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Immunoboosting and Increase in Antibody titre by Immunol Tablets

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ABSTRACT: Various chemical agents have been used tomodulate immune system. Ayurvedic medicines constitute a rich source of activesubstances. Various plants have been shown to modulate immune system either bystimulating specific or nonspecific immunity. Hence combination of various plants wouldtherefore have diverse actions on various aspects of the immune system[15].Earlier study shown that Immunol Tablet Simulated Phagocytic and Haemtopoietic activity. Hence current study was designed to investigate the immunoboosting and increase in antibody titreactivity of an Ayurvedic formulation, Immunol Tablets. Effect of Immunol Tablet was evaluated on haematological profile in normal and myelosuppressed mice, humoral and cellmediatedresponses and prophylactic activity in E.coli induced peritonitis. Results wereanalyzed by students't'-test at $p<0.05$.It produces significant leucocytosis with predominantneutrophilia and prevented myelosuppressive activity of cyclophosphamide. Immunol atthe dose of750 mg/kg produced significant increase in antibody titre and potentiated DTHreaction against sheep erythrocytes. Immunol at the dose of 750 mg/kg showed 85.72 %protection against E.coli induced peritonitis.Immunol tablet enhanced B and T cell proliferation as well as protected mice from E.coli induced abdominal peritonitis.

Keywords: Immunity, Immunol, Antibody, Ayurved, Immunobooster

I. INTRODUCTION:

Charaka or Sushruta ,The ancient scholars and authers of Grantha (Text books) suggested that various properties of plants such as jeevaniya, balya,vayasthapaniya or rasayana might have immunological effects. Jeevaniya means lifepromoters, balya means strengtheners and vayasthapaniya means increase life span[12]. The term rasayana includes all these activities. Rasayana is one ofthe therapeutic strategies in ayurvedic medicines which increase the body's ownresistance power to the disease causing agents [12]. Different agents ofplant origins are reported to interact with immune system in a complex way and modulatethe pathophysiological process[4].

Immunol an Ayurvedic formulation has following ingredients: Amrita (Tinosporacordifolia), Gokhsur (Tribularisterristris), Amala (Emblicaofficinalis), Vidang (Embelliribes), Tulsi (Ocimum sanctum), Shatavari (Asparagus racemosus), Katuki (Picrorrhizakurroa), Ashwaghandha (Withaniasomnifera), Trikatu (combination of Piper longum,& Piper nigrum,&Zingiber officinalis). All these plants are used ethnomedically and have been proven tohave immunomodulatory activity[7, 10, 12, 16]. But the combinations of these plants have not beenstudied earlier. Hence it was decided to evaluate Immunol for its immunomodulatoryactivity.

II. MATERIALS:

A) List of chemicals, drugs and solvents:

Cyclophosphamide injection Ledoxan, Dabur Pharmaceuticals, Carbon ink Rotring, black, Germany, ERBA haemolyses Transasia, ERBA diluent Transasia, MacConkeys agar, Hi- media laboratories, SRBC were brought from Bombay Veterinary College, Parel in Alsevers solution andstored in the same.Immunol Tablet was provided as a gift sample by Ayurchem Products, Mumbai.

B) Animals

Mice: Healthy Swiss albino mice of either sex were housed in the animal house ofBombay College of pharmacy were used. Healthy female Balb/c mice were brought from Glenmark laboratories, Mumbai andhoused in the animal house of Bombay College of Pharmacy were used.

Animal feed: Animals were fed with commercially available Amrut rat and mice feed,manufactured by

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NavMaharastraChakan Oil Mill Ltd, Pune. The animal feed containedcrude protein, crude fibre, and crude oil.Animal housing: animals were maintained under standard conditions of temperature(25°C±5°C) and relative humidity (55± 10%), and 12h/12h light /dark cycle. They werehoused in standard polypropylene cages with wire mesh top and husk bedding. Theresearch project was approved by institutional animal ethics committee vide 242/PO/RE/S/2000/CPCSEA; 01/08/2000 vide protocolapproval number as CPCSEA-BCP-/206/ 2002.

C) Experimental models:

1) 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.

2. Evaluation of immunopotentiating effect of Immunol Tablets:

2.1 Cyclophosphamide induced myelosuppression in mice: [5]

Male Swiss albino mice were divided in to four groups of 5 mice each.Group I control (Distilled water).Group II Immunol treated (500 mg/kg for 11 days).Group III cyclophosphamide treated (200 mg/kg on 11th day of vehicle treatment).Group IV Immunol treated (500 mg/kg) + cyclophosphamide (200 mg/kg)On 11th day, Group III & IV received a single dose of cyclophosphamide (200 mg/kg).On 12th day, blood was collected from retro-orbital plexuses of the individual mice fromall the groups. RBC, Total WBC, % Neutrophil count was determined. Total cell valueswere determined using Erma PC-607 cell counter. Dry smear of the blood on the slideswere stained using Field A and Field B stain for % Neutrophil count of each animal usingcompound microscope[3].

2.2 Humoral response (Antibody Production) in normal and cyclophosphamide treated mice: [1]

Female Swiss albino mice were divided in to 4 groups of 5 mice each.Group I control group (distilled water).Group II Immunol treated (750mg/kg for 1-5 days).Group III Cyclophosphamide treated 25 mg/kg i.p. 2 hours prior to sensitization withsheep erythrocytes.Group IV Immunol treated (750mg/kg for 1-5 days) + Cyclophosphamide 25 mg/kg i.p. 2hours prior to sensitization with sheep erythrocytesSensitization: All the mice were primed with 2 x 10^8 cells of SRBC on day 0. On 4th dayblood was withdrawn from animal by retro-orbital plexus, serum was separated. 25 µl oftwo fold-diluted serum was challenged with 25 µl of 0.1% v/v of SRBC suspension intitre plates. The plates were incubated at 37o C for 1 hour and then observed forhaemagglutination. The highest dilution was taken as antibody titre. The antibody titreswere expressed in a graded manner, the minimum dilution to be taken rank 1.

2.3 Delayed type hypersensitivity in mice: [1]

Animals from humoral responses were continued for the Delayed type hypersensitivityreaction.Sensitization and challenge: Delayed hypersensitivity was induced in mice using sheepred blood cells (SRBC) as antigen in Alseviers solution. Animals were primed with $2x10^8$ SRBC (day 0) and challenged on day 5 with 2 x 108 SRBC subcutaneously in thehind footpad. The right hind paw received saline alone. Paw thickness measurement weremade with Mitutoyo dial caliper at 20, 22, 24, 26, 28, 48, 72 and 96 hours after challenge.The results were expressed as the percentage increase in hind paw volume as compared tothe initial hind paw volume and % edema was calculated.

3. Evaluation of Immunoprophylactic effect of Immunol:E.coli induced abdominal peritonitis [9]

Female Balb/c mice were divided into 3 groups of 7 mice each.Group I control group (distilled water).Group II Immunol treated (750 mg/kg for 15 days).Group III Positive control (plain nutrient broth i/p).E.coli induced abdominal peritonitis was carried out in two parts. In the first part thestrength of E.coli was standardized to induce 100% mortality. In the second part theeffect of E.coli injection in mice and protection by the drug was evaluated. On day 15 th,3 hours after the last dose of Immunol, E.coli (1x 10^8 cells) was given intraperitoneallyto all the groups of mice and percentage mortality was observed from 16-19 hours as thatwas the expected time of mortality.

Evaluation: % protection of Immunol treated group with respect to control group was calculated.

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III. RESULTS:

1. Evaluation of immunopotentiating effect of Immunol:

1.1Cyclophosphamideinduced myelosuppression in mice:

Immunol treated group showed significant (P< 0.05) increase in total WBC and %Neutrophil count as compared to control animals, CYP treated group and (CYP+Immunol) treated group (Table no: 1).

Cyclophosphamide (200mg/kg) showed significant (P< 0.05) decrease in the total RBCand WBC count as compared to control and immunol treated group. Significant decreasein % Neutrophil count was observed as compared to immunol treated group and (CYP+Immunol) treated group (Table no: 1).

When Cyclophosphamide (200 mg/kg) was given along with Immunol (500 mg/kg),significant (P< 0.05) increase in total WBC count and % Neutrophil as compared to CYPtreated group (Table No. 1).

Table no 1: Effect of Immunol Tablets on haematological profile in normal and myelosuppressed mice:

Treatment Groups	Dose (mg/kg)	RBC (million/mm^3)	WBC (1000/mm^3)	% Neutrophils
Control	-	7.73 ± 1.3	8.78 ± 0.4	22.53 ± 3.7
Immunol	500	8.18 ± 0.8	14.66 ± 1.5*	33.22 ± 3.3*
CYP	200	5.73 ± 0.3*ā	6 ± 0.7*ā	21.39 ± 1.58 €¥
CYP + Immunol	200 500	7.96 ± 1.4£	9.02 ± 1.4ā£	38.58 ± 1.9□ *€

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

*: significant difference at p< 0.05 as compared to control group by students t-test.

ā: significant difference at p< 0.05 as compared to drug treated by students t-test.

£: significant difference at p< 0.05 as compared to CYP group by students t-test.

1.2 Humoral response (Antibody Production) in normal and cyclophosphamide treated mice:

Studies of Immunol with 500 mg/kg did not showed significant effect on humoral andcell mediated responses hence it was decided to increase the dose to 750 mg/kg. Fromtable no: 2 it can be seen that the antibody titre of Immunol treated animals issignificantly (p < 0.05) more than control, cyclophosphamide and (Cyclophosphamide +Immunol) treated animals.

Table no 2: Effect of immunol on humoral responses in normal and cyclophosphamide treated mice:

Treatment groups	Dose (mg/kg)	Mean antibody titre
Control	-	8.8 ± 0.72
Immunol	750	10.2 ± 0.32*
Cyclophosphamide	25	9.2 ± 0.64
Cyclophosphamide + Immunol	25 750	9.2 ± 0.64

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Results are expressed as the mean ± s.d. of 5 observations.
*:significant differenceat p <0.05 as compared to control, cyclophosphamide and (Cyclophosphamide + Immunol) by student's t-test.

1.3 Delayed type hypersensitivity reaction in mice:

The mean percent edema at 24 hrs for control animals was 74.44% and that of Immunoltreated group 89.74% which is significantly (p< 0.05) higher than the control group(Table no: 3).Low dose of cyclophosphamide given prior to sensitization showed potentiation of DTH(Mean percent edema 109.71% at 24 hrs) which is significantly (p< 0.01) high ascompared to control and Immunol treated group (Table no: 3).(CYP + Immunol) treated animals, showed a significant (p<0.01) reduction in meanpercent edema as compared to cyclophosphamide treatment alone. But the mean percentedema of (CYP + Immunol) treated group was significantly (p< 0.01) higher than controland Immunol treated animals.

Table no 3: Effect of Immunol on mean % edema in normal and cyclophosphamide treated mice:

Treatment groups	Dose (mg/kg)	Mean % edema			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
Control	-	74.44 ± 10.00	55.73 ± 9.44	31.21 ± 4.06	9.89 ± 2.7
Immunol	750	89.74 ± 2.56 *	59.31 ± 3.21	34.34 ± 8.49	10.85 ± 2.30
Cyclophosphamide	25	109.71 ± 4.43 *¥	58.88 ± 3.42	40.19 ± 6.79	20.85 ± 4.37
Cyclophosphamide + Immunol	25 750	96.44± 2.16 *¥ã	51.59 ± 4.89	38.86 ± 8.98	17.76 ± 4.03

Results are expressed as the mean ± s.d. of 5 observations.
*: significant difference at p< 0.05 as compared to control by students t-test.
¥: significant difference at p< 0.01 as compared to immunol treatment by students t-test.
ã: significant difference at p< 0.01 as compared to CYP group by students t-test.

2. Evaluation of Immunoprophylactic effect of Immunol:E.coliinduced abdominal peritonitis in mice:

100% mortality observed in control animals. Immunol at the dose of 750 mg/kg/dayorally for 15 days reduced percentage mortality to 14.28 % as compared to controlanimals (Table no: 4). Positive control i.e. only plain broth was given and there was nomortality observed. Thus any mortality observed was due to E. coli infection.

Table no 4: Effect of immunol on E.coli induced abdominal peritonitis in mice:

Treatment groups	Dose (mg/kg)	Percentage mortality
Control	-	100

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Positive control (plain broth)	-	0
Immunol	750	14.28

Results are expressed on the basis of 7 observations.

IV. DISCUSSION:

Results of earlier studies indicate that immunol had an immuno-stimulating effect in normal mice. Immunol stimulated monocyte-macrophage lineage as well as stimulated haematopoiesis. So the potentiating effect of immunol was evaluated against myelosuppression induced by cyclophosphamide in mice and against hypersensitivity induced by antigen.

Bone marrow is a site of continued proliferation and turnover of blood cells and is a source of cells involved in immune reactivity. A high degree of cell proliferation renders bone marrow a sensitive target, particularly to cytotoxic drugs [1]. Cytotoxic drugs like cyclophosphamide and azathioprin act at various levels on cells involved in defence against foreign invaders. The suppression of bone marrow activity reflecting myelosuppression bycyclophosphamide was significant as can be seen by decrease in blood cell counts and %Neutrophils (table no: 1). Combined treatment of myelosuppressive drug and Immunolresulted in a restoration of bone marrow activity as compared to cyclophosphamide alone. Immunol treated animals showed significant increase in bone marrow activity ascompared to cyclophosphamide alone and even control animals. Hence Immunol mighthave prevented myelosuppressive activity of cyclophosphamide and could be used as anadjuvant in cancer therapy.

In specific immunity macrophages regulate both humoral and cellular immune responses i.e. the regulation of B and T cells. As the results of our study showed stimulation of monocyte-macrophage lineage by immunol, it was decided to study the effect on humoral responses i.e. on B cells. Humoral responses were initiated by sensitizing and challenging mice with sheep erythrocytes as foreign antigens. Antibody titres were determined using haemagglutination method [8]. The haemagglutination antibody titre test is indicative of thedegree of humoral responses. The humoral immunity involves interaction of B-cells withantigen and their subsequent proliferation and differentiation into antibody secretingplasma cells. The augmentation of the humoral response to sheep erythrocytes indicatesthe enhanced responsiveness of the macrophages and T and B lymphocyte subsetsinvolved in the antibody synthesis [11]. Macrophages also play a pivotalrole in coordinating the processing and presentation of antigen to T and B cells[6]. Thepresent study showed stimulation of monocyte-macrophage lineage by Immunol and thusenhancement of humoral effect by facilitating such responses.

Control of disease by immunologic means has two objectives: the development of immunity and the avoidance of undesired immune reactions. Modification of immune functions by pharmacological agents is emerging as a major area of therapeutics in those cases where undesired immunosuppression is the result of therapy. Such efforts were previously being carried out by using glucocorticoids in combination with cytotoxic drugs like cyclophosphamide. On other hand, undesired immunostimulation (i.e. hyper-reactivity) is a common side effect with drugs like quinine, salicylates, indomethacin, etc. Whether or not experimentally induced hyper reactivity is restored back to normal was checked by using immunol in the delayed type hypersensitivity animal models[1].

Delayed type hypersensitivity (DTH) is a typical T-cell mediated immune response in the skin. DTH begins with the first exposure of specific antigen to macrophages, resulting in antigen specific $CD4^+$ T-cell activation, expansion and differentiation into effector and memory T-cells. Upon second exposure of same antigen, the specific memory T-cells are stimulated to proliferate and to release lymphokines. The continual release of lymphokines from sensitized T-cells results in the accumulation of large number of activated macrophages that become epitheloid cells [2].

Immunol showed a potentiating effect on DTH in normal mice whereas the potentiatingeffect on DTH of cyclophosphamide was reduced by Immunol. Cyclophosphamide at thedose of 25mg/kg showed maximum potentiation of DTH because cyclophosphamidedamaged short lived suppressor T cells in immune regulatory system. This is also inaccordance with earlier reports [9]. Thus Immunol showsmodulating effect on the immune system. It stimulates immune system in

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normalconditions whereas it suppresses immune system in hypersensitized conditions. Thus itcan be concluded that Immunol stimulated cell mediated immunity, by having stimulatingeffect on T lymphocytes and accessory cell types required for the expression of the DTHreaction.

The intestinal tract harbours a large number of bacteria which under normal condition are not able to invade the peritoneal cavity. However, if the defense barriers get broken down under diseased conditions or trauma, bacteria have an access into the peritoneal cavity and produce sepsis [13]. Intraabdominal sepsis continues to be a major cause of morbidity and mortality following trauma and abdominal surgery for bowel perforations[14]. Treatment of this condition has always been focused on appropriate surgery supplemented with antibiotics and good nutritional support. In spite of such therapy fatal complications often occur. Nowadays stimulating cellular immune function and increase in resistance to infection has been more emphasized[13]. Immunol has stimulated macrophage count and its phagocytic capability. It also producedsignificant leucocytosis along with predominant neutrophilia, which probably occurs dueto secretion of IL-1 and GM-CSF from activated macrophages [13].Thus immunol showed stimulation of non-specific defense system which appears to bethe underlying mechanism of protection against E. coli induced peritonitis.

V. CONCLUSION:

From this study it can be concluded that Immunol Tablets has Immuno-boosting activity from the following effects:

- Stimulated haematopoiesis and bone marrow.
- Stimulated B cell proliferation. Increased antibody titre and hence also as an adjuvant in vaccination.
- Stimulated T cell and accessory cells types.
- Enhanced responsiveness of macrophages and T and B lymphocytes subsets involved in antibody synthesis.
- Can be used as an adjuvant in cancer chemotherapy.
- Can be used with antibiotics for treating infections.

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