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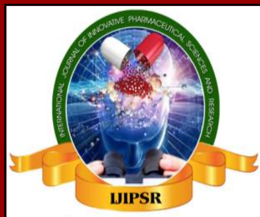

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STIMULATION OF PHAGOCYTIC AND HEMATOPOIETICACTIVITY BY IMMUNOL TABLETS

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Abstract

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 is recognized in the Ayurveda that the immune system is involved in the etiology and pathophysiologic mechanism of many diseases. Immunology is thus probably one of the most rapidly developing areas of biomedical research and has great promises with regard to prevention and treatment of a wide range of disorders. Inflammatory diseases of the skin, gut, respiratory tract, joints and central organs as well as in infectious diseases are now primarily consider immunological disorders, while neoplastic diseases may involve an immunosuppressive state. Immunol Tablets was evaluated for Immunomodulatory effect using various models. Immunol Tablets Increased Phagocytic process.

Keywords: Immunity, Immunol, Ayurveda, Phagocytosis, Ayurchem, Monocyte.

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INTRODUCTION

The function and efficiency of the immune system may be influenced by many exogenous and endogenous factors like food, drugs, physical and psychological stress, hormones etc., resulting in either immunosuppression or immunostimulation [1]. The healthy state is believed to be based on a sophisticated fine-tuning of immunoregulatory mechanisms [2]. Immunomodulation is any procedure which can alter the immune system of an organism by interfering with its function; if it results in an enhancement of immune reactions is named as immunostimulation and primarily implies stimulation of non-specific system i.e. stimulation of the function and efficiency of granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances [3]. Immunosuppression implies mainly to reduced resistance against infections, stress and may be because of environmental or chemotherapeutic factors [4]. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence Immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over. Apart from specific stimulative or suppressive activity certain agents have been shown to posses' activity to normalize or modulate the pathophysiological processes in the underlying immune response and hence the terms immunomodulation or immunomodulatory agents now used [4]. Four classes of immunosuppressives are currently used in clinical practice: (1) cyclosporine and tacrolimus, (2) adrenocortical steroids, (3) cytotoxic drugs and (4) antibody reagents [5]. Four classes of immunostimulants are :(1) Natural adjuvants (2) Immune globulin, (3) synthetic agents, Levamisole and Isoprinosine, and (4) Cytokines, Interferon alfa and Interleukin- [5]. Most of the chemical agents which have effect on immune system are immunosuppressants and cytotoxic agents. These drugs have serious side effects such as nausea, vomiting, alopecia, mucosal ulceration, pulmonary fibrosis, cardiac and hepatic toxicity [6].

One of the therapeutic strategies of Indian herbal medicines is to increase the body's natural resistance to the disease-causing agent. This concept would mean enhancement of immune responsiveness against a pathogen by specifically or nonspecifically activating the immune system using immunomodulatory agents of plant origin [7].

MATERIALS AND METHODS

Chemicals and Drug Sample: Cyclophosphamide (Inj, Dabur), EDTA, Sodium Chloride, Neutral red pH indicator, Ethanol (SD fine chem), Immunol Tablet (Ayurchem Products)

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Animals

Mice: Healthy Swiss albino mice of either sex were housed in the animal house of Bombay College of Pharmacy. Healthy female Balb/c mice were brought from Glenmark laboratories and housed in the animal house of Bombay College of Pharmacy.Animals were fed with commercially available Amrut rat and mice feed, manufactured by NavMaharastraChakan Oil Mill Ltd, Pune. Animals were maintained under standard conditions of temperature ($25^oC±5^oC$) and relative humidity (55± 10%), and 12h/12h light /dark cycle. They were housed in standard polypropylene cages with wire mesh top and husk bedding. The research project was approved by institutional animal ethics committee.

Experimental models:

1. Effect of Immunol on peritoneal macrophages in mice [8]

Female albino mice of strain Balb/c were divided into 5 groups of 5 mice each. Group I served as control group and received distilled water orally. Drug was finely suspended in distilled water and sonicated for 2 mins. Group II received Immunol at the dose of 500mg/kg orally for 5 days. Group III received Immunol at the dose of 500mg/kg orally for 10 days. Group IV received Immunol at the dose of 500mg/kg orally for 15 days. At the end of respective treatment period mice were sacrificed by cervical dislocation. Peritoneal fluid was collected in phosphate buffer saline. The macrophage count and cell size in peritoneal was counted with ocular micrometer in 45x. The data obtained was analyzed using student's t- test.

2. Carbon clearance assay [9]

Female Swiss albino mice were divided into 2 groups of 5 mice each. Group I served as control group and received distilled water orally. Group II received Immunol at the dose of 500mg/kg orally for 10 days. On 10th day of treatment 0.3 ml of Carbon ink was administered i/v. to all the animals of both groups. Blood samples were collected into small centrifuge tubes from retro-orbital plexuses at 3, 6, 9, 12 and 15 minutes. Immediately 25 µl of the blood was lysed in 3ml of distilled water and the optical density was measured spectrophotometrically at 650 nm. The graph of absorbance against time was plotted and phagocytic index was calculated. The stimulation of phagocytic rate was obtained as the ratio of slope of regression line of the drug [k (Sample)] to the slope of regression line of the control [k (Control)]. According to Jurcie indices, values 0 were considered to represent - absence of activity. Values between 1.3 and 1.5 are active and values >1.5 are very active.

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3. Effect of Immunol on hematological profile in mice [8]

Male Swiss albino mice were divided into 2 groups of 5 mice each.Group I served as control group and received distilled water orally.Group II received Immunol at the dose of 500mg/kg orally for 10 days. On 10th day blood was withdrawn from retro-orbital plexuses into vials containing anticoagulant EDTA. Haematological parameters: the blood samples were studied for following parameters, (i) Haemoglobin content (ii) Haematocrit value (iii) RBC (iv) Total WBC. The total cell count was determined using Erma PC-607 cell counter. Whereas Differential leukocyte count (DLC) was done by making smear of blood samples. Statistical analysis: the data obtained was analyzed using student's t- test.

RESULTS

Selection of the dose of Immunol

The dose of Immunol was calculated from the human dose. The human dose was 2.3 grams i.e. 4 Immunol tablets. This dose was for a 60 kg individual and hence considering the conversion factor 12.3 for a mouse, the dose calculated was 500 mg/kg. Preliminary studies were carried using 500 mg/kg. This dose was found to show activity and hence this dose was selected for the rest of the study.

Table 1: Effect of Immunol on peritoneal macrophages size and count in mice

Days of Immunol treatment (500 mg/kg)	0	5	10	15
Macrophage Cells/mm^3	923 ± 229.15	1093.2 ± 418.82	1126.7 ± 104.41	5429.4 ± 2969.65
Cell size (range in µm)	14-18	18 - 22	28 - 32	40 – 46
T-test	-	0.09173 $^{n.s.}$	0.079444 $^{n.s.}$	0.011661*

Results are expressed as the mean ± s.d. of 5 observations.
'*': significant differenceat p <0.05 as compared to 0th day by students t-test.
n.s.: nonsignificant difference at p<0.05 as compared to 0th day by students t-test.

Table 2: Effect of Immunol on phagocytic activity by macrophages in mice:

Time in minutes	Control group	Immunol treated
	Mean absorbance (650nm)	Mean absorbance (650nm)
0	0 ± 0	0 ± 0
3	0.4232 ± 0.09	0.2395 ± 0.07
6	0.3109 ± 0.09	0.1476 ± 0.05
9	0.2516 ± 0.10	0.1062 ± 0.03
12	0.1714 ± 0.08	0.0782 ± 0.04
15	0.1358 ± 0.08	0.0684 ± 0.03
Mean phagocytic index / mean slope	0.0029 ± 0.019	0.0039 ± 0.006

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Fig.1: Effect of Immunol on carbon clearance activity in mice:

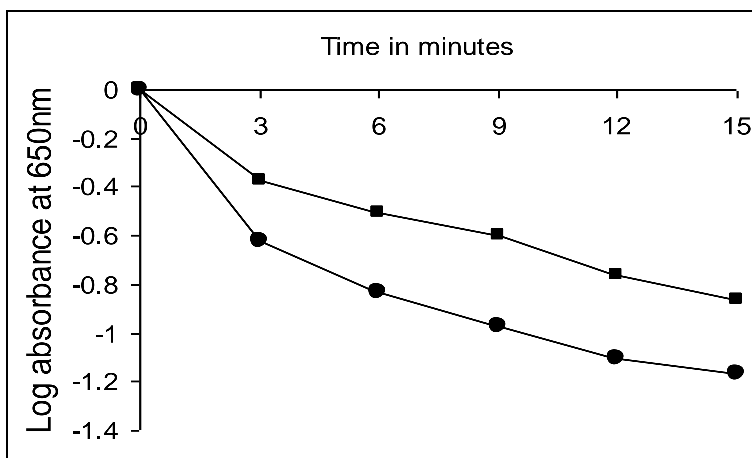

Table 3: Effect of Immunol on hematological profile in mice

Groups	Dose (mg/kg)	RBC millions/mm3	HCT (%)	MCV (um^3)	WBC 1000/mm^3	Hgb gm%	% Neutrophils
Control	-	7.73 ± 1.3	32.96 ± 6.7	42.46 ± 2.0	8.78 ± 0.4	12.56 ± 2.1	22.53 ± 3.8
Immunol	500	8.18 ± 0.8^{ns}	31.88 ± 2.9 ^{ns}	38.96 ± 0.5 ^{ã}	14.66 ± 1.5^{ã}	14.9 ± 0.9^{ns}	33.23 ± 3.3 ^{ã}

Results are expressed as the mean ± s.d. of 5 observations.

ã:significant differenceat p <0.01 as compared to control group by students t-test.

ns: nonsignificant differenceat p <0.01 as compared to control group by students t-test.

DISCUSSION

Macrophages play an important role in nonspecific and specific immune response. It is well-known that macrophages are able to ingest and kill microorganisms. Macrophages also regulates both humoral and cellular immune responses. Here the effect of Immunol at a dose of 500 mg/kg/day orally was evaluated on mouse peritoneal macrophage count and its size. Peritoneal macrophage count was taken because peritoneal macrophages are representative of other macrophage populations and are also readily available in large numbers in mice [10]. Table no: 1 shows that Immunol caused increase in the peritoneal macrophage count progressively up to 15 days. The cell size also increased progressively up to 15th day. Maximum cell count was seen on 15th day of the Immunol treatment i.e. (5429.4 ± 2969.65) cells which is significantly more than the 0th day count. It is known that when macrophages are in the activated state they are larger, adhere better, spread faster on glass and more phagocytic [11]. Hence it can be concluded that Immunol caused activation of macrophages as seen from the bigger size of macrophages. From

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the above results it was clear that Immunol stimulated the production of macrophages and also caused increased in its size. As macrophages arise from a common precursor in the bone marrow, which give rise to blood monocyte. From the blood, monocytes migrate into various tissues and transforms into macrophages [4]. Hence Immunol might have effect on bone marrow and may stimulate monocyte-macrophage lineage.

In this study Immunol was given at the dose of 500 mg/kg/day orally for 10 days to assess the phagocytic capability of the macrophages in mice by carbon clearance assay. Carbon clearance assay is used to assess the rate of removal of injected colloidal carbon particles from the bloodstream, which correlates reticuloendothelial phagocytic activity. When colloidal carbon particles are injected intravenously, the carbon particles are removed by sessile intravascular phagocytes in the liver and spleen. The kupffer cells of the liver take up approximately 90% and the splenic macrophages 10% [12]. When carbon ink was injected into control animals it was seen that more amount of the carbon particles were present in the blood, as evidenced by high absorbance readings and which can be clearly seen from Table no: 2. It was seen that the mean absorbance at 650nm for control animals reached its peak value of 0.4232 at 3 minutes after the injection of carbon ink and progressively decreased up to 15 minutes, which indicates that lesser amount of carbon particles were phagocytosed. Whereas when carbon ink was injected into Immunol treated animals it was seen that less amount of the carbon particles were present in the blood, as evidenced by low absorbance readings. The mean absorbance at 650nm for Immunol treated animals was found to reach its peak value of only 0.2395 at 3 minutes after the injection of carbon ink and progressively decreased up to 15 minutes. The observations show that more amount of carbon particles were phagocytosed as compared to control animals. Therefore Immunol has stimulated phagocytic capability of the macrophages (Table no: 2). To confirm the increase in the phagocytic capability of macrophages by Immunol treated animals, slope of the control and Immunol treated group was calculated from figure no1. It was found that slope of control group was 0.0534 whereas for Immunol treated group slope was 0.0707. Increase in the phagocytic capability by macrophages was confirmed by phagocytic rate. The stimulation of phagocytic rate is obtained as the ratio of slope of regression line of the Immunol treated group to the slope of regression line of the control group. The phagocytic rate was found to be 1.323. According to *Wagner et al*, phagocytic rate having values 0 are considered to represent an absence of phagocytic activity whereas values between 1.0 – 1.5 are active and values more than

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1.5 are very active [12]. Phagocytic rate obtained in the present study being 1.32 indicates stimulation of phagocytosis.

Drugs which increase the phagocytic capacity by rapid clearance of colloidal carbon from blood stream there is decrease in the half life of colloidal carbon particles [13]. Half life calculated, (figure no: 1) for control group was 12.98 minutes whereas half life for drug treated group was 9.80 minutes. Since half life is decreased for Immunol treated group it can be said that Immunol increased phagocytic capability of macrophages. The reticuloendothelial system (RES) plays an important role in host defence. It is responsible for clearing the blood of particulate matter, bacteria and endotoxins. The system is populated by cells of the monocyte-macrophage lineage. RES functions can be evaluated by carbon clearance test. This test also indirectly reflects the activity of kupffer cells of the liver as they constitute 80-90% of the cells of the RES [14]. The stimulation of monocyte-macrophage lineage and increase in clearance of particulate matter from blood by Immunol are indications of stimulation of reticuloendothelial system. Haemopoetic system is the major indicator for the changes in the immune system. Hematopoiesis is the process of generation and maturation of the blood cells. The cellular constituents of the immune system arise from the common precursor cell ie pluripotent stem cell in bone marrow. The production of immune cells is one component of haematopoiesis, a process by which all cells get differentiated into particular lineage. The proliferation and maturation of precursor cells in the bone marrow are stimulated by cytokines. In the absence of infection, bone-marrow stromal cells are the major source of haematopoietic cytokines. In the presence of infection, cytokines produced by activated macrophages and T-helper cells induce additional haematopoietic activity [4]. Immunol caused significant increase in leucocyte count and % neutrophils. Increase in leucocyte count is due to secretion of interleukin-1 and colony stimulating factor (GM-CSF) from activated macrophages [15]. Earlier models shown that Immunol stimulated monocyte-macrophage lineage. Hence it can be concluded that leucocytosis is due to activated macrophages by Immunol. Immunol had therefore stimulated haematopoiesis (table 3). This suggests that even for a normal healthy individual, treatment with Immunol will serve as an immuno-stimulating agent.

CONCLUSION

From this study it can be concluded that Immunol Tablets have stimulate monocyte macrophage lineage. Further studies on Humoral and T-cells would be planned to understand the immunomodulatory effect of Immunol Tablets.

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