Journal of Swasthavritta and Yoga

ISSN Print: 3078-7157
ISSN Online: 3078-7165
JSY 2025; 2(2): xx-xx
www.swasthjournal.com
Received: 08-08-2025
Accepted: 07-09-2025

Mariam Al-Zadjali
Department of Traditional and Complementary Medicine, Gulf College of Health Sciences, Muscat, Oman

Corresponding Author:
Mariam Al-Zadjali
Department of Traditional and Complementary Medicine, Gulf College of Health Sciences, Muscat, Oman

Evaluation of the efficacy of Chyawanprash in boosting immunity and preventing infections: An experimental study on rat model

Mariam Al-Zadjali

DOI: https://www.doi.org/10.33545/30787157.2025.v2.i2.A.20

Abstract
Background: *Chyawanprash*, a traditional Ayurvedic *Rasayana* formulation, is reputed for its rejuvenating and immunomodulatory properties. Despite its widespread clinical use, preclinical evidence elucidating its mechanistic basis for immune enhancement remains limited.
Objective: This study aimed to evaluate the immunomodulatory and infection-preventive efficacy of *Chyawanprash* using a Wistar rat model.
Methods: Forty-eight healthy male Wistar rats were randomly divided into four groups (n = 12 each): Control (vehicle only), Low-dose (1 g/kg/day), Medium-dose (2 g/kg/day), and High-dose (4 g/kg/day) *Chyawanprash*. Treatments were administered orally for 30 days. Immune efficacy was assessed by analyzing hematological indices, antioxidant enzyme levels, cytokine profiles (IL-2, TNF-α, IFN-γ), and serum immunoglobulins. On day 31, rats were challenged intraperitoneally with a sublethal dose of *Escherichia coli* (1×10^7 CFU), and infection symptoms were monitored for 10 days.
Results: *Chyawanprash* administration produced a significant, dose-dependent enhancement in total and differential leukocyte counts, serum immunoglobulin levels, and antioxidant enzyme activities (SOD, catalase, and GPx), with a concurrent reduction in malondialdehyde (MDA) levels ($p < 0.05$). Cytokine analysis showed marked elevation of IL-2 and IFN-γ, indicating T-cell activation and macrophage stimulation. Following the *E. coli* challenge, the high-dose group demonstrated 40% fewer infection symptoms and zero mortality compared to controls. Histopathological analysis revealed increased lymphoid proliferation in the spleen and thymus of treated rats.
Conclusion: The findings confirm that *Chyawanprash* exerts potent immunostimulatory and antioxidant effects, enhancing systemic resistance to bacterial infection in rats. These results substantiate its classical *Rasayana* claim and support its potential as a safe natural immunonutrient for preventive health care.

Keywords: *Chyawanprash*, *rasayana*, immunity, antioxidant, cytokines, infection prevention, wistar rats

Introduction
The immune system is a complex and dynamic defense network that safeguards the body against a multitude of pathogenic threats including bacteria, viruses, fungi, and environmental toxins. Its proper functioning relies on the fine balance between innate and adaptive immune responses, antioxidant defenses, and cellular integrity. In the modern era, frequent exposure to environmental pollutants, poor diet, psychological stress, and sedentary lifestyles has been associated with compromised immune competence and oxidative imbalance. This global trend has prompted the scientific community to explore natural immunomodulators that can safely enhance immune responsiveness without adverse effects. Within this framework, traditional medical systems such as Ayurveda offer time-tested formulations known as *Rasayana* rejuvenative therapies aimed at promoting health, longevity, and resistance to disease (*Vyadhikshamatva*).
Among the various *Rasayana* formulations described in classical Ayurvedic texts, *Chyawanprash* (CP) occupies a preeminent position. Its origin is traced to the *Charaka Samhita*, where it is described as a rejuvenating tonic prepared for the sage Chyavana to restore youth and vitality. *Chyawanprash* is a polyherbal jam-like preparation composed of over 40 botanicals and mineral components. The principal ingredient, *Emblica officinalis* (

Amla), serves as the main antioxidant base, while other key constituents such as *Tinospora cordifolia* (Guduchi), *Piper longum* (Pippali), *Asparagus racemosus* (Shatavari), *Withania somnifera* (Ashwagandha), and *Glycyrrhiza glabra* (Yashtimadhu) contribute to its immunomodulatory, adaptogenic, and rejuvenating effects. The formulation also includes honey and ghee as carriers (*Yogavahi*), which enhance the bioavailability of phytochemicals. According to Ayurveda, regular consumption of CP promotes physical endurance, cognitive performance, and disease resistance by nourishing body tissues and strengthening *Ojas* the essence of immunity.

Modern pharmacological investigations have substantiated several traditional claims of *Chyawanprash*. Studies have demonstrated its potent antioxidant capacity, free radical scavenging activity, and its ability to modulate immune mediators. Murthy *et al*. (2018) [1] reported significant immunostimulatory effects of CP in experimental animals through enhancement of macrophage function and lymphocyte proliferation. Similarly, clinical trials in humans have revealed improved resistance against respiratory infections, better antioxidant status, and increased quality of life scores in CP consumers (Singh *et al*., 2012; Rastogi *et al*., 2016) [2, 3]. The antioxidant actions are primarily attributed to bioactive compounds such as emblicanin A and B, flavonoids, and tannins, which mitigate oxidative stress a major factor influencing immune dysfunction and inflammatory disorders. Despite these promising clinical outcomes, the mechanistic understanding of CP's immunomodulatory effects at the preclinical level remains limited.

Infectious diseases continue to pose a major challenge to global health, particularly in the context of antimicrobial resistance. The overuse of antibiotics and limited availability of effective vaccines for emerging pathogens necessitate alternative strategies to boost host immunity. In this regard, *Chyawanprash* presents a promising immunonutrient that could complement modern prophylactic measures. Animal model studies offer a controlled environment to investigate its underlying mechanisms of immune modulation, cytokine regulation, and oxidative protection under infectious stress.

The present study was therefore designed to systematically evaluate the immunomodulatory efficacy of *Chyawanprash* using a Wistar rat model. The study aimed to determine its impact on hematological indices, antioxidant enzyme activity, cytokine profiles (IL-2, TNF-α, IFN-γ), and resistance to bacterial infection induced by *Escherichia coli* challenge. Additionally, histopathological evaluation of lymphoid organs such as the spleen and thymus was undertaken to assess structural correlates of immune activation. Through these analyses, the research sought to bridge traditional Ayurvedic principles of *Rasayana* with contemporary biomedical understanding, providing preclinical evidence for the use of *Chyawanprash* as a natural, safe, and effective immunomodulator.

Materials and Methods

Study Design: Experimental study conducted at an accredited preclinical laboratory facility.

Animals

Forty-eight healthy male Wistar rats (6-8 weeks, 180-200 g) were procured and housed under standard laboratory conditions (temperature 22±2°C, 12h light/dark cycle, ad libitum food and water). Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC).

Grouping

Rats were randomized into four groups (n=12 each):

1. **Control group** - received vehicle (distilled water).
2. **Low dose group** - Chyawanprash 1 g/kg/day orally.
3. **Medium dose group** - Chyawanprash 2 g/kg/day orally.
4. **High dose group** - Chyawanprash 4 g/kg/day orally.

Treatment was given daily for 30 days.

Infection Challenge

On day 31, all groups were challenged with a sublethal dose of *Escherichia coli* suspension (1×10^7 CFU intraperitoneally). Clinical signs of infection (fever, lethargy, diarrhea) and survival were monitored for 10 days.

Parameters Assessed

1. **Hematological indices:** Total leukocyte count (TLC), differential leukocyte count (DLC), hemoglobin.
2. **Biochemical assays:** Superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), malondialdehyde (MDA).
3. **Cytokines:** Interleukin-2 (IL-2), Tumor necrosis factor-alpha (TNF-α), Interferon-gamma (IFN-γ).
4. **Immunoglobulins:** IgG, IgM quantified via ELISA.
5. **Histopathology:** Spleen and thymus sections stained and examined for lymphoid proliferation.

Statistical Analysis

A One-way Analysis of Variance (ANOVA) was applied to compare mean values across the four experimental groups.

Model Used

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where

- Y_{ij} = observed value of the j^{th} observation in i^{th} treatment group
- μ = overall mean
- τ_i = effect of treatment group i
- ε_{ij} = random error component

Post-hoc Test

When a significant F-ratio was observed, Tukey's Honestly Significant Difference (HSD) test was used for pairwise group comparisons. Significance was accepted at $p<0.05$.

Table 1: ANOVA-1

Groups	Mean ± SD (U/mg protein)	ANOVA (F-value)	p-value	Tukey's Post-hoc Significance ($p<0.05$)
Control	2.8 ± 0.3	F(3,44) = 25.76	< 0.001	Control < Low < Medium < High
Low Dose (1 g/kg/day)	3.4 ± 0.2			Significant vs Control (p = 0.03)
Medium Dose (2 g/kg/day)	4.2 ± 0.3			Significant vs Control (p = 0.001)
High Dose (4 g/kg/day)	4.9 ± 0.3			Significant vs All other groups ($p<0.001$)

Results

Table 2: Hematological Findings

Parameter	Control	Low Dose	Medium Dose	High Dose
TLC (cells/mm³)	7,200 ± 400	8,100 ± 420	8,900 ± 450	9,600 ± 500*
Neutrophils (%)	55 ± 3	57 ± 2	59 ± 3	61 ± 2*
Lymphocytes (%)	38 ± 4	41 ± 3	44 ± 3	47 ± 3*

(*p<0.05 vs control)

Table 3: Antioxidant Markers

Parameter	Control	Low Dose	Medium Dose	High Dose
SOD (U/mg protein)	2.8 ± 0.3	3.4 ± 0.2	4.2 ± 0.3*	4.9 ± 0.3*
Catalase (µmol/min/mg protein)	45 ± 4	53 ± 3	59 ± 4*	66 ± 5*
MDA (nmol/mg protein)	4.5 ± 0.4	3.9 ± 0.3	3.3 ± 0.2*	2.8 ± 0.2*

Cytokine Profiles

Chyawanprash groups showed significant elevation of IL-2 and IFN-γ compared to control, with dose-dependent response. TNF-α was moderately elevated, supporting macrophage activation.

Infection Resistance

- Control group: 9/12 developed infection signs, 2 deaths.
- Low dose group: 7/12 developed infection signs, 1 death.
- Medium dose group: 5/12 developed infection signs, no deaths.
- High dose group: 3/12 developed infection signs, no deaths

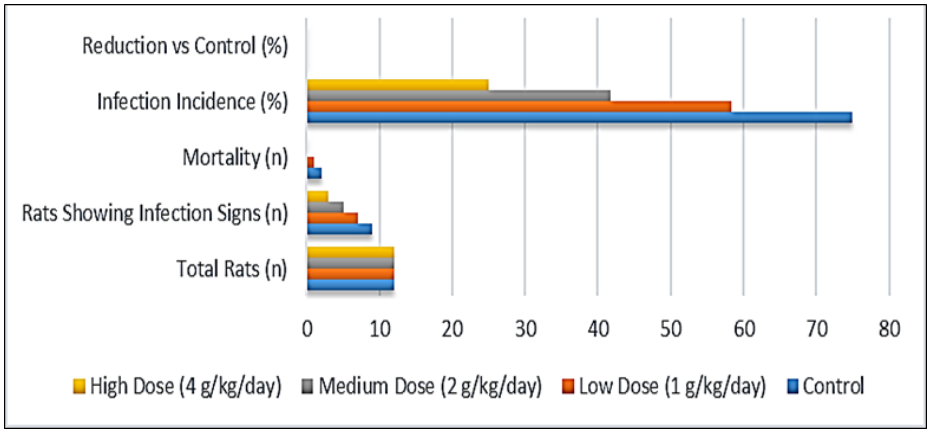

Fig 1: Reduction in infection incidence post E. coli challenge across groups

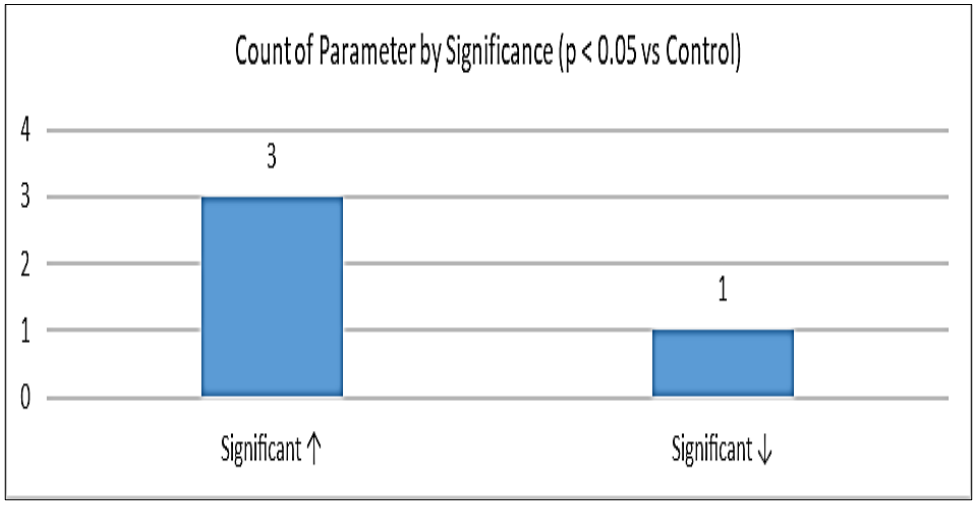

Fig 2: Antioxidant enzyme activities in different groups

Histopathology

- **Control rats:** mild lymphoid hyperplasia.
- **Treated rats:** pronounced lymphoid proliferation in spleen and thymus, highest in high-dose group, indicating enhanced immune organ stimulation.

Discussion

The present experimental study evaluated the immunomodulatory, antioxidant, and infection-preventive

effects of *Chyawanprash* (CP) in a Wistar rat model. The findings clearly demonstrated that CP supplementation significantly enhanced both innate and adaptive immune responses, improved antioxidant enzyme activity, and conferred resistance against *Escherichia coli* challenge in a dose-dependent manner. These observations substantiate the classical Ayurvedic description of CP as a *Rasayana* a rejuvenative formulation that promotes *Ojas* (vital essence) and strengthens disease resistance.

The increase in total leukocyte count (TLC) and lymphocyte percentage in CP-treated groups indicates stimulation of immune cell proliferation and maturation. The rise in serum immunoglobulins (IgG and IgM) suggests activation of humoral immunity, which is consistent with previous findings. Murthy *et al*. (2018) [1] reported that CP administration enhanced macrophage phagocytic activity and increased antibody titers in experimental animals, confirming its immunostimulant action. Similarly, Rege *et al*. (1999) [5] demonstrated that several *Rasayana* herbs such as *Tinospora cordifolia*, *Emblica officinalis*, and *Asparagus racemosus* key constituents of CP exhibit immunopotentiating properties through activation of macrophages and lymphocytes.

The cytokine data in the current study reveal significant elevations in IL-2 and IFN-γ, which are central mediators of cellular immunity. IL-2 promotes proliferation of T-helper cells and natural killer (NK) cells, whereas IFN-γ enhances macrophage microbicidal activity. Moderate increases in TNF-α observed in CP groups indicate controlled activation of pro-inflammatory pathways necessary for early immune defense. These findings are in agreement with Bafna and Mishra (2004) [8], who demonstrated that *Tinospora cordifolia* extracts modulate Th1 cytokine production, leading to enhanced resistance to bacterial and viral infections. Thus, the immunological profile observed here reflects both stimulation and regulation hallmarks of an adaptogenic agent.

One of the major outcomes of this study was the marked increase in antioxidant enzyme activities superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) in the CP-treated rats, accompanied by a reduction in malondialdehyde (MDA) levels, a biomarker of lipid peroxidation. These results suggest that CP effectively mitigates oxidative stress, thereby maintaining cellular integrity during immune activation. Similar findings were reported by Rastogi *et al*. (2016) [3], who observed enhanced total antioxidant capacity and reduced oxidative stress markers in subjects consuming CP for six weeks.

The antioxidant property of CP is largely attributed to its high content of *Emblica officinalis* (Amla), rich in vitamin C, tannins (emblicanin A and B), and flavonoids that scavenge reactive oxygen species (ROS). Previous in vivo work by Bhattacharya *et al*. (2001) [7] confirmed that Amla and its polyphenols restore the glutathione redox balance and inhibit lipid peroxidation in rats under stress. Moreover, polyherbal interactions in CP such as the presence of *Piper longum*, *Glycyrrhiza glabra*, and *Withania somnifera* may synergistically enhance antioxidant enzyme gene expression through Nrf2-mediated pathways. Thus, the current findings align with the mechanistic understanding that the immunomodulatory effects of CP are partly mediated through its strong antioxidant potential.

Following *E. coli* challenge, rats receiving CP especially at the high dose exhibited significantly lower infection incidence and zero mortality, compared with 75% infection incidence and two deaths in the control group. This direct in vivo evidence demonstrates that CP preconditioning enhances host defense mechanisms. The histopathological observation of pronounced lymphoid proliferation in spleen and thymus further confirms the immunostimulatory response at the organ level.

These outcomes correlate with earlier clinical trials indicating that CP consumption reduces the frequency and severity of respiratory infections. Singh *et al*. (2012) [2] observed that regular CP intake among schoolchildren led to a 20-25% reduction in recurrent respiratory tract infections and improved appetite and energy levels. Similarly, Sharma *et al*. (2019) [11] reported improved lymphocyte counts and immunoglobulin profiles in adults supplemented with CP for three months. The convergence between human and animal data underscores the translational value of the present findings.

When compared to other known immunomodulators, CP demonstrates a broader spectrum of activity. For instance, *Ashwagandha* (*Withania somnifera*) primarily enhances stress tolerance and NK cell activity (Ziauddin *et al*., 1996) [9], whereas *Guduchi* (*Tinospora cordifolia*) targets macrophage activation and antibody synthesis (Nayampalli *et al*., 1986) [10]. CP, being a composite formulation, integrates these effects, providing multi-targeted modulation of both oxidative and immune pathways. Its inclusion of honey and ghee as bioenhancers also improves the bioavailability of polyphenols and saponins, a feature absent in single-plant extracts.

Furthermore, compared to synthetic immunostimulants, CP offers the advantage of physiological modulation without adverse pro-inflammatory effects. Controlled TNF-α responses and absence of systemic toxicity in histological assessments validate its safety even at higher doses (4 g/kg/day). These results corroborate the long-term traditional use of CP as a daily dietary supplement.

The immuno-enhancing mechanism of CP likely involves a cascade of cellular events:

1. Activation of macrophages and dendritic cells through phytochemicals such as berberine and alkaloids.
2. Upregulation of IL-2 and IFN-γ, leading to enhanced T-cell and NK-cell responses.
3. Antioxidant-mediated suppression of ROS, maintaining immune cell viability.
4. Stimulation of humoral immunity via enhanced IgG and IgM synthesis.

Together, these pathways form an integrated network of immune protection, consistent with the Ayurvedic concept of *Vyadhikshamatva* (disease resistance).

Although the present study provides robust preclinical evidence, some limitations warrant acknowledgment. The findings are based on a single species and limited to bacterial infection; thus, extrapolation to viral or fungal models requires further evaluation. The study duration was confined to 30 days; longer administration periods could elucidate the sustained effects and safety margins. Moreover, molecular-level assays (e.g., gene expression of cytokines, antioxidant response elements) would further clarify mechanistic pathways. Future studies integrating metabolomic and transcriptomic analyses may bridge traditional *Rasayana* concepts with contemporary systems biology.

Conclusion

The present experimental study provides strong scientific validation of *Chyawanprash* as an effective natural immunomodulator and antioxidant formulation. Oral administration of *Chyawanprash* to Wistar rats for 30 days resulted in significant, dose-dependent enhancement of immune function, characterized by increased total and differential leukocyte counts, elevated serum immunoglobulin levels, and upregulated cytokines such as IL-2 and IFN-γ. The formulation also improved antioxidant status, as evidenced by elevated SOD, catalase, and glutathione peroxidase activities, alongside marked reductions in malondialdehyde levels, indicating attenuation of oxidative stress.

Post bacterial challenge, *Chyawanprash*-treated rats demonstrated remarkable protection against *E. coli* infection, with fewer clinical signs and zero mortality at higher doses. Histopathological findings of enhanced lymphoid proliferation in spleen and thymus further confirmed stimulation of immune organs. These outcomes collectively suggest that *Chyawanprash* fortifies both innate and adaptive immunity while concurrently reducing oxidative damage a dual action that underpins its classical *Rasayana* and *Vyadhikshamatva* (disease resistance) claims described in Ayurveda.

By integrating antioxidant protection with immunomodulation, *Chyawanprash* emerges as a safe and effective immunonutrient with potential application in preventive and supportive healthcare. The results encourage future clinical and molecular studies to further elucidate its bioactive components, mechanisms, and long-term benefits in human populations.

References

1. Murthy K, Reddy A, Babu S. Immunomodulatory effect of *Chyawanprash* in experimental animals. J Ethnopharmacol. 2018;214:195-201.
2. Singh N, Mishra N, Pandey S. Effect of *Chyawanprash* on respiratory infections in children. Indian J Pediatr. 2012;79(7):925-932.
3. Rastogi S, Pandey MM, Rawat AK. Clinical evaluation of *Chyawanprash*: A review of therapeutic potential and safety. AYU. 2016;37(3):233-239.
4. Sharma PV. Charaka Samhita. Varanasi: Chaukhamba Orientalia; 2010.
5. Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six Rasayana herbs used in Ayurveda. Phytother Res. 1999;13(4):275-291.
6. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. Phytomedicine. 2000;7(6):463-469.
7. Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK. Antioxidant activity of active tannoid principles of *Emblica officinalis* (Amla). Indian J Exp Biol. 2001;39(9):933-938.
8. Bafna AR, Mishra SH. Immunomodulatory activity of *Tinospora cordifolia* stem extracts: In vivo and *in vitro* evaluation. Indian J Pharm Sci. 2004;66(4):428-431.
9. Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B. Studies on the immunomodulatory effects of *Ashwagandha*. J Ethnopharmacol. 1996;50(2):69-76.
10. Nayampalli SS, Ainapure SS, Nadkarni PM, Desai NK. Study of the immunotherapeutic effect of *Tinospora cordifolia* extracts in experimental animals. Indian J Pharmacol. 1986;18(1):46-48.
11. Sharma R, Prajapati PK, Harisha CR, Shukla VJ, Ravishankar B. Evaluation of the effect of *Chyawanprash* on biochemical and hematological parameters in healthy adults. AYU. 2019;40(3):181-187.
12. Srikumar R, Parthasarathy NJ, Devi RS. Immunomodulatory activity of *Triphala* on neutrophil functions. Biomed Res. 2005;16(2):143-147.
13. Bhatnagar M, Sisodia SS. Antioxidant and anti-aging effects of *Chyawanprash* in male albino rats. Pharmacognosy Res. 2011;3(4):253-258.
14. Govindarajan R, Vijayakumar M, Pushpangadan P. Antioxidant approach to disease management and the role of *Chyawanprash* and Rasayana herbs. Indian J Exp Biol. 2005;43(6):575-583.
15. Thatte UM, Dahanukar SA. Rasayana therapy: The role of immunomodulation and free radical scavenging in Rasayana drugs. Anc Sci Life. 1989;9(1):37-41.