

ORIGINAL RESEARCH ARTICLE

Immuno-Modulatory Effect Of Makaradhwaja

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ABSTRACT

The term immune-modulation is used for describing the effect of various chemical mediators, hormones and drugs on the immune system. The human body has the ability to resist almost all types of organism or toxins that tend to damage the tissue and organ. This capability is called immunity. Makaradhwaja is a metalo-mineral preparation, which is most popular and effective in Kupipakwa preparation of Ayurvedic medicine which is mainly used for Rasayana purposes. In this study total **18 Swiss albino mice** of either sex weighing between 20 - 36 g were divided into **three groups**, each having 3 males and 3 females. Sample of Makaradhwaja was taken and few drops of 5% gum acacia suspension were added, it was further grounded for 5 minutes and the volume was made up with distilled water, suspension form in 5% gum acacia orally with the help of plastic tube attached to a tuberculin syringe. Makaradhwaja comes to 16.25 mg kg⁻¹ and was rounded of to 16 mg kg⁻¹ dose, for 10 consecutive days. Study suggested **that the test drug has significant CMI (cell mediated immunity) enhancing effect** but has no significant effect on humoral anti-body formation.

Key words: Makaradhwaja, Immune-modulation, CMI, Humoral anti-body formation.

INTRODUCTION:

The term "immunomodulation"ⁱ is used for describing the effect of various chemical mediators, hormones and drugs on the immune system. The human body has the ability to resist almost all types of organism or toxins that tend to damage the tissue and organ. This capability is called immunity.

The immune mechanism: Basically there are two different types of lymphoid cells T and B cells which mediate "cellular" and "serologic" or humoral immunity, respectively. Both these types of cells are present in the circulating blood and in peripheral lymphoid tissues. The recognition of the antigen by the T cells leads to proliferation of these cells, infiltration of immune cells at the site of action and cellular immunity. These reactions may be manifested as delayed hypersensitivity reactions, tissue graft rejection or organ transplant rejection. The infiltrating T cells exert their cytotoxic action by the release of various

lymphokines (transfer factor, TF; migration inhibitory factor, MIF; chemotactic factor, CF; lymphotoxin, LT; interleukin II (IL-2), interferon, IFN).The other limb of immune system involving B cells is responsible for the genesis of specific antibodies immunoglobulins (IgA, IgG, IgD, IgE, IgM).The recognition of antigen (Ag) by the B-cells lead to proliferation of these cells, conversion to plasma cells and generation of specific antibodies (Ab) (Igs). The specific antibody (Ab) binds with the specific antigen (Ag) leading to its inactivation or even phagocytosis. Besides the conventional T and B cells, the other cells of special significance are T₄ helper cells, T₈ suppressor cells, monocytes and macrophages. The existence of a heat labile serum component known as the complement system, which causes bacteriolysis and phagocytosis, is also well-known.

Mechanisms of Immuno-modulation:

Drugs may modulate immune mechanism by either suppressing or by stimulating one or more of the following steps:

- (1) Antigen recognition and phagocytosis.
- (2) Lymphocyte proliferation
- (3) Synthesis of antibodies
- (4) Antigen - antibody interaction.
- (5) Release of mediators due to immune responseⁱⁱ
- (6) Modification of target tissue response.

Makaradhwaja is a metallo-mineral preparation, which is most popular and effective in Kupipakwa preparation of Ayurvedic medicine. As per classical literature, this drug is mainly used for Rasayana purposes. These drugs are also well-known for their propensity to modulate immune system hence the present study was planned to assess the effect of test drug on Immunomodulation activity.

MATERIALS AND METHODS:

Swiss albino mice of either sex weighing between **20 - 36 g** from the animal house of I.P.G.T. & R.A., G.A.U., Jamnagar were used in the studies. They were maintained on Nav Chankan Oil Mills "Amrut" brand animals feed and tap water given *ad-libitum*. The test drug (Makaradhwaja) was prepared in the department of Rasa Shastra and Bhaishajya Kalpana Including Drug Research, I.P.G.T. & R.A., G.A.U., sample of Makaradhwaja was taken in requisite amount in separate small porcelain mortars and few drops of 5% gum acacia suspension were added, it was further grounded for 5 minutes and the volume was made up with distilled water. The drug was administered in suspension form in 5% gum acacia orally with the help of plastic tube attached to a tuberculin syringe. Dose for this experimental study was calculated by extrapolating the therapeutic dose to mice dose on the basis of body surface area ratio (conversion factor 0.0026). Calculated this way the mice dose of Makaradhwaja comes to 16.25 mg kg⁻¹ and was rounded of to 16 mg kg⁻¹ dose. The experiment was carried out in accordance with the directions of the Institutional Animal Ethics Committee (IAEC) after obtaining its permission.

(A) Effect on humoral antibody formation in miceⁱⁱⁱ:

Total eighteen Swiss albino mice including nine male and nine females were divided into three groups each having 3 males and 3 females. Dose of the test formulations (Both A and B) was calculated by extrapolating the human dose to animals (1.8 g/kg) based on the body surface

area ratio by referring to the standard table of Paget and Barnes (1969)^{iv}- **(Table-1)**

Table 1: Grouping for effect on humoral antibody formation and CMI in mice:

S.No.	Name of the group	Dose
(a)	Group A (control)	Tap water (No drug given)
(b)	Group B (Therapeutic dose)	Makaradhwaja suspension 16 mg/kg body weight of mice.
(c)	Group C (Higher dose)	Makaradhwaja suspension 32 mg/kg body weight.

SRBC solution was prepared from the Sheep blood collected from the city slaughterhouse in a sterilized bottle containing Alsever's solution^v (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride). SRBC was thoroughly washed with sterilized normal saline by centrifuging and stored in Alsever's solution in a refrigerator till experimentation. The drug was administered for 10 consecutive days, on 3rd day, 25% SRBC solution was **injected by i.p.route** in the dose of 0.1 ml/ 10 g of body weight. On the 10th day mice were sacrificed by **ether overdose** and blood was collected in separate test tubes. Blood from the same animals (Sheep) was used for both sensitization and determining antibody titre. From the collected blood, serum was separated and incubated in a serological water bath for 30 minutes at 55°C to inactivate the complement in it. Serial two fold dilutions of the serum in sterile saline solution were made in the volume of 0.1 ml in microtitre plate. 0.1 ml of thrice saline washed 2% SRBC was added to each well of the tray. The tray was covered and placed in refrigerator overnight. Antibody titre^{vi} (haemagglutination titre) was noted next day. Titre was converted to log₂ values for easy comparison. After scarifying, specimens of important organ related with Immuno-modulation activity like spleen, thymus and lymph nodes were collected from animals and their weight were recorded; tissues were transferred to 10% formaldehyde solution for fixation and histopathological study was carried out.

(B) Effect of test drug on cell mediated immunity in mice^{vii}:

Total eighteen Swiss albino mice of either sex weighing between 20 - 36 g were selected and divided into three groups, each having 3 males and 3 females. Immunological inflammation was produced in mice by injection of Triple antigen with alum precipitate in the following proportion into sub planter tissue of mice hind paw. pH of the above solution was maintained between 5.6 - 6.8 using 10% sodium carbonate.

Triple antigen : 1 ml
 Normal saline (0.9% : 4 ml
 Potash alum (10%) : 1 ml

Initially the mice were sensitized by injecting the triple antigen with alum precipitates **subcutaneously** in the nape of the neck in a dose of 0.1 ml / 10 g body weight. The test drug administration began on the day of sensitization and continued for the next five days. On 5th day, 1 hour after administration of the test drug, the mice were injected with 0.05 ml triple antigen with alum precipitates beneath plantar aponeurosis in the left hind paw.

The initial paw volume was measured in all the mice after marking in left leg and before sensitization of triple antigen in neck. Further paw volume was measured 24 and 48 hrs hour after injecting alum adjuvant. The paw volume was measured with the help of a Plethysmometer^{viii}.

Statistical analysis:

Student's t test for unpaired data has been used for analyzing the data generated during the study. A 'P' value less than 0.05 is considered as statistically significant and the value of P<0.01 or P<0.001 is considered statistically highly significant. Level of significance was noted and the results interpreted accordingly.

Observations and results:

The data pertaining to the effect of test drugs on spleen and thymus weight in SRBC sensitized mice are presented in (Table-2). The test drug did not affect spleen and thymus weight significantly. An apparent moderate 31.25% increase observed in thymus weight was found to be statistically non-significant.

(Table-3) depicts data related to the effect of test drug on antibody formation against SRBC. The apparent moderate increase observed at therapeutic dose level and slight decreases observed at higher dose level were found to be statistically non-significant.

Table 2: Effect on humoral antibody formation- Effect of Makaradhwaja in different doses on spleen and thymus weight in SRBC sensitized mice.

Treatment	Dose mg/kg(po)	Spleen mg/10g body wt.mean ± SEM	% Change	Thymus mg/10g body wt .mean ±SEM	% Change
Control	-	22 ± 0.30	-	0.16 ± 0.02	-
Therapeutic dose	16	0.21 ± 0.01	4.54 ↓	0.21 ± 0.01	31.25 ↑
Higher dose	32	0.23 ± 0.05	4.54 ↑	0.18 ± 0.03	12.5 ↑

Table 3: Effect of Makaradhwaja on antibody formation against SRBC in mice

Treatment	Dose mg/kg	Haemagglutination titre log ₂ mean ± SEM	% Change
Control	-	4.04 ± 0.21	-
Therapeutic dose	16	4.96 ± 0.45	22.77 ↑
Higher dose	32	3.60 ± 0.33	10.86 ↓

Table 4: Effect of Makaradhwaja on alum adjuvant induced immunological paw oedema in pre sensitized mice:

Treatment	Dose mg/kg	% Increased in Paw volume after alum adjuvant injection			
		24 hours Mean ± SEM	% Change	48 hours Mean ± SEM	% Change
Control	-	19.06 ± 06.34	-	30.24 ± 8.93	-
Therapeutic dose	16	45.89 ± 12.63	140.76 ↑	71.22 ± 11.26*	135.51 ↑
Higher dose	32	39.15 ± 08.78	105.40 ↑	75.63 ± 8.95 **	150.09 ↑

The following organs were subjected to histopathological study

- (I) Spleen
- (II) Thymus
- (III) Lymphnodes

Fig- 1) Fig-15 to 15b Microscopic examination of Spleen: Sections obtained from different groups of rats subjected to immuno-modulation study did not show any significant difference in the cytoarchitecture. Show photomicrographs of representative sections from different groups.

Fig- 2) Fig-16 to 16b Microscopic examination of Thymus: Scanning of thymus sections obtained from different groups showed increased cellularity in-group A, while sections obtained from group B and C did not show any change in the cytoarchitecture. Show photomicrographs of representative sections from different groups.

Fig-3) Fig-17 to 17b Microscopic examination of Lymph node: Examination of sections of lymph node from different groups did not show any changes in the cytoarchitecture of the gland, respectively show photomicrographs of representative sections from different groups.

Fig 1: 15 to 15b Microscopic examination of Spleen:

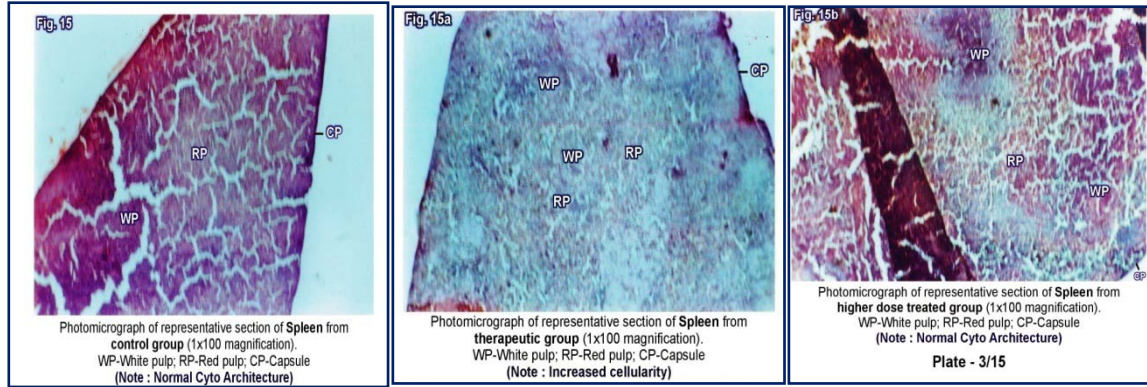


Fig 2: 16 to 16b Microscopic examination of Thymus:

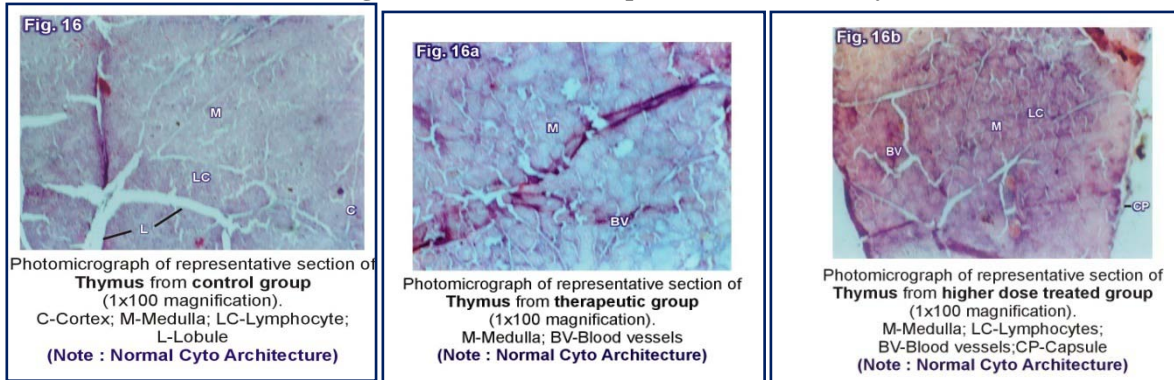
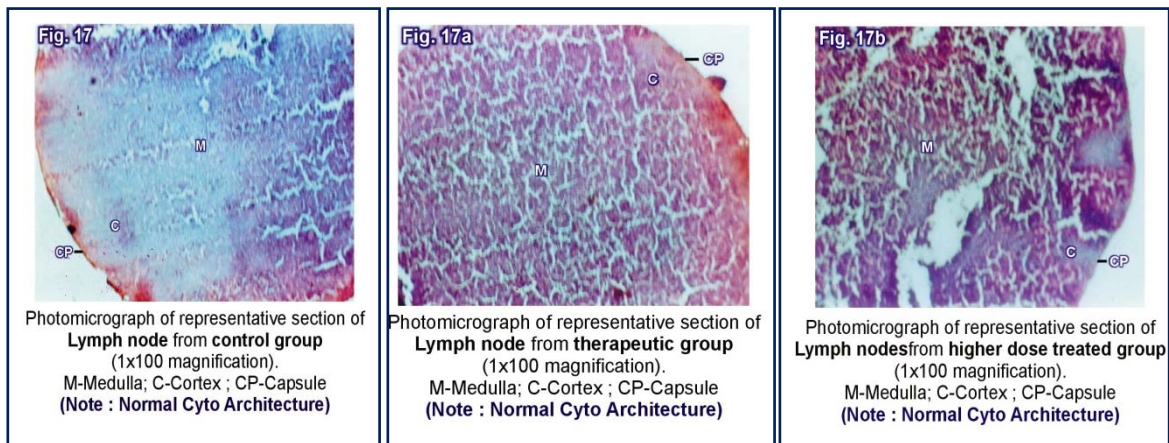


Fig 3: 17 to 17b Microscopic examination of Lymph node:



Effect on cell mediated immunity:

The data related to the effect of test drug on alum adjuvant induced immunological oedema studied at different dose level are presented in (Table-4). An apparent marked elevation in pedal oedema was observed in both the groups at both 24 and 48 hrs after the injection of the response eliciting dose of antigen. The increase with 16 mg/kg dose was 140.76% at 24h and 135.11% at 48 h. However due to variation in the data only the increase observed at 48 h was found to be statistically significant. At 32 mg/kg dose also

similar type of results were observed. At 24 h 105.40% increase and at 48h 150.09% increase was observed with only the latter being statistically significant.

DISCUSSION:

From the above presented data it can be suggested that the test drug has no significant effect on humoral anti-body formation. But has significant CMI enhancing effect. CMI as mentioned earlier is mainly mediated through T-lymphocyte. The first step, which is common to both types of

immune mechanism, is recognition of the antigen. The second step is the activation of cell involved in the CMI through elaboration of cytokines. The final mediator of this activity is the activated macrophage. The sequence of the events is that the allergen is taken up by antigen presenting cells, such as Langerhans cells in the skin, which then migrate to lymph nodes and present the allergen to T cells. This results in sensitization and proliferation of T-cells, some of which migrate via the lymph and blood to the site of allergen entry into the body. There they secrete cytokines, such as gamma interferon, which activates macrophages, and tumor necrosis factor (TNF), which stimulates an inflammatory response. Cell mediated immunity is amplified by γ -interferon by enhancing the process of antigen processing by macrophages. Macrophage migration inhibition factor inhibits movements of macrophages from the affected site. Interleukin-2 (IL2) acts on the activated T-lymphocyte and helps in their clonal expansion. It also activates cytotoxic lymphocytes and B-lymphocytes. T-lymphocytes modulate the adherence, locomotion and activation of eosinophils leading to accumulation at the site of immune reaction. Activated eosinophils further add to the tissue injury. In the light of the above it can be suggested that anyone or combination of the following mechanisms may be involved in the stimulation of CMI by the test drug:

- (1) Facilitation of the presentation to antigen by macrophages.
- (2) Stimulation of secretion of interleukin - 1 (IL-1) from macrophages.
- (3) Upgrading the IL-1 receptor either through increase in their number or increasing the reactivity of the receptors
- (4) Enhancement if release of IL-2 from activated T cells
- (5) Increase in the formation of alpha interferon or/ and facilitation its effect on T-lymphocytes
- (6) Increasing the effect of IL-2 on cytotoxic lymphocytes.

However to arrive at an unequivocal inference it is necessary to carry out further detailed studies employing appropriate experimental models.

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