

Comparative Immunomodulatory Activity of Methanolic Extract obtained from *Tabernaemontana alternifolia* Leaves and Stem

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Abstract

Tabernaemontana alternifolia, a plant of the Apocynaceae family and commonly referred to as Kampillakah, is a medicinal plant recognized for its remarkable therapeutic benefits. The plant stems, roots, and leaves are abundant in alkaloids, which have been traditionally used to treat cancer, diarrhea, and syphilis. Due to bioactive alkaloids and phenolic compounds, the current study aimed to evaluate the immunomodulatory effects of methanolic extracts from *Tabernaemontana alternifolia* leaves (TAL). It stems (TAS) in mitigating cyclophosphamide-induced myelosuppression in rats. Preliminary phytochemical analyses revealed the presence of compounds known for immunomodulatory properties. The administration of these extracts resulted in a notable recovery of hemoglobin levels and red and white blood cell and platelet counts, with TAL demonstrating greater effectiveness compared to TAS. Body weight measurements corroborated the protective effects, such as TAL and TAS significantly increased rat body weight during treatment. Furthermore, TAL and TAS enhanced neutrophil adhesion, an important marker of immune system activation. Overall, the results indicate that TAL, in particular, offers notable immunomodulatory benefits that may help mitigate the adverse effects of chemotherapy-induced myelosuppression and boost immunity against infections, delineating its potential for therapeutic use in integrative medicine.

Keywords: Cyclophosphamide; Immunomodulatory; Myelosuppression; *Tabernaemontana*.

1. Introduction

The immune system, primarily involving leukocytes and other components, is crucial for defending against infections and diseases [1] Disruptions within this

system can lead to various illnesses, underlining the importance of immunomodulation research. Immunotherapy is increasingly favored over chemotherapy and aligns with Ayurvedic principles like

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"Rasayana," which focuses on agents modulating immune responses [2]

Natural immunomodulatory compounds can complement conventional cancer treatments by enhancing immune response and patient quality of life, prompting investigations into medicinal herbs as immunomodulators [3] With synthetic compounds prevalent in pharmaceutical research, the lack of new agents necessitates exploring natural resources for immunomodulatory compounds [4] Natural alternatives are also needed due to the side effects of immunosuppressants like cyclosporine, tacrolimus, and methylprednisolone [5]

Traditional medicines, particularly plant-derived substances such as tannins, polysaccharides, flavonoids, lectins, and peptides, have shown significant immunomodulatory potential crucial for immune system modulation [6] Recent trends favor natural compounds for regulating pathological immune responses in immune-mediated diseases, though thorough efficacy and safety evaluations are necessary [7-9] The alkaloid piperine, extracted from *Piper nigrum* and *Piper longum*, has shown immunomodulatory effects in vitro by reducing pro-inflammatory cytokines, including IL-1 β , IL-6, and TNF- α [10]. Similarly, colchicine from *Colchicum autumnale* demonstrated inhibition of IL-1 β , IL-6, and TNF- α in TC-1 cells [11]. Berberine, obtained from *Hydrastis canadensis* and *Coptis chinensis*, was found to down-regulate Th1/Th2 cytokines in primary mouse splenocytes [12, 13]. Additionally, flavonoids in *Emblica officinalis* exhibit various pharmacological activities, including anticancer, antioxidant, anti-inflammatory, and immunomodulatory properties [10]

The genus *Tabernaemontana*, belonging to the Apocynaceae family, comprises approximately 100 species distributed across tropical and subtropical regions worldwide. Historically, many of these species have been used for their medicinal properties. Phytochemical analyses of *Tabernaemontana* species have revealed alkaloids and other key secondary plant metabolites like camptothecine, vincamine, and ibogaine, potentially responsible for their immunomodulatory effects [11-13] However, no research has yet explored the immunomodulatory activity of *T. alternifolia* extracts. This study evaluates and compares the immunomodulatory effects of methanolic leaf extracts (TAL). It stems (TAS) of *T.*

alternifolia in rats subjected to cyclophosphamide-induced myelosuppression, focusing on parameters such as body weight and neutrophil adhesion.

2. Materials and Methods

2.1. Plant Materials and Chemicals

The entire *T. alternifolia* plant was collected from the Ratnagiri region in Maharashtra, India. The College of Forestry in Dapoli (Ratnagiri) authenticated the plant material, and the voucher specimen was documented as BSKKV/TC/4216/10.

The leaves and stems of *T. alternifolia* were collected, thoroughly washed, and dried in an air dryer at 55°C for 48 hours. The dried plant materials were then ground into a fine powder using a Wiley mill (Model 4276-M, Thomas Scientific, USA), passed through a 20-mesh sieve, and stored in sealed plastic bags. All chemicals utilized in this study were of analytical grade.

2.2. Extract Preparation

The leaves and stems of *T. alternifolia* were dried separately in the shade and then ground into a coarse powder. These powdered materials were individually extracted with methanol as the solvent using a Soxhlet apparatus. Following the extraction process, the methanol was evaporated under reduced pressure using a vacuum rotary evaporator to obtain the methanolic extracts of *T. alternifolia* leaves (TAL) and stems (TAS). Extracts were subjected to a preliminary analysis of their phytoconstituents [14]

2.3. Experiment Animals

Swiss albino mice and rats of both sexes, weighing 20–25 g and 100–150 g, respectively, were employed for immunomodulatory studies. The animals were maintained under standard laboratory conditions throughout the experiments, receiving standard food pellets and unrestricted access to water. The pharmacological study and acute toxicity protocol were approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, with registration number CPCSEA/50/10.

2.4. Immunomodulator Activity

Experimental setup

Group I: Control group (10 ml/kg/day of saline)

Group II: Cyclophosphamide (30 mg/kg intra peritoneal)

Group III TAL (200 mg/kg/day in 1% carboxy methyl cellulose, p.o)

Group IV: TAS (200 mg/kg/day in 1% carboxy methyl cellulose, p.o.)

2.5. Cyclophosphamide induced myelosuppression

The method described by Ziauddin *et al.* (1996) was used to induce cyclophosphamide (CP) myelosuppression [18]. Albino rats were divided into four groups: a control group (Group I) and three treatment groups (Groups II to IV), each comprising six rats. The control group received a saline solution. Group II was administered CP alone at a dose of 30 mg/kg intraperitoneally. At the same time, Groups III and IV received CP along with TAL and TAS at a dose of 200 mg/kg orally for 10 consecutive days. On the 11th day, blood samples were collected from the retro-orbital plexus of each animal and analyzed for hematological parameters.

2.6. Determination of body weight

A single dose of TAL and TAS was orally administered to the animals for 20 days. Initial body weight measurements were taken one day before drug administration. On the 20th day, body weight was measured following the protocol described by Benslay and Bendele (2005) [15]

2.7. Neutrophil adhesion test and hematological parameters

The method outlined by Smith *et al.* (1979) was employed to evaluate neutrophil adhesion. In Group I, rats were the control, and 10 ml/kg of normal saline was administered. Groups II and III received different concentrations of TAL and TAS (200 mg/kg orally), respectively. On the 14th day following drug administration, blood samples were collected by puncturing the retro-orbital plexus into heparinized vials. These samples were analyzed for total leukocyte count (TLC) and differential leukocyte count (DLC). After initial counts, the blood samples were incubated with nylon fibers at 37°C for 15 minutes. Following incubation, the blood samples were re-examined for TLC and DLC to determine the neutrophil index. The percentage of neutrophil adhesion was calculated using the following formula.

$$\text{Neutrophil adhesion (\%)} = \frac{(\text{NI}_u - \text{NI}_t)}{\text{NI}_u} \times 100$$

NI_u represents the neutrophil index of untreated blood samples, and NI_t denotes the neutrophil index of treated blood samples [5, 16]

2.8. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences between the data were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Preliminary phytochemical screening

Phytochemical screening of TAL and TAS methanolic extracts identified alkaloids, phenols, amino acids, coumarins, tannins, steroids, terpenoids, and flavonoids. Given that many secondary metabolites in the TAL and TAS methanolic extracts have immunomodulatory properties, an immunomodulatory study was conducted on these extracts, as represented in **Table 1**.

Table 1. Phytochemical test of Methanolic extracts of *T. alternifolia* leaf and stem

| Chemical test | Leaves Methanolic extract | Stem Methanolic extract |
|-----------------------|---------------------------|-------------------------|
| Alkaloids | | |
| Dragendroff's Reagent | + | + |
| Mayer's reagent | + | + |
| Hager's reagent | + | + |
| Wagner's reagent | + | + |
| Glycosides | | |
| Fehling | - | - |
| Legal | - | - |
| Tannins | | |
| Gelatin | + | + |
| Lead acetate | + | + |
| Carbohydrates | | |
| Molisch | - | - |
| Fehling | - | - |
| Flavonoids | | |
| Alkali | + | + |
| Amino acids | | |
| Ninhydrin | - | - |
| Millon's | - | - |
| Phenol | | |
| Ferric chloride | + | + |
| Steroid | | |
| Salkowski | + | + |
| Gums | | |
| Hydrochloric acid | - | - |
| Resin | | |
| Water | - | - |
| Hydrochloric acid | - | - |
| Terpenoid | | |
| Salkowski | + | + |
| Lieberman | + | + |
| Saponin | | |
| Foam test | - | - |

(+ Present; - Absent)

3.2. Cyclophosphamide-induced bone marrow suppression

Figure 1 illustrates the impact of TAL and TAS on cyclophosphamide-induced bone marrow suppression. Cyclophosphamide (30 mg/kg, intraperitoneally) significantly reduced hemoglobin, red blood cell (RBC), white blood cell (WBC), and platelet counts. Concurrent administration of TAL and TAS with cyclophosphamide restored these values, which had been suppressed by cyclophosphamide alone. Platelet and WBC counts even exceeded those of the control group. Among the extracts, TAL was more effective than TAS. The results were statistically significant compared to the control group ($P < 0.05$).

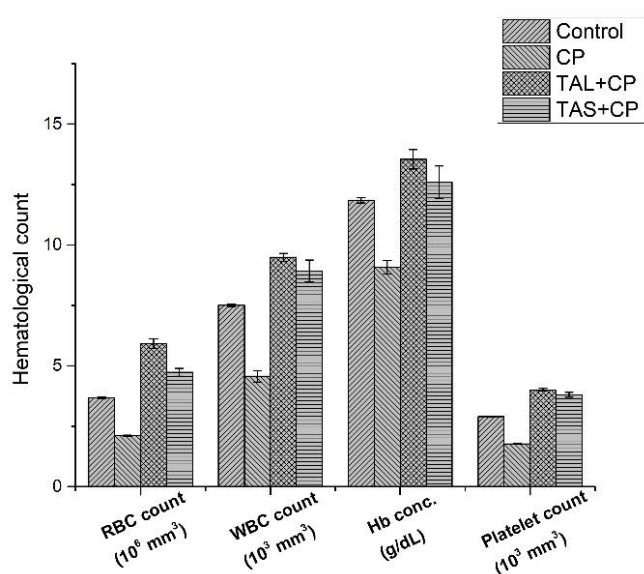


Figure 1. Effect of TAL and TAS on cyclophosphamide-induced bone marrow suppression.

3.3. Measurement of body weight

Body weight assessments before and after drug treatment showed a significant difference compared to the control

group ($P < 0.05$). TAL and TAS significantly increased the rats' body weight more than the control. Similar results were reported for *Tabernaemontana coronaria* [17] The highest body weight increase, reaching 205.0 g, was observed with TAL at 200 mg/kg after 20 days of treatment. **Table 2** represents the effect of TAL and TAS on body weight.

Table 2. Effect of TAL and TAS on Body weight.

| Groups | Dose (mg/kg) | Body Weight (g) | |
|---------|--------------|---------------------|----------------------|
| | | 0 th day | 20 th Day |
| Control | 10 | 149.33±0.4 | 180.50±0.5 |
| CP | 30 | 149.83±0.5 | 121.50 ± 0.8** |
| TAL | 200 | 148.50 ± 0.84 | 205.66 ± 0.4** |
| TAS | 200 | 148.33 ± 0.80 | 190.00 ± 0.5* |

*All values are expressed as mean ± SEM (n=6); One-way ANOVA followed by Dunnett's test; * $p < 0.05$ and ** $p < 0.01$ considered significant as compared to control.

3.4. Neutrophil adhesion test

Table 3 presents the effects of TAL and TAS on neutrophil adhesion. Neutrophils, terminal cells with limited division and protein synthesis abilities, exhibit various responses, including chemotaxis, phagocytosis, exocytosis, and antigen killing. In this study, both TAL and TAS at a dose of 200 mg/kg orally significantly enhanced neutrophil percentages, suggesting a potential increase in immunity against microbial infections. This test highlights the marginalization of phagocytic cells within blood vessels, indicating immune system activation. The neutrophil adhesion percentages were 13.99% in the control group, 17.64% for TAL, and 14.47% for TAS at 200 mg/kg. TAL and TAS demonstrated a significant dose-dependent increase in neutrophil adhesion compared to the control group.

Table 3. Effect of TAL and TAS on neutrophil adhesion.

| Group | Dose (mg/kg) | TLC | | Neutrophil adhesion | | Neutrophil Index | | % Neutrophil adhesion |
|---------|--------------|-------------|---------------|---------------------|----------------|------------------|----------------|-----------------------|
| | | UTB | TB | UTB | TB | UTB | TB | |
| Control | 10 | 7.26±0.17 | 6.84±0.13 | 56.58 ± 0.74 | 53.46± 0.32 | 417.81± 0.94 | 359.23 ± 0.80 | 13.99±0.35 |
| CP | 30 | 5.66±0.04 | 5.25± 0.07 | 46.69 ± 0.29 | 44.61± 0.35* | 264.30± 0.23 | 234.48 ± 0.20* | 12.24± 0.22 |
| TAL | 200 | 9.08±0.11** | 8.06±0.11** | 69.07 ± 0.61** | 64.34 ± 0.67** | 629.75 ± 0.11** | 518.93± 0.11** | 17.64±0.57** |
| TAS | 200 | 8.37± 0.04* | 7.84 ± 0.03** | 64.14 ± 0.5* | 58.53 ± 0.49** | 537.01± 0.68** | 459.15±0.45** | 14.47±0.55* |

*All value expressed an ± SEM (n=6); One-way ANOVA followed by Dunnett's test; * $P < 0.05$ and ** $p < 0.01$ considered significant as compared to control.

4. Conclusions

The study's findings show that TAL and TAS effectively reduce drug-induced myelosuppression side effects, evidencing their balancing and adaptogenic properties. Additionally, TAL and TAS significantly increased neutrophil percentages, with TAL displaying superior immunomodulatory activity due to its higher concentration of secondary metabolites. This effect suggests the potential for enhanced immunity against microbial infections.

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Conflict of interest

The authors have no conflicts of interest associated with this research.

Data availability

The dataset used and/or analyzed in the current study is available upon request.

Authors Contributions

Mrs. Pallavi Karanje and Dr. Ajay Namdeo conceptualized and designed the study, with Dr. Vishwajeet Ghorpade handling data collection. Dr. Jyotiram Sawale conducted data analysis and prepared the initial draft of the article. Mr. Abhijit Karanje supervised the study, Ms. Jotsana Gandhi and Dr. Rohan Phatak contributed to data analysis and interpretation, and Dr. Namdeo Jadhav provided essential revisions. All authors have reviewed and approved the final version of the manuscript.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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