IMMUNOMODULATORY ACTIVITY OF CHLOROPHYTUM BORIVILIANUM

G.S.CHAKRABORTHY[*] and VIDHU AERI[1]

School of Pharmacy and Technology Management, Faculty of Pharmacy, SVKM's,NMiMS University,Shirpur, Maharastra 425 405
[1]Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062

Summary

To investigate the immunomodulatory effect of *Chlorophytum borivilianum* in rats. *Chlorophytum borivilianum* was administered orally at a dose of 200 mg/kg to healthy rats divided into three groups consisting of six animals each. The assessment of Immunomodulatory activity was carried out by testing the humoral (antibody titre) and cellual (foot pad swelling) immune responses to the antigenic challenge by sheep RBCs and by neutrophil adhesion test. On oral administration of the extract, a significant increase in neutrophil adhesion and delayed type hypersensitivity response whereas the humoral response to sheep RBCs was unaffected. Thus *Chlorophytum borivilianum* significantly potentiated the cellular immunity by facilitating the foot pad thichness responses to the sheep RBCs in sensitized rats. With a dose of 200 mg/kg the DTH response (mean ± SEM). The responses were statistically significant when they were compared with the control. The study stated that *Chlorophytum borivilianum* shows a significant stimulation of the cell mediated immunity and no effect on the humoral immunity.

Key words: *Chlorophytum borivilianum*, Immunomodulaty Activity, Humoral Activity.

Introduction

Chlorophytum borivilianum San. and Fern. (Liliaceae) is a traditional perennial herbaceous medicinal plant commonly known as safed musli. It is endangered species (1). The centre of its origin lies in Tropical and subtropical Africa. In India it is endemic to the subcontinent. It is mainly cultivated in Rajasthan, Gujarat, Madhya Pradesh, Maharashtra and Karnataka. Traditionally safed musli was used for lack of libido male impotency and oligospermia. It is also widely used as a general health promotive tonic and for delaying the ageing process. Varying its common use for health promotion, it is also used for increasing lactation, treating various gynecological disorders, arthritic conditions and to control diabetes mellitus (2). *Chlorophytum borivilianum* contains proteins(8-9%), carbohydrates (41%), root fibers (4%), saponins (2-17%), minerals and vitamins. Saponin is the chief medicinal compound present in roots. Saponins and alkaloids present in the plant are the primary source of its significant medicinal properties. The root is a rich source of pharmaceutically active compound like steroids, stigmasterol (3), glycosides, oligofuro and spirostanosides (4) and phenols.

Thus in the present investigation the successive extraction of *Chlorophytum borivilianum* leaves and roots was screened for immunomodulatory properties using standard operating procedures.

Material and Methods

Collecction of plant material: The plant (Leaves and Roots) were collected from the Herbal Garden of Jamia Hamdard, New Delhi, India in the month of May and August 2006 respectively. Both were identified at Department of Science of Hamdard University. A voucher specimen (No1) has been deposited in the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Hamdard University.

Preparation of extracts and Standards: The plant parts (Leaves and Roots) were washed, shade dried and extracted with methanol for 36 h by hot continuous method in a soxhlet extraction unit. The mixture was filtered and evaporated to dryness. The dark green and brownish semisolid mass was obtained for leaf and root respectively. Both were stored in a well closed air tight light resistant container. The extracts of leaf and roots were subjected to preliminary phytochemical screening (5) which indicated the presence of alkaloids, glycosides, flavonoids, tannins, phenolic substances and saponins.

Animals: Adult male Wistar rats, weighing 150-200 gms, were used to study the immunomodulatory activity. The animals (six per cage) were maintained under standard laboratory conditions (light period of 12 h/day and a temperature 25 ± 2°C) with access to a standard commercial diet and water *ad libitum.* The experiment was carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the Institutional Animals Ethics Committee had approved all the procedures. Experiment studies were undertaken according to their rules and regulations (6).

Antigen: Sheep Red Blood Cells were collected in Alsever's solution, washed three times in large volumes(30 ml) of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.05 X 10^9 cells/ml for immunization and challenge.

Immunomodulatory Activity

Neutrophil adhesion test (7)**:** On the 14^{th} day drug treatment, blood samples were collected (before challenge) by puncturing the retro orbital plexus into heparanized vials and analyzed for total leucocyte count (TLC) and differential leucocyte count (DLC) by fixing blood smears and staining with Field stain I and II- Leishman,s stain. Afetr initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percentage neutrophil adhesion was calculated as shown below

$$\text{Neutophil adhesion}(\%) = \frac{NI_u - NI_t}{NI_u} \times 100$$

Where NIu = Neutrphil index of untreated blood samples
NIt = Neutrophil index of treated blood sample.

TABLE 1: EFFECT OF *CHLOROPHYTUM BORIVILIANUM* ON NEUTROPHIL ADHESION IN RATS

Groups	Neutrophil index		Neutrophil Adhesion (%)
	UB	FTB	
I (Untreated)	277.8 ± 56.21	230.1 ± 46.21	
II (200 mg/kg, p.o. MLECB)	298.6 ± 44.31	245.7 ± 23.41*	17.71
III (200 mg/kg, p.o.MRECB)	310.7 ± 40.41	240.6 ± 45.12*	22.61

The values are mean ± SEM of 6 rats in each group. One way ANOVA followed by Dunnett's test, *p, 0.001 Vs Grouo I, Ub= untreated blood: FTB= Fibre treated Blood: MLECB= Methanloic Liaf extract of *Chlorophytum borivilianum*; MRECB= Methanloic Root extract of *Chlorophytum borivilianum*.

Haemagglutinating antibody (HA) titre (8)**:** Rats of group II and III were pretreated With CB for 14 days and each rat was immunized with 0.05 X 10^9 SRBC/rat by i.p. route including control rats. The day of immunization was referred to as day 0. The animals were treated with CB for 14 more days and blood samples were collected from each rat on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBC (0.025 X 10^9 cells). The micro titre plates were incubated at room temperature for 2 hours and examined visually for agglutination. The highest number of dilution of serum showing haem agglutination has been expressed as HA titre.

Delayed Typr Hypersensitivity (DTH) Response (8): Six animals per group (Control and treated) were immunized on day 0 by i.p. administration of 0.05 X 10^9 SRBC/rat and challenged by subcutaneous administration of 0.025 X 10^9 SRBC/ml in to right hind foot pad on day 14. The extract of CB was administered orally from day 14 untill day 13. DTH responses were measured at 24 h after SRBC challenged on day 14 and expressed as mean percent increase in paw volume (plethysmometrically)

TABLE 2: EFFECT OF *CHLOROPHYTUM BORIVILIANUM* ON HA TITRE AND DTH RESPONSE TO ANTOGENIC CHALLENGE BY SHEEP RED BLOOD CELLS IN RATS

Groups	HA Titre	DTH response (% increase in paw volume)
I (Untreated)	4.34 ± 0.87	6.23 ± 1.74
II (200 mg/kg, p.o. MLECB)	5.43 ± 1.17	12.45 ± 2.12*
III (200 mg/kg, p.o.MRECB)	5.76 ± 0.39	15.32 ± 1.65*

The values are mean ± SEM of 6 rats in each group. One way ANOVA followed by Dunnett's test, *p,0.001 Vs Group I

Statistical Analysis: The data was analyzed using one way analysis of variance (ANOVA) follwoed by Dunnett's Test. P values ,0.001 were considered as significant.

Estimation of total phenolics: The total phenolic contents of methanol extract was determined with Folin-Ciocalteu reagent according to Slinard & Singleton (10) and slightly modified. The stock solution of extract 1mg/ml in water was prepared. From the stock solution, 5 ml was transferred to a 25 ml volumetric flask and made up with distilled water. Out of this 5 ml of sample and 2 ml of standard was taken in 25-ml volumetric flask, to this 10 ml of distilled water, and 2ml of phenol reagent (20%v/v) was added, and then the volume was made up with 29% sodium bicarbonate. The mixture was kept in the dark for 20 min. and the absorbance was read at 760 nm. The total phenolic content was calculated as gallic acid and expressed as percent of gallic acid detected. Standard used was gallic acid.

Results And Discussion

It has been observed that the immunomodulatory agents obtained from the plant and animal source serves as the best for immune responsiveness of any organism against a pathogen by activating the system. In the present investigation CB (*Chlorophytum borivilianum)* when administered orally, significant increased in the adhesion of the neutrophila to the nylon treated fibers which interelated the process of margination of cells in blood vessels. It was alos found to be highly significant when compared with the control group. The HA titre showe dvery mild increase with CB administration. The DTH response directly correlated the cell mediated immunity and was found to be significant. Thus in this process it was observed that T-lymphocytes were sensitized when they were challenged by an antigen which thereby converted to lymphoblasts and secreted the lymphokines and attracted the scavenging cells to the specific site of reaction. The increase in the response indicated by CB had a stimulating effect on the lymphocytes. Thus it can be also be correlated with the free radical oxidative stress which has a major role in the pathogenesis of a wide range of clinical disorders resulting from different natural antioxidant defenses So it can be concluded that methanolic extract of leaf and roots of CB was found to be highly stimulating agent for both the responses. The standards exhibited IC 50 values 74.66 ± 1.52 and 55.00 ± 0.77 µg/mL respectively.

References

1. Nayar MP. Shastry. *Chlorophytum* borivilianum. In Nayar and Shastry (Ed), Red data Book of Indian Plants, Published by Botanical Survey of India, Calcutta, India.
2. Purohit SS. Prajapati ND. Agro's Colour Atlas of Medicinal Plants, Agrobios Publication, Jodhpur, India.
3. Tandon M. Shukla YN. Thakur RS. Steroid glycoside from *Asparagus adscendens*. *Phytochemistry*, 1990, 29, 9, 2957-2959.
4. Sharma SC. Sharma HC. Oligofuro and spiro-stanosides of *Asparagus adscendens, phytochemistry*, 1984, 23, 3, 645-648.
5. Harbone JB. Phytochemical Methods-A Guide to Modern Technique of Plant Analysis, Chapman and Hall, London.
6. Zimmermann M. Ethical guidelines for investigation of experimental pain in conscious animals, *Pain*, 1983, 16, 10.
7. Wilkonson PC. Neutrophil adhesiontest: In Vane JK Ferreria Sh, 1st Ed, Handbook of Experimental Pharmacology, Springer-Verlag Berlin, Heidelberg, New York.
8. Puri A. Saxena R. Saxena RP. Saxena KC. Delayed type reactions in rats,*j Nat Prod*,56, 995-999.
9. Slinard K, Singleton VL, Total phenol analysis automation and comparison with manual methods. *Am J Enol Vitic* 1977, 49–55.