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## Research Article



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### **‘Neutrophil Adhesion Test’ as a tool for assessment of immunomodulatory activities of blended formulation of *Chlorophytum borivillianum*, *Withania somnifera*, *Wagatia spicata* Dalz., *Picrorrhiza kurroa* and *Spilanthes paniculata***

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## Abstract

Immunomodulatory activities of blended formulations of *Chlorophytum borivillianum*, *Withania somnifera*, *Wagatia spicata* Dalz., *Picrorrhiza kurroa* and *Spilanthes paniculata* were studied in three different groups of ‘Swiss Albino Mice’ each having three males and three females, such as Control having no treatment, Standard having treatment of FDA approved Ayurvedic immunomodulator and Test group treated with blended formulations of the selected plant derivatives. Parameter used for assessment was ‘Neutrophil Adhesion Test’. Following Animal study with approval of ‘Animal Ethics Committee ‘vide study code no 169 significant increase in percent neutrophil count was observed in Test group. At the same time significant increase in mean corpuscular haemoglobin concentration was observed in Standard group. Blended formulations of *Chlorophytum borivillianum*, *Withania somnifera*, *Wagatia spicata* Dalz., *Picrorrhiza kurroa* and *Spilanthes paniculata* exhibited immunomodulatory activities in Swiss Albino mice in terms of ‘Neutrophil Adhesion Test.’

**Keywords:** Neutrophil Adhesion Test, Immunomodulator, *Chlorophytum borivillianum*, *Withania somnifera*, *Wagatia spicata* Dalz., *Picrorrhiza kurroa* and *Spilanthes paniculata*

## Introduction

Since ancient time, Ayurvedic Medicines are used for maintaining human health<sup>9,10,12</sup>. Many researchers gave experimental evidences for immunomodulating features of plants<sup>2</sup>. Immunocomprised condition including AIDS and many of autoimmune diseases are characterized by dysfunction of immune system or declined state of immune system<sup>12</sup>. Allopathic medication of such diseases has remained expensive and reported for serious side effects. Potential use of immunomodulatory principles of plants for the treatment of such diseases requires careful experimental validation in ‘Animal Models’. *Chlorophytum borivillianum* belongs to Family Agavaceae. It has been described as an immunomodulator<sup>3,7,9</sup>, sperm enhancer<sup>3,4</sup> and

rejuvenator<sup>9</sup>. *Withania somnifera* belongs to Family Solanaceae. It is common in North Western Hilly regions of India. It has been described as sperm enhancing, disease protective agent in literature. *Wagatia spicata* Dalz. belongs to Family Leguminosaceae and is multipurpose medicinal base as described by literature<sup>6</sup>. *Picrorrhiza kurroa* belongs to Family Scrofulariaceae and biopolymeric fraction significantly increases lymphocyte growth, IL- 4 and IFN- $\gamma$  in serum<sup>1</sup>. It is also reported as sperm enhancer<sup>5</sup>. *Spilanthes paniculata* belongs to Family Asteraceae and is used as Diuretic and immunomodulatory<sup>12</sup>. Depressed Neutrophil superoxide production was observed in human immunodeficiency virus infection<sup>11</sup>. The Wilkinson method[1978] is routinely

used for evaluating the effect of herbal extracts on neutrophil adhesion. Many medicinal plants could be better exploited by using synergistic action which often delivers better experimental output.

## Materials and Methods

### Materials:

*Chlorophytum borivilianum*, *Withania somnifera* were collected from Pavan Agro Farm, Pune, *Wagatia spicata* Dalz. was collected from hilly regions of Malakapur (Dist.Kolhapur), *Picrorrhiza kurroa* was collected from licenced ayurvedic supplier and brought from Himalayan region. *Spilanthes paniculata* was collected from hilly regions of Mulashi (Pune). All plant derivatives were identified using standard identification keys and the identification of dubious plant was confirmed by 'Botanical Survey of India', Pune. *Chlorophytum borivilianum* (Root), *Withania somnifera* (Root), *Wagatia spicata* Dalz (Stem), *Picrorrhiza kurroa* (Root) and *Spilanthes paniculata* (Root) were sun dried. The plant derivatives were separately ground to smaller particle size of  $< 1\mu$ .

### Methods:

#### Neutrophil Adhesion Test:

'Neutrophil Adhesion Test' was conducted in Albino Mice vide IAEC approval and study code no. 169 NTC Pune. Three different groups of Swiss Albino Mice, each carrying 3 males and 3 females were selected for the study as Control (No Treatment Group), Standard (The group treated with FDA approved Cytonini 1 O.D. Morning and Cytomaw 1 O.D. Evening) and Test (Treated with blended dose of Preparation no.1 as 1 O.D. Morning and blended dose of Preparation no.2 as 1 O.D. in Evening). Preparation No.1 carried 100 mg. of *Wagatia spicata* Dalz (STEM), 50 mg. of *Picrorrhiza kurroa* (ROOT) as a single human dose and was converted to animal dose considering weight fluctuations in Swiss Albino Mice. Preparation No.2 was the blended dose of 250 mg. of *Withania somnifera* (ROOT), 50 mg. of *Wagatia spicata* Dalz (STEM), 20 mg. of *Spilanthes*

*paniculata* (ROOT) as a single human dose and was similarly converted to animal dose considering weight fluctuations in Swiss Albino Mice. In Standard and Test groups, respective treatment was continued for 14 days. After fourteen days, 0.5 ml blood sample was collected from all the three groups of animals, in heparinized vials by 'retro orbital puncture'. In each vial, 20 mg of nylon fiber was added and vials were incubated at 37°C for 15 min. Using 'Coulter Type Blood Cell Counter' haematological parameters were studied. Count of fiber bound neutrophil was taken by dispensing the fibers in fresh 1 ml saline.

### Statistical analysis:

Since the distribution of data for three groups was unknown, non parametric counter part of ONE WAY ANNOVA was used followed by 'Kruskal Wallis Test' [P- value  $< 0.05$ ], Post-hoc Test, Man-Whitney Test [P- value  $< 0.025$ ].

## Results

As compared to Control and Standard groups, significant increase in percentage neutrophil count was observed in Test group (Table 1). 'Kruskal Wallis Test' within Control, Standard and Test group gave the P- value = 0.031, which was  $< 0.05$ . In Post-hoc Test; Standard and Test Groups were compared with control group. P -value of Standard FDA approved medicine (0.067 which is  $> 0.025$ ) had no significant effect on percentage neutrophil count in Albino mice. With 'Test group' following 'Man-Whitney Test', P-value was found to be 0.010, which is  $< 0.025$ . Thus it can be concluded that synergistic action of *Chlorophytum borivilianum*, *Withania somnifera*, *Wagatia spicata* Dalz., *Picrorrhiza kurroa* and *Spilanthes paniculata* Wall. Ex DC had a significant effect on percentage neutrophil count in Albino mice. Gender wise statistical analysis following 'Man-Whitney Test' showed the P-value of 0.359, which is  $> 0.05$ . This indicates that immunomodulating activities of the plants do not differ in males and females. The experiment also reported for increase in MCHC values in Standard group.

**Table No.1: Percentage Neutrophil Count**

Sample No.	Group	% Neutrophil
1	Control Male	14
2	Control Male	16
3	Control Male	12
4	Control Female	14
5	Control Female	12
6	Control Female	05
7	Standard Male	61
8	Standard Male	Haemolysed sample
9	Standard Male	26
10	Standard Female	08
11	Standard Female	54
12	Standard Female	30
13	Test Male	32
14	Test Male	29
15	Test Male	21
16	Test Female	20
17	Test Female	33
18	Test Female	14

**Table No.2 : Haematological parameters :**

Group	HB	Platelet	RBC $\times 10^6$ / $\mu\text{l}$	PCV	MCV	MCH	MCHC
Control Male	12.30	1005000	7.30	41.90	57.30	16.90	29.50
Control Male	13.10	705000	7.96	46.80	58.80	16.50	28.10
Control Male	12.40	1117000	7.11	42.80	60.20	17.40	28.90
Control Female	12.90	806000	7.40	45.00	60.90	17.40	28.60
Control Female	13.80	743000	7.41	47.90	64.70	18.70	28.90
Control Female	12.20	1000000	7.26	41.60	57.40	16.80	29.40
Standard Male	10.80	761000	6.70	36.60	54.60	16.00	29.40
Standard Male	haemolysed	haemolysed	haemolysed	haemolysed	haemolysed	haemolysed	haemolysed
Standard Male	14.00	1117000	8.87	43.00	48.50	15.80	32.70
Standard Female	12.60	976000	6.96	42.40	60.90	18.10	29.70
Standard Female	13.10	1128000	8.10	40.20	49.70	16.10	32.50
Standard Female	14.00	1023000	8.95	44.30	49.50	15.60	31.60
Test Male	12.80	790000	8.28	42.20	50.90	15.50	30.40
Test Male	14.70	945000	8.84	48.80	55.30	16.60	30.10
Test Male	14	1735000	8.56	48.00	56.10	16.40	29.20
Test Female	13	978000	8.10	43.80	54.10	16.10	29.70
Test Female	10.30	1158000	6.82	36.30	53.30	15.10	28.30
Test Female	13.50	831000	8.50	46.50	54.70	15.90	29.10

**Table No.3: Comparative statistical analysis of RBCs, Platelets and Mean Corpuscular Haemoglobin Concentration**

Blood Parameters	P value following Kruskal – Wallis Test	Standard Vs. Control (P value following Mann-Whitney Test )	Test Vs. Control (P value following Mann-Whitney Test)	Gender wise study (P value following Mann-Whitney Test)
Platelet Count	0.506 ( > 0.05 )	NA	NA	NA
RBC Count	0.280 ( > 0.05 )	NA	NA	NA
MCHC	0.024( < 0.05 )	0.013( < 0.025 )	0.149( > 0.025 )	0.772( > 0.05 )

## Discussion

‘Neutrophil Adhesion Test’ is one of the tests used for the assessment of immunomodulatory activities of plant derived substances<sup>8</sup>. Neutrophil is a prime phagocytic cell which carries multi targeted enzymes keeping ability of every existing chemical bond. Following viral infection neutrophils shows significant release of superoxide radicals and in HIV infection the neutrophils reported decline in the level of superoxide radicals<sup>11</sup>. Microbial infections including viruses often show low count of percentage neutrophil. Neutrophil extravasation process involves conformational changes in the LFA-1 and MAC-1 in presence of IL-1 and MIP-1 in the neutrophil membrane and thus increases their affinity towards ICAM- 1 of endothelial cells. Nylon fiber serves as an ICAM analogue and thus allows binding of neutrophils which carries modified intergrins. Thus ‘Neutrophil Adhesion Test’ serves as a tool for assessment of immunomodulatory activities. The results obtained indicates that, synergistic action of *Chlorophytum borivilianum*, *Withania somnifera*, *Wagatia spicata* Dalz., *Picrorrhiza kurroa* and *Spilanthes paniculata* Wall. Ex DC increases percentage neutrophil count in males and females with respect to study in the animal model. This study consolidate the claims for immunomodulatory principles of respective plant derivatives described in medical literature<sup>7,8,9,10,12</sup>. The study also compared the immunomodulatory principles of FDA approved medicines with blended formulations.

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