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## Study of immunomodulatory activity of *Putranjiva roxburghii* Wall. using cyclophosphamide induced immune suppressed mice. (*In vivo* study)

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### Abstract

Modulation of immune functions by using medicinal plants and their products as a possible therapeutic measure has become fundamental principle of therapeutic approach. Plants and minerals have been used since ancient times for the treatment of many ailments and diseases. It is now being recognized that immunomodulation of immune response could provide an alternative to conventional chemotherapy for a variety of disease conditions, especially when host's defense mechanism has to be activated under the conditions of impaired immune responsiveness or when a selective immunosuppression has to be induced in situation like autoimmune disorders and organ transplantation.

In the present research work, immunomodulatory activities of *Putranjiva roxburghii* Wall. is evaluated by studying their protective effects against leucopenia/ neutropenia produced by a single dose of cyclophosphamide on Swiss albino mice. (*In vivo* study) and the results are compared with that of the known immunomodulators, *Tinospora cordifolia* Miers. (Rasayana plant) and lithium carbonate (Synthetic drug).

**Keywords:** *Putranjiva roxburghii* Wall., cyclophosphamide, *Tinospora cordifolia* Miers, Lithium carbonate

### 1. Introduction

Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of 'Rasayana' is based on related principles [1]. Rasayana, listed as a class in the texts of traditional Indian medicine literature, consists of a number of plants reputed to promote physical and mental health, improve defense mechanisms of the body and enhance longevity. A number of medicinal plants as Rasayanas have been claimed to possess immunomodulatory activities. Some of the Rasayana plants used as immunomodulatory agents are *Withania somnifera* Linnaeus., *Tinospora cordifolia* Miers, *Asparagus racemosus* Willd. And *Mangifera indica* Linnaeus [2-5] are well known for their traditional uses. Furthermore, medicinal plants used for immunomodulation can provide potential alternatives to conventional chemotherapies for a variety of diseases, especially when the host defense mechanism has to be activated under the conditions of impaired immune response. *Putranjiva roxburghii* Wall. Is described in Charak Samhita as a "rasayana" plant and is thus expected to show immunomodulatory activity [6, 7].

The present study was undertaken to evaluate the effect of aqueous extract of leaves of *Putranjiva roxburghii* Wall. On the immune system using Swiss albino mice as the experimental model to substantiate the traditional claim.

### 2. Materials and Methods

#### 2.1 Plant Materials

*Putranjiva roxburghii* Wall. Leaves were collected from 'Keshav Shrushti', Mumbai, India. And authenticated from Botanical Survey of India, Pune. (No. BSI/WC/Tech/2008/177). An authentic sample of stem powder of *Tinospora cordifolia* Miers. was procured from the Ayurvedic retail shop.

#### 2.2 Chemicals

Cyclophosphamide monohydrate (purity 99.5%) and lithium carbonate (purity  $\geq$  99%) were procured from Sigma-Aldrich Chemie (Schnellendorf, Germany).

#### 2.3 Mice as the animal model

The Swiss albino mice, weighing about 20-23 grams, three- four weeks old were purchased

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from Haffkine Bio- Pharma. Corpn. Ltd. (A Govt. of Maharashtra undertaking), Parel, Mumbai, India.

**3. Drug administration**

The study was conducted on 24 male Swiss albino mice, divided into four groups of six mice per group, Group I was observed as the control group and all six animals belonging to

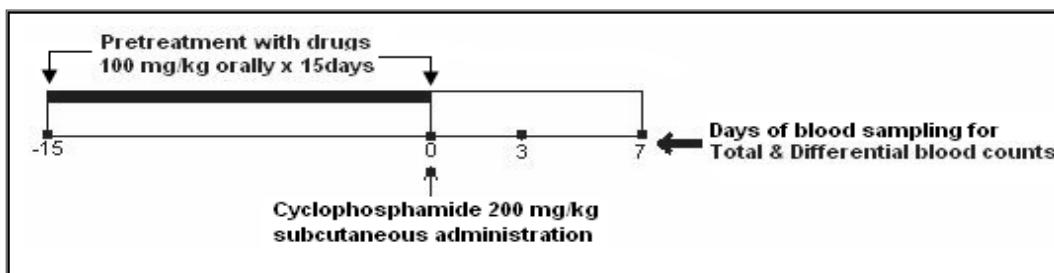
the group were fed with normal diet and were given 1.0 mL of distilled water orally by a gavage, for first 15 days prior to cyclophosphamide administration. Similarly, animals belonging to Group II to Group IV were also fed with normal diet and were pretreated for 15 days with the drug orally by a gavage as mentioned in table 1. This protocol of pretreatment was followed as prescribed in Ayurveda [8].

**Table 1:** Drug treatment administered to various groups

Group n = 6/ group	Drug Treatment	Concentration of Dose & Duration
I (Control Group)	Distilled water	1.0 mL of distilled water/animal, for 15 days
II (Positive Control)	Lithium Carbonate	100 mg/ kg bodyweight/ animal, in 1.0 mL of distilled water, for 15 days
III (Positive Control)	Stem powder of <i>Tinospora cordifolia</i> Miers.	
IV	Leaf powder of <i>Putranjiva roxburghii</i> Wall.	

n- Number of animals

**Dosage regimen**



**Fig 1:** Schedule for the present study

In the present study all the animals (mice) were rendered neutropenic by subcutaneous injection of single dose of cyclophosphamide of concentration 200 mg/kg bodyweight/ animal after administration of respective drugs for 15 days as shown in the dosage regimen (Figure 1). The day on which cyclophosphamide was administered was labeled as zero (0) day.

Blood samples (~200 µl) were taken from the retro-orbital plexus in heparinized capillary tubes, on day -15, day 0, and day 3 as shown in Figure 1. Total and Differential cell counts (neutrophils, lymphocytes and monocytes) were performed

for each blood sample using Neubauer chamber followed by microscopic examination of Wright-stained smears with 100 X objective.

**4. Result and Discussion**

The results of blood counts viz. mean total leukocyte counts and mean absolute neutrophil counts for six animals belonging to respective groups are tabulated in Table 2. The total leukocyte counts and the absolute neutrophil counts in the drug treated group were compared with the values of the control group.

**Table 2:** The Total Mean Leukocyte and Mean Absolute Neutrophil count of control and drug treated animals.

Days for blood counts	-15 <sup>th</sup> day		0 day		3 <sup>rd</sup> day		7 <sup>th</sup> day	
	Counts (cells/mm <sup>3</sup> )		Counts (cells/mm <sup>3</sup> )		Counts (cells/mm <sup>3</sup> )		Counts (cells/mm <sup>3</sup> )	
Group.	Mean Leukocyte	Mean Absolute Neutrophil	Mean Leukocyte	Mean Absolute Neutrophil	Mean Leukocyte	Mean Absolute Neutrophil	Mean Leukocyte	Mean Absolute Neutrophil
I	5800.0	3102.9	6053.3	3174.2	2966.7	1310.3	15566.7	9573.5
II	6483.3	3166.0	9316.7	4518.6	6400.0	2901.3	15108.3	9367.2
III	6133.3	2923.6	9203.3	5215.2	6816.7	3681.0	16066.7	9934.6
IV	5433.3	2198.5	10016.7	5728.6	5699.0	2342.9	16450.0	9722.5

After fifteen days of drug treatment to the animals belonging to Group II to Group IV, on “Day 0” it was observed that there was a rise in the total leukocyte count and the absolute neutrophil count, as compared to the blood counts measured on -15<sup>th</sup> Day. Thus all four drug treated groups showed a significant leukocytosis (increase in total leukocyte count) with predominant neutrophilia (increase in absolute neutrophil count). The drug treated mice, belonging to Group II to Group IV thus showed an increased number of cell counts revealing the immune stimulated condition.

The administration of single subcutaneous dose of cyclophosphamide, at concentration of 200 mg/kg

bodyweight of animals to the drug treated groups produced a fall in total leukocyte count and absolute neutrophil count on “3<sup>rd</sup> Day” of blood count measurement as shown in Figure 2 and Figure 3.

However, it was observed that the total leukocyte counts and the absolute neutrophil counts of the drug treated animals belonging to Group II to Group IV were not as low as that of Control animals (Group I). This effect occurred due to the counter activity of the respective drugs administered for the first fifteen days to mice belonging to Group II to Group IV, against the immunosuppressive action of cyclophosphamide. Thereafter an increase in the total leukocyte count and

absolute neutrophil count was observed on Day 7, in all the animals of drug treated groups, similar to that of the control

group. This increase in blood counts is attributed to the irreversible toxic effects produced by cyclophosphamide.

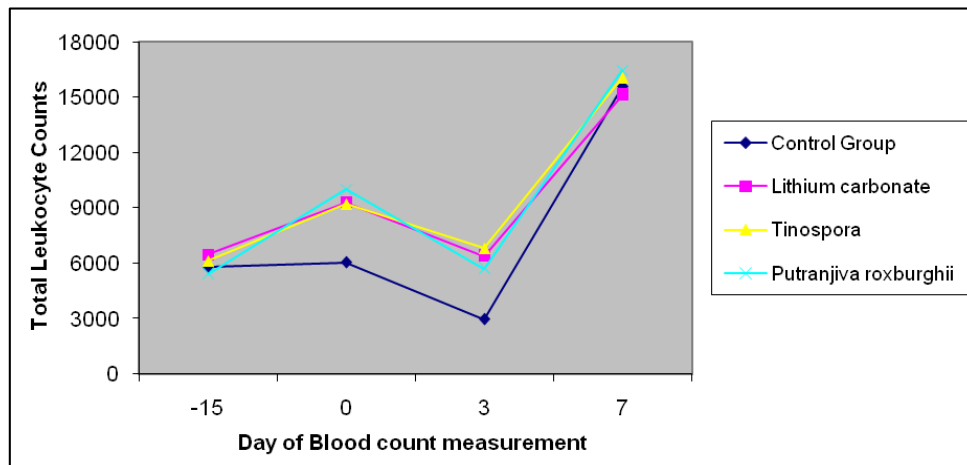


Fig 2: Mean graph of Total leukocyte counts against the day of blood count measurement

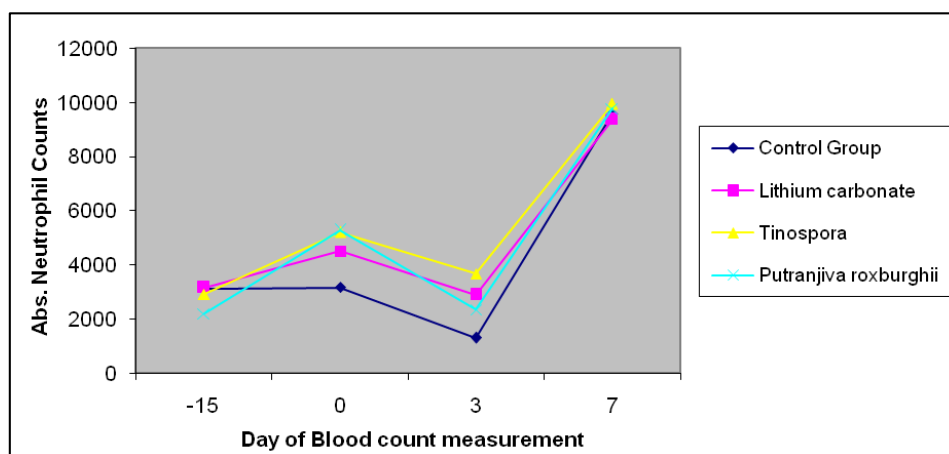


Fig 3: Mean graph of Absolute neutrophil counts against the day of blood count measurement

### 5. Conclusion

The leaf powders of *Putranjiva roxburghii* Wall. Produced leucocytosis with neutrophilia on pretreatment. Also when compared to control group the total aqueous extract of leaf powders of *Putranjiva roxburghii* Wall. Prevented, leucopenia and neutropenia produced by cyclophosphamide in mice. Therefore on the basis of results of the present study it is concluded, that the plant is potent immunostimulant with effects comparable to known immunostimulants, lithium carbonate and *Tinospora cordifolia* Miers. The leaf powders of *Putranjiva roxburghii* Wall. Can thus be recommended for use in certain herbal formulations with immune enhancing activity.

### 6. Acknowledgement

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