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EFFECT OF IMMUE-21 ON MURINE PERITONEAL MACROPHAGES AND SPLENIC LYMPHOCYTES

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ABSTRACT: *Effect of Immue-21 (an herbal immunomodulator) was studied on different immunopharmacological models. Immue-21 significantly enhanced the mobilization and functional capacity of peritoneal macrophages of experimental animals. An elevated blastofenic response of splenic lymphocytes was observed in Immue-21 treated mice. The potential to modulate the immunity in general by Immue-21 assumes considerable therapeutic and/or prophylactic significance.*

INTRODUCTION

In recent years, the field of immunomodulation attracts attention of scientists, in view of the growing awareness regarding the need to modulate the host immune system to achieve the desirable effects of preventing an infection rather than treating it at an advanced stage.

Different immunomodulators of chemical origin are currently in use to enhance the non-specific host resistance against infections. However, the major drawbacks of these substances are their prohibitive cost and development of local reactions with associated side effects. The use of herbal products to restore and rejuvenate positive health and to maintain organic balance, has been in vogue since ancient times[1].

Immue-21, a herbal formulation of **Indian Herbs,** Saharanpur, containing immunoactive plant principles of *Ocimum Sactum* and *Withania somnifera* has been reported to potentiate non-specific defence mechanism of the host and thus helps to overcome infective processes[2].

Immunomodulators have been shown to produce their effects through different ways. We have investigated the possible mechanism of action of Immue-21 through different experimental models in our present study.

MATERIALS AND METHODS

Immunomodulatory effect of Immue-21 was assessed using adult male albino mice (Approx.30gm). The animals were acclimatized to laboratory conditions for seven days prior to experimentation. They were grouped in polycarbonate cages in a centrally air conditioned room at an ambient temperature of $22^0 \pm 1^0$ C with 12 hour light and dark cycle. The animals were maintained on pellet diet (JDB Agency, New Delhi) and water *ad libitum*.

The experimental animals were divided into three groups of six animals each. The animals of Group-I served as vehicle treated control. The animals of Group-II and III were treated with Immue-21 at the dose level of 20 and 200 mg/kg body weight,

respectively, for 15 days (20 mg is recommended dose and 200 mg is ten times higher dose to observe adverse effects, if any). Test product was dissolved in distilled water (10 mg/ml) and given by intragastric route using tuberculin syringe equipped with blunted 21 gauge needle tipped with polyethylene tubing daily at 10.00 AM.

All the animals were challenged (I.P) with same quantity of non-pathogenic *Staphylococcus* sp. On 13^{th} day of experiment of non-specific antigen. Peritoneal cells from each animal were collected on 16^{th} day. Macrophages were isolated by adhering on glass surfaces[3] and counted using haemocytometer.

Phagocytic activity was assessed using reduction of nitroblue tetrazolium by macrophages in terms of formazan produced by $1 * 10^6$ cells, expressed as optical density (OD) measured at 515 nm[4].

Spleen from each animal was collected aseptically. Spleenocytes were prepared in cold RPMI-1640 as described elsewhere[5]. Cell counts were made using haemocytometer. Percentile blastogenic transformation of spleenic lymphocytes was assessed by morphological identification of blast. Number of blasts cells were calculated as medium and large size lymphocytes with dividing stage of nuclei[6].

Statistical analysis of the data generated during the course of study was done by student's-t test[7].

RESULTS

Effects of Immue-21 on peritoneal macrophages of the experimental animals are presented in Table-I. Significant increases in the number and phagocytic activity of peritoneal macrophages was observed in mice treated with Immue-21 at the dose of 20 mg/kg. No significant alteration regarding the number and activity of macrophages was noticed in the mice treated with Immue-21 at higher dose level.

Immue-21 treatment did not significantly alter the weight of spleen of the experimental animals. The cell counts in spleen showed in increase in lymphocyte number in the animals (Group-II) as compared to control (Table-II). The viability of the cells were not altered when tested by trypan blue method. Percentile count showed increased number of lymphoblasts in the spleen of product treated animals (Group-II).

DISCUSSION

Macrophages are the major constituent of mononuclear phagocyte system of an individual. They play a central role in modulating cell mediated immunity[8]. In our present study, increase in the number of peritoneal macrophages in product treated animals (20 mg/kg) suggests that the component(s) of Immue-21 directly or indirectly stimulate the proliferation of macrophages. The results also indicate the Immue-21 stimulates the phagocytic activity of macrophages at 20 mg/kg dose level. Activated macrophages can release different cytokines which are having immunomodulatory actions like antigen presentation, lymphocyte activation and tumoricidal activity[9,10]. Very high dose of Immue-21 (200 mg/kg) did not significantly influence the number and activity of macrophages.

Activation of macrophages by Immue-21 may be due to the fact that glycosides from *W. somnifera* (one of the major constituents of this product) produce significant mobilization and activation of peritoneal

macrophages with increased phagocytic activity and lysosomal enzyme secretion by activated macrophages[11]. Increased phagocytic activity of the peritoneal macrophages of mice treated with *W. somnifera* over the control animals was previously reported. It was suggested that *W. somnifera* primarily activated macrophages which in turn secrete cytokines to stimulate other immunocytes like neutrophils[12,13]. In an earlier experiment[2], identical modulatory effect of Immue-21 on microbicidal activity of neutrophils was noticed. The present study supports the previous one by showing the influence of the product on functional properties of macrophages suggesting a possible role in immunomodulation.

Lymphocytes are the key cells, generally referred to as immunocytes and once sensitized with antigen become committed for the production of antibodies. In our earlier study it was found that the number of lymphocytes was increased in product treated animals[2]. The lymphocytes are primarily stored in the spleen, a secondary lymphoid organ that controls immue response of an individual.

In he present study, increase in the number of spleenic lymphocytes and conversion of small lymphocytes to blast cells of the product treated mice (20 mg/kg) after antigenic challenge provided the basis for immunopotentiation by this compound. A high count of lymphoblasts indicates that the lymphocytes have undergone transformation and confirm their sensitivity to antigen, suggesting that Immue-21 improves the T-cell memory of the individuals.

Based on the present study, it can safely be presumed that Immue-21 at recommended dose level is a potent immunostimulator with considerable therapeutic significance. At a very high dose, it has no significant effect on host immue system. It is hoped that these observations, which are quite preliminary, will stimulate further investigations of this product with a view to discover a non-toxic immunomodulator that may have unique potential to treat or prevent immue-based diseases.

Table – I Effect of IMMUE-21 on Peritoneal Macrophages

Treatment	Number of macrophages per ml. of peritoneal exudates (*10^6)	Phagocytic activity of macrophages (1*10) (O.D. of formazan)
I. Vehicle	7.68 ± 0.38	0.472 ± 0.015
II. Immue-21 (20 mg/kg)	12.59 ± 0.27 *	0.601 ± 0.019 *
III. Immue-21 (200 mg/kg)	7.19 ± 1.27	0.460 ± 0.009

* Significant difference (P<0.01) compared to control animals.

Table – I Effect of IMMUE-21 on Peritoneal Macrophages

Group / Treatment	Number / gm of Spleen (*10^6)	Percent blast cells
I. Vehicle control	247.2 ± 3.57	18.29 ± 0.37
II. Immue-21 (20 mg/kg)	270.3 ± 7.32*	24.71 ± 0.52*
III. Immue-21 (200 mg/kg)	248.0 ± 7.95	19.21 ± 0.48

* Significant difference (P<0.01) compared to control animals.

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