

Immunomodulatory Effect of Triphala during Experimentally Induced Noise Stress in Albino Rats

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Stress is a term that generally has a negative connotation, which results in immune dysfunction. In this study, immunomodulatory effect of Triphala (equal proportion of *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis*) during noise-stress in male albino rats was evaluated by analyzing the antibody titer, cytokines IL-2-Interleukin (2), IL-4 and IFN-Interferon (gamma) and Pan T, CD4⁺/CD8⁺ lymphocyte phenotype in spleen. Four groups of rat were employed namely control, Triphala (1 g/kg body weight), noise-stress (100 dB/4 hr/15 days), Triphala + noise-stress and rats were immunized with sheep red blood cells (5×10^9 cells/ml). Results indicate that noise-stress induced elevation in the serum antibody titer and IL-4 levels associated with decreased IL-2, IFN-gamma, and reduction in Pan T, CD4⁺/CD8⁺ lymphocyte phenotype in spleen were significantly prevented in Triphala treated noise-stress exposed group. This study showed the immunomodulatory effect of Triphala during noise-stress and suggests its therapeutic usefulness.

Key words — Triphala, noise-stress, cytokines, antibody titer

INTRODUCTION

The modulation of immune response with the aid of various medicinal plants in order to alleviate certain diseases is an active area of interest. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy

for a variety of diseases, especially when the host defense mechanism has to be activated under the conditions of impaired immune response. Triphala is an Ayurvedic herbal formulation, consisting equal parts of three medicinal plants namely *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis*. Triphala credited with diverse beneficial properties like anti-stressor, anti-oxidant and immunostimulant properties.^{1,2)}

Stress is a familiar aspect of modern life, being a stimulator for some, but having a negative impact for many others. During daily life, people are exposed to potentially hazardous noise levels related to work environment, urban traffic, household appliances, discos *etc.*³⁾ World Health Organization estimated that approximately 20% of the population are exposed to noise generated by urban traffic greater than 65 dB, a level regarded as a maximum safety threshold, whereas 40% of the population are exposed to noise levels between 55–65 dB, which might be responsible for several disorders.⁴⁾ Now there is compelling evidence that stress responses can cause clinically relevant immunosuppression and immune dysfunction.^{5,6)}

In an effort to search for a new immunomodulators during stressful conditions, the Triphala screened for its immunomodulatory effect by examining the levels of serum antibody titer, cytokines (IL-2, IL-4 and IFN-gamma) and Pan T, and CD4⁺/CD8⁺ lymphocyte phenotype in spleen during noise-stress.

MATERIALS AND METHODS

Animals — The study was conducted on male Wistar strain albino rats (170–190 g). All the protocols approved by Institute's Animal Ethical Com-

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mittee of the University of Madras (Institute Animal Ethical Committee (IAEC) No. 08/006/02) and confirmed to National Guidelines on the Care and Use of Laboratory Animals. The animals were maintained under standard laboratory conditions: temperature $25 \pm 2^\circ\text{C}$ and photoperiod of 12 hr. Commercial pellet diet and water were given *ad libitum*.

Drug and Dosage — *T. chebula*, *T. bellerica* and *E. officinalis* were collected and authenticated by The Chief Botanist, Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Ltd. (Chennai City, India). The seedless fruits were dried under shade and made into fine powder. Equal proportion (1:1:1) of weighed powder from each fruits were dissolved in saline (1 ml) and administered orally at the dose of 1 g/kg/body weight for 48 days.

Experimental Groups — Albino rats were divided into four groups of six rats each: Group I (Control), received normal saline (1 ml); Group II (Triphala), received Triphala (1 g/kg/body weight for 48 days) orally by dissolved in saline (1 ml); Group III (Noise-stress), exposed to noise-stress 100 db/4h/15days and Group IV (Triphala + noise-stress) subjected to noise-stress on the 33rd day of the Triphala pretreatment. Groups I, II and IV animals were immunized by injecting 1 ml of 20% of fresh SRBC suspension intraperitoneally on 44th day of the regimen and Group III animals were immunized on 11th day of the regimen.

Noise-stress — Rats were exposed to broad band (White) noise produced by a white noise generator and amplified by an amplifier (40 W) connected to a loudspeaker located at 30 centimeter above the animal cage (3 rats/cage). The intensity of the sound was monitored by a sound level meter (Cygnat systems-D 2023 Serial No. F02199, Chandigarh City, India) and maintained at 100 dB intensity.¹⁾

Immunization — Sheep red blood cells (SRBC) collected in Alsever's solution were washed three times in pyrogen free 0.9% normal saline and adjusted to a concentration of 5×10^9 cells/ml for immunization. The animals were immunized by injecting 1 ml of 20% of fresh SRBC suspension intraperitoneally on day 0. On the 5th day, the experimental parameters were carried. Blood samples (approximately 2 ml) were drawn from jugular vein and ether was used to anaesthetize the animals to avoid stress.⁷⁾

Antibody Titer — Antibody titer was carried out according to the protocol of Puri *et al.*⁸⁾ In briefly, two-fold dilution of sera was performed in 0.15 M Phosphate buffered saline (PBS) and aliquoted in

“U” bottomed microtiter plates. 1% SRBC suspended in PBS was dispensed into each well and mixed thoroughly. The plates were incubated for 2 hr at 37°C and then observed visually for haemagglutination. The highest dilution of the test serum giving haemagglutination was taken as antibody titer.

Quantification of Cytokine (IL-2, IL-4 and IFN-gamma) — Cytokines such as IL-2 (BMS634), IL-4 (BMS628) and IFN-gamma (BMS606) was quantified by Enzyme Linked Immuno Sorbent Assay (ELISA) procedure using kits from Bender MedSystem (Vienna City, Austria), according to manufacturer's protocols. Briefly, 50 μl of ELISA diluents is pipette into antibody coated wells (anti-IL-2, anti-IL-4 and anti-IFN-gamma), followed by 100 μl of each standard and 50 μl of test samples (serum), shaken for 5 seconds to mix the contents in the wells, covered with plate sealer and incubated for 2 hr at room temperature. After incubation, contents of the wells were aspirated and washed three times with wash buffer. After complete removal of the wash buffer in the final wash, 100 μl of detection solution was added, covered with plate sealer and incubated for 1 hr. The wells were washed three times with wash solution and, 100 μl of substrate reagent was added and incubated for 30 minutes in dark. The color development was arrested by adding 100 μl of stop solution and the absorbance was recorded at 450 nm with a reference wavelength of 570 nm.

Lymphocyte Phenotype in Spleen (Pan T, CD4⁺/CD8⁺) — The spleen samples were teased on a wire mesh, for single cell suspension in PBS (including 0.05% Ethylene Diamine Tetra Acetic Acid (EDTA) and excluding Mg^{2+} and Ca^{2+}). Splenic erythrocyte was lysed by two consecutive incubations (5 and 3 minutes at 37°C) of the suspension in ammonium chloride (0.83% NH_4Cl in 0.01 M Tris-HCl, pH 7.2). Cells were washed, re-suspended in cold PBS. The Pan T or CD4⁺ or CD8⁺ expressed lymphocytes from spleen suspension (1×10^7) were isolated by incubating with anti-T or anti-CD4⁺ or anti-CD8⁺ conjugated magnetic microbeads (Miltenyi Biotech, Bergisch-Gladbach, Germany). After a wash with PBS, cells were passed through a separation column, placed in a magnetic field. The retained cells were collected by flushing out using a plunger to obtain enriched Pan T or CD4⁺ or CD8⁺ lymphocytes phenotype and enumerated.

Statistical Analysis—Data were expressed as mean \pm S.D. and statistical analysis was carried out using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS, Cary, NC, U.S.A.). When there was a significant difference, Tukey's multiple comparisons were performed by fixing the significance level at $p < 0.05$.

RESULT AND DISCUSSION

The prime objective of the study was to investigate the immunomodulatory effect of Triphala during noise-stress. One of the basic tenets of herbal medicine is that interactions between different constituents occur, enhancing activity or reducing the likelihood of adverse effects.⁹⁾ Present study, Ayurvedic herbal formulation Triphala was selected for its immunomodulatory effect during noise-stress exposure in albino rats. With our different dosages and time-course of Triphala administration (data not shown), it was found that 1 g/kg/body weight for 48 days significantly prevents the noise-stress induced changes, in addition food and water intake and animal body weight remains unaltered.¹⁾ Noise-stress was selected, since it has a high environmental and clinical relevance and appears to be a mild stressor with respect to neuroendocrine activation.¹⁰⁾ To determine the best potential impact of Triphala intake on an immune response, the highly immunogenic, non-infectious SRBC used as a stimulating agent in

this study.¹¹⁾

The cellular and humoral immunity is regulated by distinct subsets of helper T-cells (Th), former is regulated by Th1 cells and later is controlled by Th2 cells.¹²⁾ Two subsets of effector Th cells have been defined on the basis of their distinct cytokine secretion patterns and their immunomodulatory effects: Th1 cells produce predominantly IL-2 and IFN-gamma, which is required for cell-mediated immune response, Th2 cells secrete predominantly IL-4 which mediate B cell activation and differentiation of humoral immune response.¹³⁾

In the present study elevated antibody titer (Fig. 1) and IL-4 levels (Table 1) during noise-stress

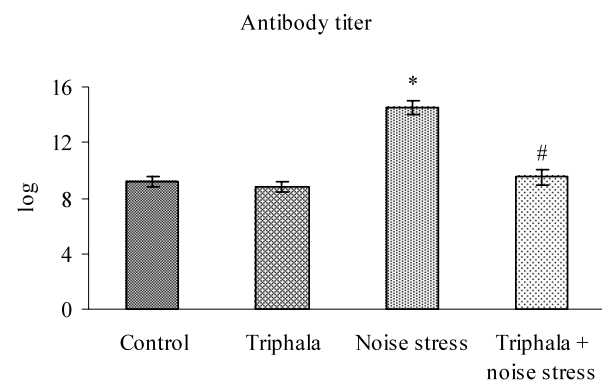


Fig. 1. Effect of Triphala (1 g/kg/body weight) on Antibody Titer Against Sheep Red Blood Cells in Male Albino Rats Exposed to Noise-stress

Each column represents the mean \pm S.D. of six animals. * compared with control; # compared with noise-stress. The symbols, *,# represent statistical significance at $p < 0.05$.

Table 1. Effect of Triphala on Cytokines Levels IL-2, IL-4 and IFN-gamma in Male Albino Rats Exposed to Noise-stress

Parameters	Control	Triphala	Noise-stress	Triphala + Noise-stress
IL-2 (pg/ml)	470 \pm 31	475 \pm 32	273 \pm 21*	348 \pm 27*.#
IL-4 (pg/ml)	24 \pm 2	23 \pm 2	59 \pm 5*	36 \pm 3*.#
IFN-gamma (pg/ml)	280 \pm 23	332 \pm 32*	153 \pm 14*	199 \pm 21*.#

Values are expressed as mean \pm S.D. of six animals. * compared with control. # compared with noise-stress. The *,# symbols represent statistical significance at $p < 0.05$.

Table 2. Effect of Triphala on Spleen Lymphocyte Phenotype Pan T, CD4⁺/CD8⁺ in Male Albino Rats Exposed to Noise-stress

Parameters	Control	Triphala	Noise-stress	Triphala + Noise-stress
Pan T %	48 \pm 3	49 \pm 4	36 \pm 3*	43 \pm 3*.#
CD4 ⁺ %	33 \pm 3	36 \pm 2	22 \pm 2*	27 \pm 2*.#
CD8 ⁺ %	18 \pm 0.98	19 \pm 0.94	7 \pm 0.39*	13 \pm 0.52*.#

Values are expressed as mean \pm S.D. of six animals. * compared with control; # compared with noise-stress. The *,# symbols represent statistical significance at $p < 0.05$.

were significantly prevented in Triphala administered group may speculate us the balance between Th1:Th2 ratio was augmented towards Th2 during noise-stress was significantly prevented. In addition noise-stress induced decrease in IL-2 and IFN-gamma (Table 1) was significantly prevented in Triphala administered group were further conformed that Triphala prevents the noise-stress induced shift in Th2. By preventing the noise-stress induced shift in Th2 balance during Triphala administration might specifically decrease the susceptibility of the host to intracellular infections.¹⁴⁾ Noise-stress induced decline in Pan T, CD4⁺/CD8⁺ lymphocytes phenotype were significantly prevented in Triphala administered group (Table 2) may be due its strong antioxidant property which effectively prevents the lymphocytes from oxidative stress induced apoptosis.²⁾

In conclusion, finding of this study has shown the immunomodulatory effect of Triphala during noise-stress. Further detailed studies with exact mechanism are required to establish a possible use during stress induced immune dysfunction.

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