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Immunoprophylactic Activity of Immunol, a Polyherbal Formulation Against Dexamethasone Induced Immunosuppression in Rats

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Abstract: The objective of this study was to evaluate the immunoprophylactic activity of immunol, a polyherbal formulation against dexamethasone induced immunosuppression in rats. The rats were administered oral doses of 1, 5 and 10 mL kg^{-1} b.wt., orally for 28 days following which immunosuppression was induced using dexamethasone (5 mg kg^{-1}, twice daily for 3 days, intraperitoneally). Immunol at the dose of 10 mL kg^{-1} b.wt. exhibited prophylactic effect against dexamethasone induced immune suppression by way of both cell mediated and humoral mediated immunity.

Key words: Humoral mediated immunity, cell mediated immunity, dinitrochlorbenzene, plaque forming cell, ayurveda, immunomodulation

INTRODUCTION

Ayurveda is one of the traditional systems of medicine practiced in India and Sri Lanka and can be traced back to 6000 BC (Samhita, 1949). Great emphasis has been placed on research of herbal formulations which can be helpful in the management of stress related disorders. One of the main approaches in Ayurvedic medicine is to increase the body's natural resistance to disease/stress causing agents rather than directly neutralizing the agent itself. This has been achieved by using extracts of various plant materials known as Rasayana [Rejuvenation] (Pallabi *et al*., 1998). Side effects and higher expenses often associated with allopathic drugs have provoked the need for research into drugs, which are devoid of side effects and which are more cost effective, particularly drugs belonging to the traditional systems of medicine like Ayurveda, Homeopathy, Unani, Siddha etc. Emphasis is also laid on the integration of indigenous healthcare systems with modern medical practices (Ziauddin *et al*., 1996). It is observed that acute stress may enhance immune response whereas chronic stress may suppress the immune system (Quinn, 1990).

Immunomodulatory therapy could provide an alternative to conventional chemotherapy for a variety of diseased conditions, especially when the host's defense mechanisms have to be activated under the conditions of impaired immune responsiveness or when a selective immunosuppression has to be induced in a situation, like inflammatory diseases, auto-immune disorders and organ/bone marrow transplantation (Upadhyay, 1997).

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Plant extracts used in traditional therapy are being reviewed for their chemoprotective and Immunomodulatory activities. Latorre *et al.* (2009) investigated *Ipomoea carnea* aqueous fraction and swainsonine for immunomodulatory potential and reported that swainsonine has immunostimulant effect in mice. Pretreatment with the ethanolic extract of *Lagenaria siceraria* fruits ameliorated the stress-induced variations in this biochemical parameters-serum glucose, triglyceride, cholesterol, BUN and cortisol levels, blood cell counts and organ weights in forced swimming endurance test (physical stress) and acute heat induced stress rat models and therefore suggests an antistress and adaptogenic property of *Lagenaria siceraria* (Lakshmi and Sudhakar, 2009). Ethanolic leaf extract of *Ocimum gratissimum* was found effective in inhibiting/preventing the disease condition after infection and capable of reducing excessive breakdown of red blood cells and neutralizing toxin produced by the organism (Oladunmoye, 2006). Prophylactic use of Echinacea purpurea extract resulted in stimulation of granulocytes chemiluminescent and lymphocytes proliferative response (Bang *et al*., 2003). Otsuki *et al.* (2010) observed that *Carica papaya* leaf extract can mediate a Th1 type shift in human immune system and suggested that the *Carica papaya* leaf extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, various allergic disorders and may also serve as immunoadjuvant for vaccine therapy. Several studies have shown that Septilin, a polyherbal formulation possess immunomodulatory effect, such as stimulation of the leucocyte production and increased differentiation (Kumar *et al*., 1992), protect mice from cyclophosphamide-induced leucopenia (Kumar *et al*., 1995) and antibody producing cells in the spleen and activation of antibody-dependent complement-mediated cell lysis (Kumar *et al*., 1997). Pallabi *et al.* (1998) demonstrated the immunoprophylactic and immunopotentiating activity of Immu-21, polyherbal ayurvedic formulation in rats. Other study by Kumar *et al.* (2002) indicated that pre-treatment with Immu-21 selectively increased the proliferation of splenic leucocytes to B-cell mitogens, Lipopolysaccharide and cytotoxic activity against K562 cells in mice. Immunomodulators are biological response modifiers which exert their antitumour effects by improving host defense mechanisms against the tumour. They have a direct anti-proliferative effect on tumour cells and also enhance the ability of the host to tolerate damage by toxic chemicals that may be used to destroy cancer. Most of these preparations are widely advertised, are available over the counter and are used without medical supervision. Sale of spurious herbal drugs with disastrous effects on health and well being has brought the natural remedies into disrepute. It hence, becomes necessary to study the safety of such herbal preparations and to ensure that the benefits outweigh the risks (if any) of using such preparations and also to study the prophylactic and/or therapeutic effects.

The present study was undertaken to evaluate the immunoprophylactic potential of immunol against dexamethasone induced immunosuppression in rats.

MATERIALS AND METHODS

Experimental Period

The research project was performed at the Department of Safety Assessment, Advinus Therapeutics, Bangalore during February to April 2007. The research facility is certified for Good Laboratory Practice (GLP) compliance by the Monitoring Authorities of Germany (BfR), The Netherlands (W and V inspectie) and the National GLP Compliance Monitoring Authority of India and is accredited for animal care and use by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. The experimental project was approved by Institutional Animal Ethics committee (IAEC).

Animals

Wistar rats, in-house bred at Toxicology Department of Advinus Therapeutics Private Limited, Peenya Industrial area, Bangalore-560 058, were used in the experiments. Animals of 8 to 10 weeks old were randomly selected and were acclimatized to experimental conditions for 5 days. Animals were housed under standard laboratory conditions, air conditioned with 12-15 filtered fresh air changes h^{-1}, environmental temperature 17-23°C, relative humidity 30-70%, with 12 h fluorescent light (6.00 am to 6.00 pm) and 12 hours dark cycle. Rats were housed individually in standard polypropylene cages (size: approximately L 410×B 280×H 140 mm), with stainless steel top grill having facilities for pellet food (Tetragon chemie Ltd., Bangalore, India) and Aquaguard filtered cum-purified water.

Test Material and Drugs

Immunol® (Batch No. H276006; Manufacture date: August 2006; Expiry date: August 2009) manufactured by The Himalaya Drug Company, Makali, Bangalore, India, was the test product used for the experiment. Each milliliter of immunol contains extracts of *Tinospora cordifolia* (14.5 mg), *Withania somnifera* (7 mg), *Boerhaavaia diffusa* (7 mg), *Asparagus racemosus* (7 mg), *Trigonella foenum-graecum* (4.8 mg), *Terminalia chebula* (3.5 mg) and *Tylophora asthamatica* (4.8 mg). Dexamethasone sodium phosphate (Dexacip®), manufactured by Cipla Ltd., Mumbai, India) was used to induce immunosuppression.

Experiment Design

Five groups of rats consisting of 15 males and 15 female rats per group were used, designated as Distilled water control group (G1-10 mL kg^{-1} distilled water), dexamethasone control (G2-10 mL kg^{-1} distilled water), immunol-low (G3-1 mL kg^{-1}), mid (G4-5 mL kg^{-1}) and high (G5-10 mL kg^{-1}) dose groups and were administered distilled water or immunol respectively for 28 consecutive days. Following this, immunosuppression was induced in the rats of G2 to G5 group using dexamethasone (5 mg kg^{-1}, twice daily for 3 days, intraperitoneally). The animals were observed twice daily for clinical signs and mortality, weekly food consumption and body weight, subjected to weekly clinical examinations. The fifteen animals/sex/group were divided into 3 groups for the following investigations: Five animals/sex/group were subjected to (1) clinical laboratory investigations-hematology and clinical chemistry, gross necropsy and histopathology, (2) immunotoxicity assessment using Plaque Forming Cell assay and (3) Delayed Type Hypersensitivity test.

Clinical Laboratory Investigations

Blood was collected from the abdominal aorta at sacrifice. Blood was collected into EDTA and lithium heparinized tubes for haematology and clinical chemistry respectively. Red Blood corpuscles, White Blood Corpuscles, Differential Leukocyte Counts, Haematocrit, Haemoglobin and Platelets were analyzed using ADVIA 2120 Haematology System (Bayer HealthCare LLC, USA). Plasma was separated in a refrigerated centrifuge and analysed for Glucose, Alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP), Blood Urea Nitrogen (BUN), Albumin, Globulin, Albumin/globulin ratio and Total Proteins in Roche/Hitachi 902 Automated Analyser (Hitachi Science Systems Ltd., Japan), using Diagnostic kits (Roche Diagsnostics GmbH, Germany).

Determination of B-Lymphocyte Function

To determine the effect of immunol on humoral immunity, the plaque forming cell response of splenic lymphocytes to sheep erythrocytes was assessed using the method described by Talwar and Gupta (1992). Five males and five females selected from each group

were used for Plaque forming cell assay. The rats were immunized by intravenous injection of 5% Sheep RBC (males: 2 mL, females: 1.5 mL) on the 24th day of treatment. At the end of the treatment period + 3 days immunosuppression (day 32), the animals were euthanized, spleens collected from each animal, homogenized and suspended in chilled Hank's Balanced Salt Solution and the number of lymphocytes per spleen were counted and 0.25 mL of spleen cell suspension was incubated with 0.1 mL of 20% SRBC suspension in 2.0 mL of agarose in Petri plates. After the agar set, the plates were incubated at 37±1°C for 1 h in a humidified atmosphere. After incubation, 2 mL of 1:10 guinea pig complement was added to each plate and the plates were again incubated in the incubator for 30 min. After the 2nd incubation, the complement from each plate was poured out and the number of plaques (clear zones) per spleen were calculated and taken as a measure of humoral immunity.

Determination of T-Lymphocyte Function

To determine the effect of immunol on cell mediated immunity, delayed hypersensitivity to 2,4-dinitrochlorbenzene (DNCB) was assessed using the delayed type hypersensitivity (DTH) skin test described by Rajan *et al*. (1981). Five males and five females selected from each group were used DTH assay. On day 14 an area of 3 cm in diameter was marked on the left flank of the animal using a metallic ring. The hair on the marked site was clipped using an electrical hair clipper. On day 15 primary sensitization was done using 0.4 mL of 2% DNCB in acetone applied drop by drop on the marked area keeping the metallic ring on the site marked. The solution was allowed to evaporate quickly by blowing gently during each application. After 14 days of the primary sensitization a challenge dose of 0.25 mL of 2% DNCB was applied at the same site. The skin thickness was measured before and after primary sensitization and also at 24 and 48 h after challenge. The increase in the skin thickness was taken as a measure of delayed hypersensitivity to DNCB.

Gross Necropsy, Organ Weights and Histopathology

At completion of the experimental period, the animals were fasted overnight (water allowed), anaesthetised, weighed and were subjected to detailed necropsy. Thirty five tissues were collected from each animal and preserved in 10% buffered neutral formalin for further processing for histopathology. Spleen, thymus, kidneys, adrenals, liver, lymph nodes (axillary and mesenteric lymph nodes) were weighed and organ weights ratios as percentage of body weights were determined. Histopathological evaluation of bone marrow cellularity, thymus, lymph nodes (axillary and mesenteric lymph nodes), lungs, liver, bone marrow, spleen and peyer's patches was performed. The tissues were processed for routine paraffin embedding and 5 micron sections were stained with Mayer's Haematoxylin Eosin stain.

Statistical Analysis

All the grouped data were statistically evaluated with SYSTAT Statistical software package version 12.0. The results were expressed as Mean±SD. Differences between control and the treatment groups were assessed by hypothesis testing methods included one-way analysis of variance followed by Dunnett's t-test. All analyses and comparisons were evaluated at 5% ($p \leq 0.05$) level.

RESULTS

There were no clinical signs of toxicity or mortality in immunol treated or dexamethasone control animals in both sexes. Immunol treatment did not affect the body weight gain (Table 1) and food consumption (Table 2) in any of the dose groups of both sexes. The

Table 1: Effect of immunol on the body weights of rats upon administration for 28 days

	Body weight (g) at week					Net body weight gain (g) at
Groups	Pre-dose	1	2	3	4	termination
Males						
G1	252±14.4	273±18.3	281±19.7	291±16.6	306±26.5	54±8.5
G2	289±4.6	301±2.0	326±9.0	347±15.5	367±24.1	78±19.5*
G3	295±4.7	316±11.3	326±6.8	336±5.6	346±10.4	51±3.1
G4	297±9.3	306±15.2	325±11.6	341±13.6	352±24.1	55±28.6
G5	298±6.8	313±2.8	330±6.8	344±18.7	359±18.3	61±24.4
Females						
G1	185±16.3	199±18.5	207±17.8	214±19.7	216±18.0	31±6.7
G2	202±11.5	207±11.5	211±4.0	217±8.5	220±15.1	18±28.7*
G3	194±8.1	201±11.1	203±11.0	206±8.1	208±8.5	14±6.4*
G4	200±7.4	208±3.7	212±2.5	213±4.2	215±6.4	15±9.0*
G5	191±15.7	203±14.5	210±11.8	218±7.3	222±5.6	31±7.9

Values are Mean±SD. *$p \leq 0.05$ vs. control group

Table 2: Effect of immunol on the food consumption of rats upon administration for 28 days

	Food consumption (g/rat/day) at week				Food conversion
Groups	1	2	3	4	efficiency
Males					
G1	23.0±1.30	22.7±1.33	23.0±0.98	23.6±1.15	58.0±13.8
G2	22.4±0.65	23.9±1.40	24.2±1.88	24.6±1.98	82.6±27.6*
G3	25.2±1.47	24.1±0.85	24.8±2.08	24.7±1.82	51.3±12.7
G4	23.3±1.53	22.8±1.38	23.6±1.69	23.6±1.70	58.2±23.8
G5	23.6±1.80	24.3±1.11	25.1±1.72	25.4±1.01	61.8±16.1
Females					
G1	16.7±1.23	16.7±0.61	17.1±0.86	17.2±0.72	45.2±7.3
G2	18.2±1.59	18.1±2.26	18.6±2.10	19.6±1.69	46.7±10.9
G3	17.6±0.99	16.3±1.11	17.2±1.97	17.1±1.56	51.8±6.9
G4	18.5±0.79	16.4±0.94	17.0±1.27	18.3±0.44	57.8±10.5
G5	17.2±1.86	16.7±1.12	17.0±1.30	17.6±0.86	68.7±8.7*

Values are Mean±SD. *$p \leq 0.05$ vs. control group

dexamethasone control group males showed an unusual higher net body weight gain and FCE. In contrast, the dexamethasone control females and the low and mid dose immunol+dexamethasone treated groups showed significantly lower body weight gain in comparison with the distilled water group. The animals of the high dose immunol group animals gained body weight. comparable with the distilled water control group however, they showed a higher FCE in both sexes (statistically significant in females.

The leucocyte count of the dexamethasone control and low and mid dose immunol+ dexamethasone treated groups were 4.55±0.43, 4.22±0.59 and 4.48±1.63 G L^{-1}, respectively, which were significantly lower in comparison with the distilled water treated control group (5.55±0.56 G L^{-1}) (Table 3). The neutrophil counts were higher and lymphocyte counts were lower in all the immunol treated groups when compared to the distilled water treated control group.

Glucose, BUN, total proteins, AST, ALT, ALP, albumin, bilibrubin, cholesterol and Triglycerides were higher in dexamethasone control and Immunol + Dexamethasone treated groups, in comparison with control group (Table 4). These changes were comparable to the dexamethasone treated group indicating the effect of dexamethasone on the liver in protein synthesis and stress induction. It also indicated that immunol did not have any ameliorating effect in reducing/preventing these changes.

The mean plaque (antibody) forming cells in the male sex of the control group (G1), dexamethasone control (G2), dexamethasone induced immunosuppression group treated with

Table 3: Prophylactic effect of immunol on the hematology in dexamethasone induced immunosuppressed rats

	Groups				
Parameters	G1	G2	G3	G4	G5
Males					
Leucocytes (G L^{-1})	5.55±0.56	4.55±0.43*	4.22±0.59*	4.48±1.63*	4.8±0.88
Erythrocytes (T L^{-1})	8.81±0.12	8.47±0.14	8.28±0.09	8.63±0.24	8.44±0.38
Hemoglobin (g L^{-1})	161±6.1	154±6.35	155±9.62	153±7.4	153±2.07
Hematocrit (l L^{-1})	0.47±0.018	0.48±0.014	0.50±0.021	0.50±0.01	0.51±0.014
Mean corpuscular volume (Fl)	53.5±1.61	57.1±1.35	60.7±2.49	57.5±2.19	60.2±1.07
Mean corpuscular hemoglobin (pg)	18.3±0.56	18.1±0.88	18.7±1.13	17.7±0.92	18.1±0.61
Mean corpuscular hemoglobin conc. (g L^{-1})	342±6.1	318±13.66	308±8.65	308±9.67	300±4.32
Platelet (G L^{-1})	845±71.26	986±151	945±18.74	819±51.54	927±113.11
Prothrombin time (sec)	16.1±1.22	15.9±1.32	17±0.56	15.1±0.15	16.2±2.18
Neutrophil (%)	20.8±3.11	84±2.18*	86.1±2.26*	81±4.74*	83.8±2.57*
Lymphocyte (%)	75.6±3.57	8.2±0.82*	8.6±2.73*	11±4.79*	9.1±2.43*
Monocyte (%)	1.9±0.47	7.6±1.71	4.8±0.3	7.4±1.24	6.3±0.25
Eosinophil (%)	1.2±0.33	0.1±0.11	0.3±0.16	0.3±0.13	0.6±0.05
Large unstained cell (%)	0.2±0.07	0.2±0.11	0.1±0	0.1±0.12	0.3±0.21
Basophil (%)	0.2±0.16	0.1±0.05	0.1±0	0.1±0.08	0.1±0.05
Reticulocyte (%)	2.58±0.26	3.68±0.24	2.98±0.6	3.12±0.09	3.46±0.24
Females					
Leucocytes (G L^{-1})	5.26±1.19	4.67±0.7	4.9±0.86	4.92±1.15	5.41±1.2
Erythrocytes (T L^{-1})	8.38±0.48	7.85±0.29	7.68±0.2	8.19±0.43	7.91±0.46
Hemoglobin (g L^{-1})	158±9.61	144±2.7	139±2.83	148±3.96	146±8.84
Hematocrit (l L^{-1})	0.47±0.025	0.46±0.007	0.44±0.008	0.47±0.017	0.46±0.023
Mean corpuscular volume (Fl)	55.7±1.61	58.4±1.57	57.8±0.37	57.5±1.02	58±0.5
Mean corpuscular hemoglobin (pg)	18.9±0.38	18.4±0.52	18.1±0.23	18.1±0.59	18.4±0.27
Mean corpuscular hemoglobin conc. (g L^{-1})	340±14.59	315±1.22	313±2.49	315±5.13	318±5.4
Platelet (G L^{-1})	824±78.87	949±34.95	868±74.83	884±52.63	930±47.68
Prothrombin time (sec)	16.2±1.05	16.2±0.43	15.7±0.52	16±0.68	15.1±0.37
Neutrophil (%)	14.9±4.46	82.5±2.2*	80±0.8*	80.7±3.87*	79.9±4.28*
Lymphocyte (%)	80.2±5.28	8.0±1.32*	10.0±0.74*	8.0±2.99*	8.6±2.36*
Monocyte (%)	1.9±0.72	8.8±0.73	9.3±0.47	10.6±1.06	9.7±2.65
Eosinophil (%)	2.5±1.19	0.5±0.28	0.5±0.29	0.6±0.17	1.3±0.61
Large unstained cell (%)	0.3±0.11	0.1±0.08	0±0.05	0.2±0.13	0.4±0.29
Basophil (%)	0.2±0.13	0±0.05	0±0.05	0±0	0.1±0.04
Reticulocyte (%)	2.16±0.49	2.22±0.27	2.15±0.74	3.06±0.8	2.77±0.26

Values are Mean±SD. *$p \leq 0.05$ vs. control group

Table 4: Prophylactic effect of immunol on the clinical chemistry in dexamethasone induced immunosuppressed rats

	Groups				
Parameters	G1	G2	G3	G4	G5
Males					
Glucose (mmol L^{-1})	7.72±1.38	12.89±1.5*	11.04±0.8*	11.33±2.37*	11.43±0.78*
Urea Nitrogen (mmol L^{-1})	1.98±0.34	2.43±0.48	2.84±0.27*	2.87±0.24*	2.61±0.07
Total Protein (g L^{-1})	61.7±1.97	77.5±4.75*	81.3±4.21*	76.7±1.8*	78.1±1.17*
Aspartate amino transferase (U L^{-1})	61±11.3	91±11.59	113±18.25*	76±0.55	94±17.76
Alanine amino transferase (U L^{-1})	30±6.76	66±24	78±20.84*	55±5.5	66±2.07*
Alkaine phoshatase (U L^{-1})	91±8.04	105±18.64	82±11.67	94±19.93	101±1
Albumin (A) (g L^{-1})	40.5±1.68	44.6±1.18*	45.2±2.24*	42.4±0.95	45±1.57*
Globulin (G) (g L^{-1})	21.2±0.58	32.9±4.83	36.1±2.21*	34.3±0.96*	33.1±0.42
A/G ratio	1.9±0.08	1.4±0.22*	1.2±0.05*	1.2±0.04*	1.4±0.05*
Females					
Glucose (mmol L^{-1})	6.07±0.82	12.44±0.57*	12.17±0.86*	11.66±0.99*	12.08±1.03*
Urea nitrogen (mmol L^{-1})	2.66±0.59	2.63±0.51	3.34±0.19	2.68±0.40	3.41±0.61
Total protein (g L^{-1})	68.5±3.96	79.7±3.64*	83.1±4.85*	80.1±2.18*	80.0±3.60*
Aspartate amino transferase (U L^{-1})	51±6.53	115±23.02*	106±37.80	74±16.01	86±15.97
Alanine amino transferase (U L^{-1})	17±3.19	97±44.0	84±58.39	54±25.0	70±29.15
Alkaine phoshatase (U L^{-1})	34±7.89	77±40.38*	47±3.65	59±15.02*	52±2.51*
Albumin (A) (gL^{-1})	44.9±2.42	47.2±3.30	46.7±1.86	46.8±0.86	45.2±0.29
Globulin (G) (g L^{-1})	23.6±2.31	32.5±3.21*	36.4±3.00*	33.3±1.76*	34.8±3.61*
A/G ratio	1.9±0.15	1.5±0.19*	1.3±0.05*	1.4±0.07*	1.3±0.14*

Values are Mean±SD. *$p \leq 0.05$ vs. control group

Table 5: Prophylactic effect of immunol on the humoral immunity (plaque forming cell response of splenic lymphocytes to sheep erythrocytes) in dexamethasone induced immunosuppressed rats

	Males			Females		
Groups	Spleen cells ($\times 10^5$) mL^{-1}	No. of plaques/plate	Plaque/10^6 spleen cells	Spleen cells ($\times 10^5$) mL^{-1}	No. of plaques/plate	Plaque/10^6 spleen cells
G1	48.8±7.5	538±35.8	112±18.4	46.6±9.02	514±17.0	113±18.8
G2	59.3±5.72*	300±26.4*	51±1.5*	67±13.26*	410±4.0*	63±12.0*
G3	51.7±3.05	282±131*	55±26.4*	56±7.81	220±10.5*	40±7.9*
G4	48.3±6.39	302±78.0*	64±18.4*	50.2±3.03	327±25.4*	65±2.0*
G5	50.3±2.05	418±20.4	85±4.1	45.8±6.22	407±48.5	91±21

Plate: 0.1 mL spleen cells; Values are Mean±SD. *$p \leq 0.05$ vs. control group

Table 6: Prophylactic effect of immunol on the cellular immunity (delayed hypersensitivity to dinitrochlorbenzene) in dexamethasone induced immunosuppressed rats

	Skin thickness (mm)				
	Sensitization		Challenge		
Groups	Pre-dose	24 h	24 h	48 h	72 h
Males					
G1	1.08±0.05	1.08±0.05	1.54±0.05	1.64±0.13	1.72±0.11
G2	1.11±0.01	1.11±0.01	1.00±0.00*	0.97±0.06*	1.00±0.00*
G3	1.07±0.06	1.09±0.08	0.97±0.06*	1.00±0.00*	1.00±0.00*
G4	1.10±0.1	1.10±0.1	1.40±0.10*	1.43±0.06*	1.47±0.06*
G5	1.10±0.1	1.10±0.1	1.47±0.06	1.47±0.06	1.50±0.10*
Females					
G1	0.77±0.06	0.77±0.06	1.10±0.19	1.32±0.16	1.32±0.16
G2	0.72±0.02	0.72±0.02	0.71±0.02*	0.71±0.02*	0.71±0.03*
G3	0.77±0.03	0.77±0.03	0.75±0.05*	0.75±0.04*	0.75±0.04*
G4	0.79±0.03	0.79±0.03	1.03±0.06	1.03±0.06*	1.07±0.06*
G5	0.79±0.05	0.79±0.05	1.23±0.06	1.23±0.06	1.23±0.06

Values are Mean±SD. *$p \leq 0.05$ vs. control group

low, mid and high dose immunol were 112, 51, 55, 64 and 85/106 splenic lymphocytes (Table 5). Similarly, in females the plaque (antibody) forming cells were 113, 63, 40, 65 and 91/106 splenic lymphocytes. Splenic lymphocytes and the plaque (antibody) forming cells in the low and mid dose immunol treated groups were significantly lower than that of control group, but these values were comparable to dexamethasone treated control group. In the high dose immunol (10 mL kg^{-1} b.wt.) treated group the splenic lymphocytes and the plaque forming cells were comparable with the distilled water treated control group. This indicated that immunol treatment at the dose of 1 and 5 mL kg^{-1} b.wt. failed to prevent the immunosuppressive effect of dexamethasone. However, prophylactic effect was observed at 10 mL kg^{-1} b.wt.

The challenged skin response measurement in low dose immunol treated males and females (1.0 and 0.8 mm) showed significantly lower measurement, when compared to distilled water treated control group (1.7 and 1.3 mm), but the decreased responses were comparable to dexamethasone treated control group (1.0 and 0.7 mm) (Table 6), whereas, the high dose immunol treated male and female animals (1.5 and 1.2 mm) showed significantly increased skin thickness when compared to dexamethasone treated group. The skin thickness measurement in the mid dose immunol treated males and females (1.5 and 1.1 mm) was significantly lower, when compared to the distilled water treated control group (1.7 and 1.3 mm), but these values were closer to the high dose immunol treated group. This indicated that the high dose of immunol (10 mL kg^{-1}) prevented the suppression of CMI response of dexamethasone treatment and the mid dose of immunol (5 mL kg^{-1}) has shown a potential trend in preventing the CMI suppression by dexamethasone which however was not as effective as at the high dose.

At necropsy, there were no gross pathological lesions and no changes in the organ weights and their ratios. Histopathological changes such as accumulation of glycogen in hepatocytes (liver), lymphoid atrophy characterized by decreased size and cellularity of white pulp area (spleen), lymphoid atrophy characterized by severe depletion of cortical and medullary lymphocytes (thymus), lymphoid atrophy characterized by lymphoid depletion in cortex with loss of lymphoid follicles (mesenteric lymph node) and acinar cell vacuolation in exocrine portion (pancreas) were observed in the dexamethasone and dexamethasone+immunol treated animals. The adrenals, Ileum with payer's patch and others tissues did not show any histopathological changes.

DISCUSSION

The present study was performed to assess the immunoprophylactic potential of immunol, a polyherbal formulation in dexamethasone induced immunosuppression rat model. The dexamethasone control group males showed an unusual higher net body weight gain. In contrast, the dexamethasone control females and the low and mid dose immunol+dexamethasone treated groups showed significantly lower body weight gain in comparison with the distilled water group. Treatment with immunol at the high dose aided in regaining of the net body weight gain. Similar results were reported by Van Leeuwen (1998) in which the dexamethasone treatment reduced the body weights in rats.

The leucocyte count of the dexamethasone control and immunol+dexamethasone treated groups was slightly lower in comparison with the distilled water treated control group. Immunol prophylactic treatment at lower doses of 1 and 5 mL kg^{-1} b.wt. both did not alleviate the total leucocyte reduction. However, at a higher dose of 10 mL kg^{-1} b.wt. Immunol offered protective (prophylactic) action in regards to the total leucocyte reduction caused by dexamethasone. The findings of this study are in partial agreement with the findings of Ziauddin *et al*. (1996), who observed that myelosuppressed mice treated with an extract of Ashvagandha (one of the constituents of immunol), displayed a significant increase in hemoglobin concentration, red blood cell count, white blood cell count, platelet count and body weight as compared to controls, as well as increased hemolytic antibody responses towards human erythrocytes. In the present study, there were no such increase in the hemoglobin concentration, red cell count, platelet count but there were increase in white blood cell count in the high dose.

The neutrophil counts were higher and lymphocyte counts were lower in all the immunol treated groups when compared to the distilled water treated control group. The changes in the values were comparable to the dexamethasone treated control group, indicating that the immunol did not prevent the immunosuppressive effect of dexamethasone. The increase neutrophil count and the decrease lymphocyte count may be due to the effect of dexamethasone. Corticosteroids affect circulating white blood cells. Administration of glucocorticoid leads to decrease number of circulating lymphocytes, eosinophils, monocytes and basophils. In contrast, glucocorticoids increase circulating neutrophils as result of increase from the marrow, diminished rate of removal from the circulation and increased demargination from vascular walls (Schimmer and Parker, 1996).

Glucose, BUN, total proteins, AST, ALT, ALP, albumin, bilibrubin, cholesterol and Triglycerides were all higher. These changes were comparable to the dexamethasone treated group indicating the effect of dexamethasone on the liver in protein synthesis and stress induction. It also indicated that immunol did not have any ameliorating effect in reducing/preventing these changes.

Immunol treatment at the dose of 1 and 5 mL kg^{-1} b.wt. failed to prevent the immunosuppressive effect of dexamethasone. However, prophylactic effect was observed at 10 mL kg^{-1} b.wt. The results of the present study were in disagreement with the findings of Daswani and Radha Yegnanarayan (2002) who found an increase in Plaque Forming Cells (PFC) with a polyherbal formulation Septilin in rats. High dose of immunol (10 mL kg^{-1}) prevented the suppression of CMI response of dexamethasone treatment and the mid dose of immunol (5 mL kg^{-1}) has shown a potential trend in preventing the CMI suppression by dexamethasone.

In the present study immunol at the dose of 10 mL kg^{-1} b.wt. had prophylactic effect against dexamethasone induced immune suppression by way of both cell mediated and humoral mediated immunity. The humoral immune response in the present study was measured by plaque forming cells, which are otherwise antibody producing cells. Immunol causes stimulation of spleen and generate more antibody producing cells and enhances antibody titre to any antigenic stimulation. Similar findings were reported by Rao *et al.* (1994) with septilin in cyclophosphamide induced immunosuppression , wherein they found that rats pretreated with septilin, a polyherbal formulation significantly protected cyclophosphamide-induced humoral suppression and another study by Pallabhi *et al.* (1998) found that Immu-21, a polyherbal product pre-treatment protected the cyclophosphamide and cyclosporine A induced immunosuppression.

The results of the present study is in agreement with the findings Gautam *et al.* (2009) with *Asparagus racemosus* (one of the constituents of immunol) which produced humoral and cellular (delayed type of hypersensitivity) responses in a dose dependent manner and optimum activity was observed at 100 mg kg^{-1} (doses ranged from 25, 50, 100 and 200 mg kg^{-1}) and these responses were comparable with levamisole.

Immunol has been successfully used in treating various infectious diseases in animals and human beings. Immunol is developed to potentiate the immunity and to be used an adaptogen. Immunol has been shown to inhibit the growth of many gram positive and gram negative organisms (Vishwakarma, 1979). Shrivastava (1985) reported that immunol improves phagocytosis and minimizes the use of antibiotics and Varshney and Verma (1990) indicated that the use of immunol in combination with the appropriate antibiotic resulted in more appreciable response. These observations support the findings of the present study and its usefulness in the clinical applications. The findings of the present study are in not in agreement with Daswani and Yegnanarayan (2002), who found that Septilin showed dual effects on immune system, with lower doses showing greater stimulant and higher doses showing predominantly suppressive effects. The findings of the present study is supported by Sharma and Ray (1997), who found that Septilin, enhances both primary and secondary response, in mice immunized with sheep red blood cells and counteracts IgG and IgM suppression induced by prednisolone. They opined that immune protection of septilin may probably be due to the increased number of activated macrophages as a result of septilin treatment. Similar, immunopotentiating and immunoprophylactic activities were observed with Immu-21 by Pallabhi *et al.* (1998). They observed that Immu-21 significantly protected cyclophosphamide, cyclosporine A and UV rays induced humoral immunosuppression in rats and also showed positive immunoprophylactic activity against E.coli induced peritonitis in mice.

Mehrotra *et al.* (2002) observed that the plant and root extract of *Boerhaavia diffusa* (one of the constituents of immunol) exhibited immunosuppressive potential in *in vitro* tests. The findings of Daswani and Yegnanarayan (2002) showed that Septilin had dual effects on

the immune system as it stimulates some of the immune functions but suppresses others. The immunomodulatory activity of drugs is known to vary with the dose level and most of the immunosuppressants show immunostimulation at lower dilutions (Patwardhan *et al*., 1990; Van Dijk and Voermans, 1978). Abrams *et al*. (1993) observed that administration of low doses of cyclophosphamide, an immunosuppressant, to volunteers with advanced malignancies enhanced the lymphokine activated killer cell activity induced by co-administration of interleukin-2. Similar immunostimulating properties have been found to be associated with other immunosuppressants such as glucocorticoids and 6-thioguanine (Van Dijk and Voermans, 1978).

The adrenals, Ileum with payer's patch and others tissues did not show any histopathological changes. The prophylactic treatment of immunol failed to protect the thymus, spleen, mesenteric and axillary lymph nodes against the immunosuppressive effect of dexamethasone while the adrenals, Ileum with payer's patch and others tissues did not show any histopathological changes in all groups. Van Leeuwen (1998) reported that the thymus from the dexamethasone treated rats for 13 weeks showed atrophy of the medullary and cortical tissues and .the adrenal cortex was narrowed due to loss of the regular structuring of the cells or cell columns, in addition to a reduction in lipids. In the present study, similar findings were seen in the thymus, but not in the adrenals. The differences observed may be due to difference in the doses tested and also the duration of the treatment. These findings indicated that immunol did not offer any protection in ameliorating the effect of dexamethasone.

Stresses such as injury, infection and disease result in the increased production of cytokines, a network of signaling molecules that integrate actions of macrophages/monocytes, T lymphocytes and B lymphocytes in mounting immune responses. Among these cytokines, interleukin (IL)-1, IL-6 and tumor necrosis factor-α (TNF-α) stimulate the HPA axis, with IL-1 having the broadest range of actions. IL-1 stimulates the release of CRH by hypothalamic neurons, interacts directly with the pituitary to increase the release of ACTH and may directly stimulate the adrenal gland to produce glucocorticoids. The increased production of glucocorticoids, in turn, profoundly inhibits the immune system at multiple sites. Factors that are inhibited include components of the cytokine network, including interferon-γ (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukins (IL-1, IL-2, IL-3, IL-6, IL-8 and IL-12) and TNF-α. Thus, the HPA axis and the immune system are capable of bidirectional interactions in response to stress and these interactions appear to be important for homeostasis. Interaction between the immune system and the central nervous system is the profound influence of stress on immune reactivity. Stress is a risk factor for a number of diseases and is an important predictor of health in general. Herbal medicines have been used as adaptogens to regulate and improve the stress response and there is evidence to support the use of herbal medicines for this purpose (Seely and Singh, 2006). Stress is an extremely broad term that serves to define a wide variety of phenomena that humans are exposed to throughout their lives. The facets of stress are essentially limitless; however, broad categories include exposure to environmental pollutants, drugs and chemicals, food, hormones physical exertion, emotional upset, persistent psychological pressure, existential crisis and the residual effects of emotional trauma to name just a few. Most herbal remedies have their roots in traditional medicine and polyherbal preparations are expected to combine the beneficial effects of the individual ingredients to provide relief sans the side effects which have become so much a part of allopathic remedies. Immunol raises the general defense mechanism of the

body and thus helps to overcome the infection and inflammatory process (Ross, 1984). It is well recognized in Ayurveda that adaptogenic drugs help an organism to cope better during stressful situations and retard aging. One of the main strategies in Ayurveda is to increase body's natural resistance to the disease/stress causing agent rather then directly neutralizing the agent itself. Immunomodulatory agents of plant origin enhance the immune responsiveness of an organism against pathogens by non-specifically activating the immune system. Immunomodulatory therapy could provide an alternative to conventional chemotherapy for a variety of diseased conditions, especially when host defense mechanisms have to be activated under the conditions of impaired immune responsiveness or when a selective immunosuppression has to be induced in situations like inflammatory diseased, autoimmune disorders and organ/ bone marrow transplantation (Upadhyay, 1997). Macrophages play an important role in non-specific and specific immune response. As regulators of phagocytic and inflammatory functions. In addition to cell-to-cell interactions, macrophages also influences the immune system by secretion of cytokine functions in both autocrine and paracrine manner to protect host.

Immunol has shown promising results as a prophylactic in dexamethasone induced immunosuppression rat model when tested on two arms of the immune system i.e., the humoral and the cell mediated immunity.

CONCLUSIONS

Immunol, a polyherbal formulation at the dose of 10 mL kg^{-1} b.wt. had prophylactic effect against dexamethasone induced immune suppression by way of both cell mediated and humoral mediated immunity.

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