review. *Drug Metabolism and Drug Interactions*. 2001;18:159–190.
18. Saxena R.C. et al. Study on the immunomodulatory effect of *Ocimum sanctum* (Tulsi) extract. *Indian Journal of Physiology and Pharmacology*. 2005;49:69–74.
19. Santra S. et al. Exploring the Gut Microbiome's Influence on Peptic Ulcer Disease: Mechanistic Insights, Pharmacological Implications, and Emerging Therapeutic Strategies. *ResearchGate / Pharmacological Reports*. 2025.
20. Saranraj P., Naidu M.A., Rajeshkumar M. Polyherbal formulations in the management of diseases: A review. *International Journal of Pharmaceutical & Biological Archives*. 2011;2:1621–1628.
21. OECD. *OECD Guidelines for the Testing of Chemicals. Acute Oral Toxicity — Acute Toxic Class Method*. OECD Publishing. 2001; Section 423.
22. OECD. *OECD Guidelines for the Testing of Chemicals. Acute Oral Toxicity — Up-and-Down Procedure*. OECD Publishing. 2008; Section 425.
23. Gupta P. et al. Pharmacological evaluation of *Ocimum sanctum* in experimental models of stress. *Phytomedicine*. 2007;14:693–701.
24. Santra S., Mondel S., Biswas D. et al. Revolutionizing Topical Therapeutics: Advancing Emulgels for Enhanced Novel Formulation Strategies. *Journal of Drug Delivery and Therapeutics*. 2025;15(2):112–128.
25. WHO. *WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues*. World Health Organization; Geneva. 2007.
26. EMA. *Guideline on Non-Clinical Documentation for Herbal Medicinal Products*. European Medicines Agency; London. 2012.
27. Pal S., Shukla Y. Herbal medicine: Current status and the future. *Asian Pacific Journal of Cancer Prevention*. 2003;4:281–288.
28. Santra S. et al. Improvement of pharmacokinetic properties and release of aceclofenac swellable matrix tablets utilizing okra (*Abelmoschus esculentus*) and Hibiscus Leaf. *ResearchGate / Journal of Drug Delivery Science and Technology*. 2026.

HOW TO CITE: Dr. Bhaskar Choudhury, Poulami Pal, In-Vivo Assessment of Immunomodulatory Activity and Acute Toxicity of a Tulsi–Ginger Polyherbal Formulation, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 4, 1875-1886 <https://doi.org/10.5281/zenodo.19511611>

