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Preliminary Screening of Imunocin for Immunomodulatory activity

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The immunomodulatory activity of the Imunocin (30 mg/animal, p.o.) was studied on delayed type hypersensitivity reaction and humoral antibody titre in mice. Treatment of animals with Imunocin significantly increased Sheep Red Blood Corpuscles (SRBC)-induced delayed type hypersensitivity reaction in mice by 23.46% as compared to the control. It also significantly increased the humoral antibody titre by 37.5% as compared to the control. The results of the preliminary investigation showed that Imunocin exerts immunomodulatory activity by stimulating cellular and humoral immunity.

Ayurveda, the Indian traditional system of medicine, lays emphasis on promotion of health- a concept of prevention of diseases and strengthening of both physical and mental health[1]. It was recognized in the Ayurveda that the immune system was involved in the etiology and pathophysiologic mechanisms of various inflammatory diseases of the skin, gut, respiratory tract, joints and central organs as well as in infectious diseases. It held the doctrine that modulation of the immune response would alleviate the diseases and the concept of Rasayana in Ayurveda[2] was based on related principles. Ayurvedic medicine thus constitutes a rich source of active substances for immunotherapy based on herbal preparations. Among the wide range of drugs claimed to possess the Rasayana effect, the most popularly used were, *Withania somnifera, Ocimum sanctum, Azadirachta indica, Curcuma longa and Tinospora cordifolia*[3].

The efficacy of *Withania somnifera* as an antistress agent and adaptogen has been reported[4]. It has even been found to have a growth inhibitory effect on transplantable mouse tumor[5]. Recent studies have demonstrated that *Withania somnifera* could prevent myelosuppression in mice treated with immunosuppressive drugs and could significantly raise the white blood cell count in normal mice[6].

Ocimum sanctum possesses adaptogenic and antistress activity. It has also been reported to have an antitumor activity[7]. *Azadirachta indica* has been reported to have immunostimulatory properties and to activate selectively the T_H 1 component of the lymphocyte population to elicit an enhanced cell-mediated immune response to subsequent mitogenic or antigenic challenge[8]. It has also been reported to enhance the humoral-immune responsiveness (both primary and secondary) in normal rats and to attenuate the stress-induced suppression of the secondary antibody response to Sheep Red Blood Corpuscles (SRBC). It also induces *in vitro* production of IL-1, Interferon, TNF and GM-CSF. Treatment with *Azadirachta indica* also increases white blood cell and lymphocyte counts without showing any cytotoxic effects in the body[9].

Tinospora cordifolia has been shown to produce significant leucocytosis and predominant neutrophilia in animal models[10]. The plants *Piper longum* and *Boerhaavia diffusa* have also been demonstrated to possess the ability to rejuvenate the host and increasing immunity[11]. These plants may thus act by stimulating either the non-specific or specific immunity or both. Combination of all

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these plants would therefore have diverse actions on various aspects of the immune system.

Imunocin, a herbal formulation containing a combination of immunoactive plant principles, *Withania somnifera (1 g), Ocimum sanctum (1 g), Azadirachta indica (1 g), Curcuma longa (0.50 g), Tinospora cordifolia (1 g), Boerhaavia diffusa (0.4 g) and Piper longum (0.1 g),* was based on the traditional uses of these plants in the Ayurvedic system of medicine and encouraged by the recent findings on these plants. The preparation has been indicated for strengthening the host defense system. Since all the above mentioned plants act on different arms of the immune system, the objective of the present study was to evaluate the preliminary immunomodulatory activity of Imunocin tablets (effect of the plants in conjunction) on cell-mediated and humoral immunity in mice.

MATERIALS AND METHODS

Imunocin Tablets were obtained from M/s Gufic Healthcare Ltd., Mumbai. The test extract was prepared by suspending 1 tablet in 100 ml 1% carboxymethyl cellulose to make a uniform suspension.

Swiss albino mice weighing 20± 5 g were obtained from Haffkine Biopharmaceuticals Ltd. Mumbai. The animals were housed in standard environmental conditions (22±1°, relative humidity 60±5%, 12 h light/dark cycle) and fed with standard mice feed (Amrut Gold Mohur, India) and water *ad libitum.* Cyclophosphamide (Endoxan) was purchased from Khandelwal Laboratories Pvt. Ltd., Mumbai. Sheep blood was obtained from Haffkine Biopharmaceuticals Ltd., Mumbai and stored in Alsever's solution. All other reagents used were of analytical grade.

Effect of Imunocin on delayed type hypersensitivity reaction[12]:

Animals were divided into three groups each containing six mice. Imunocin 30 mg/animal p.o., was administered in two divided doses; one in the morning and one in the evening of each day from day 0 to day 17. The second group of mice were treated with cyclophosphamide, 50 mg/kg, p.o. from day 15 to day 17 while the third group of mice received vehicle. The mice in all the groups were primed with 0.1 ml of SRBC suspension containing 1 x 10^8 cells intraperitoneally on day 11 and challenged on day 17 with 0.1 ml of 1 x 10^8 SRBC in the right hind foot pad. The contralateral paw received an equivalent volume of saline. The thickness of the foot pad was measured at 24 and 48 h after challenge using a Dial Caliper (Mitutoyo Company, Japan). The difference in the thickness of the right hind paw and the left hind paw was used as a measure of DTH reaction.

Effect of Imunocin on humoral antibody response[13]:

Animals were divided into three groups each containing six mice. The treatment schedule was similar to that of the delayed-type hypersensitivity reaction. The mice in all the groups were primed with 0.1 ml of SRBC suspension containing 1x10^8 cells intraperitoneally on day 11. Blood samples were collected from individual animals from the orbital plexus on day 17. The antibody levels were determined by haemagglutination technique. Twenty five microlitres of two fold-diluted sera in saline was mixed with 25 µl of 0.1% v/v SRBC suspension in microtitre plates. The plates were incubated at 37° for 1 h and antibody titre was measured. The value of the highest serum dilution causing visible haemagglutination was taken as the antibody titre. The antibody titres were expressed in a graded manner, the minimum dilution (1/2) being ranked as 1 and the mean ranks of different groups were compared for statistical significance.

Statistical analysis:

The results are expressed as the mean ± S.D. Data was analyzed by Student's t-test, $p<0.05$ was considered to be statisically significant.

RESULTS AND DISCUSSION

The use of immunostimulants has an important place in the current development of immunotherapy. Many plants have been traditionally used in India to stimulate immunity. Imunocin, an herbal formulation containing plant extracts of *Withania somnifera, Ocimum sanctum, Azadirachta indica, Curcuma longa, Tinospora cordifolia, Boerhaavia diffusa* and *Piper longum* was prepared with a view to have a product with diverse actions on the immune system. Individually though the immunostimulant activity of the above mentioned plants is known, their effect in combination has not been investigated.

In the present study, it was found that Imunocin augmented the delayed-type hypersensitivity reaction. The mean % edema of the Swiss albino mice in the control group at 24 h was 48.33 ± 6.12. The mean % edema at 24 h for the cyclophosphamide-treated mice was

TABLE 1 : EFFECT OF IMUNOCIN ON DELAYED TYPE HYPERSENSITIVITY REACTION

Treatment	Mean % edema 24 h	Mean % edema 48 h	% change in DTH reaction 24 h
Control	48.33 ± 6.12	20.83 ± 11.09	—
Cyclophosphamide 50 mg/kg	34.33 ± 12.29*	8.44 ± 6.57*	- 28.97
Imunocin 30 mg	59.67 ± 7.95*	18.82 ± 5.98^{NS}	+ 23.46

n=6 per group; Results are expressed as mean± S.D. *p<0.05 as compared to control; ^{NS}Non-significant as compared to control. '+' sign indicates potentiation of DTH reaction. '-' sign indicates inhibition of DTH reaction.

34.33±12.29 which was significantly less than the mean % edema for the control group (p<0.05). The mean % edema at 24 h for the Imunocin-treated mice was 59.67± 7.95 which was significantly greater than the mean % edema for the control group (p<0.05).

The Imunocin-treated mice demonstrated a potentiation of the DTH reaction of 23.46% while the cyclophosphamide-treated mice demonstrated an inhibition of the DTH reaction of 28.9% Table 1. Delayed hypersensitivity is crucially involved in host defense against viruses, fungi, mycobacterium and other organisms that replicate intracellularly. Macrophages act as principal effector cells of the DTH response[14]. Treatment with Imunocin for 17 days resulted in an enhanced response 24 h after the SRBC challenge. The effect could be attributed either to effective macrophage function or to the enhanced synthesis of lymphokines.

TABLE 2 : EFFECT OF IMUNOCIN ON HUMORAL ANTIBODY TITRE

Treatment	Mean haemagglutination antibody titre	% change in haemagglutination antibody titre
Control	8±0.63	—
Cyclophosphamide 50 mg/kg	5.33±0.82*	-33.38
Imunocin 30 mg	11±0.63*	+37.5

n= 6 per group; Results are expressed as mean ± S.D. *p<0.05 as compared to control; '+' sign indicates potentiation of haemagglutination antibody titre and '-' sign indicates inhibition of haemagglutination antibody titre

Treatment with Imunocin also augmented the humoral response to SRBC as was evidenced by the increased haemagglutination antibody titre in mice. The mean haemagglutination antibody titre for the control group on the 18th day was 86±0.63. The mean haemagglutination antibody titre on the 18th day for the cyclophosphamide-treated mice was 5.33±0.82, which was significantly less than the mean haemagglutination antibody titre for the mice in the control group (p<0.05). The mean haemagglutination antibody titre on the 18th day for the Imunocin-treated mice was 11± 0.632 which was significantly greater than the haemagglutination antibody titre for the control group (p<0.05). Imunocin (30 mg/animal, p.o.) for 17 days was found to enhance the production of circulating antibody titre by 37.5% whereas, cyclophosphamide decreased the production of circulating antibody titre by 33.38% Table 2.

The above observations suggest that the test drug may stimulate both the cellular and the humoral immunity. In view of the pivotal role played by macrophages in coordinating the processing and presentation of antigen to T and B cells and also by the various lymphokines synthesized by the T cells, the augmentation of the cell mediated and humoral response to SRBC reveals that Imunocin may enhance the effect by facilitating these processes. Further studies to elucidate the exact mechanism of action of the test drug are essential.

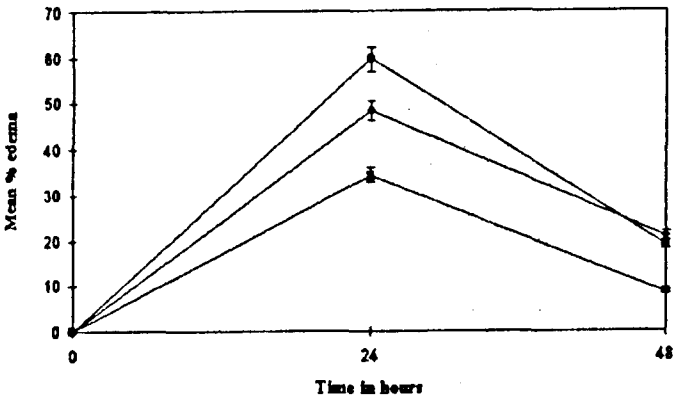

Fig. 1 : Effect of Imunocin on delayed-type hypersensitivity reaction % Edema measured at 24 h and 48 h after challenge with SRBC on day 17 for control (-♦-), cyclophosphamide (-■-) and Imunocin (-●-). Values are expressed as mean ±S.D. of six observations

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