

## EFFECT OF HERBAL PREPARATION ON IMMUNE RESPONSE OF IMMUNOSUPPRESSED MICE

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**Abstract :** The present study was undertaken to study the effect of a herbal formulation (Septilin)<sup>(R)</sup> as immunomodulator, on immune response in mice.

The study of this formulation in respect to humoral and cell mediated immune response has suggested that, oral administration of Septilin<sup>(R)</sup> (500 mg/kg) alone or in combination with an immuno-suppressive drug (prednisolone 4 mg/kg), enhances both primary and secondary immune response, in mice immunized with sheep red blood cells (SRBC).

**Key words :** humoral immune response                      immuno-modular  
cell-mediated immune response

### INTRODUCTION

Septilin<sup>(R)</sup> (Himalaya Drug Co. Private Ltd., India) is herbal preparation containing *Balsamodendron mukul Extr Maharasnadi guath*, *Rubia cordifolia*, *Tinospora cordifolia*, *Saussurea lappa* *Trikuta*, *Phyllanthus emblica* and *Glycyrrhiza glabra*. It has been reported to possess anti-bacterial, anti-inflammatory and anti-exudative properties (1-3) and is used in the treatment of acute/chronic infections prolonged use of antibiotics suppresses immune system (4). Septilin<sup>(R)</sup> has been reported to increase the phagocytic coefficient (5), which, corresponds with clinical improvement in chronic infections, resistant to the commonly used broad spectrum antibiotics.

In the present study, we have attempted to evaluate the effect of Septilin<sup>(R)</sup> in immunized mice in respect of both humoral and cell-mediated immune response.

### METHODS

Experiments were performed on male albino mice weighing between 20 to 30 gm. The mice were acclimatized in the laboratory for 2 weeks. The animals were provided standard animal feed (Hindustan Lever Ltd., India) and tap water *ad libitum*. There were 4 treatment groups. Group I was vehicle treated and acted as control group. Group II, III and IV were administered, orally, Septilin<sup>(R)</sup> (500 mg/kg), prednisolone (4 mg/kg) and Septilin<sup>(R)</sup> plus prednisolone, respectively. These treatments were carried

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out for 4 weeks. Prednisolone served as the reference standard.

#### Experimental procedure

To study the effect of Septilin<sup>(R)</sup> on IgM and IgG antibodies, primary and secondary immunisation was done. For immunization sheep red blood cells (SRBC) washed thoroughly and finally suspended in normal saline were injected in control and drug treated mice intraperitoneally in 0.1 ml doses. Approximately  $25 \times 10^6$  cells were administered in the primary and  $50 \times 10^6$  in the secondary immunization.

*1. Assessment of cell-mediated immune response:* Both in primary and secondary immunization, magnitude of localised delayed hypersensitivity was assayed by measuring increase in footpad thickness of mice after 24 hrs of intradermal injection of antigen (SRBC). A maximal volume of 0.03 ml is injected and footpad thickness was measured by dial caliper. Each measurement is a mean of six readings, three each by 2 different individuals.

*2. Humoral response:* Blood samples were collected by cardiac puncture from chloroform anaesthetized mice on the 7th day after primary immunization and 5th day after secondary immunization. Serum was inactivated by heating at 56°C for 30 minutes. Serial two fold dilutions of inactivated serum sample was made in 25 µl of normal saline containing 0.1% Bovine serum albumin (BSA) in V-bottomed microtitration plates and were mixed with 25 µl of 0.1% SRBC suspension in phosphate buffered saline (PBS). After mixing, SRBC was allowed to stand at 25°C, until control wells showed an unequivocally negative pattern (a small button). The value of

highest serum dilution carrying visible hemagglutination was taken as the antibody titre (6).

Titration was also carried out with antisera preincubated with 0.1M 2-mercaptoethanol (2-ME) at 37°C for 60 minutes for the estimation of IgG antibodies. The antibody titres are expressed as log 2 of the reciprocal of the first dilution where no visible agglutination was observed.

All the experiments were carried out in duplicate or triplicate. The average was used in the determination of mean  $\pm$  standard deviation (SD). Results of drug treated groups were compared with respective control group. Significance of the difference was assessed by student 't' test and Tukey test and "P" value of 0.05 or less was considered to be significantly different from control.

#### RESULTS AND DISCUSSION

Measurement of body weight, before and after primary immunization of mice showed a significant increase ( $P < 0.01$ ) in weight in control and Septilin<sup>(R)</sup> treated group and insignificant ( $P > 0.05$ ) increase in Septilin<sup>(R)</sup> plus prednisolone treated group. However, decrease ( $P > 0.05$ ) in body weight was observed in prednisolone treated mice. Spleen/body weight ratio also showed increase in Septilin<sup>(R)</sup> treated group and decrease in prednisolone treated group when compared to control. After secondary immunization, significant increase in body weights of control and Septilin<sup>(R)</sup> treated animals was observed. However, increase in body weight was less in Septilin<sup>(R)</sup> plus prednisolone treated animals. A significant increase in spleen/body weight ratio in Septilin<sup>(R)</sup> treated animals was also observed



while in prednisolone treated animals, significant decrease in spleen/body weight ratio was found when compared to control (Table I). It may be due to proteolytic (catabolic) or immunosuppressive effect of prednisolone.

Cell-mediated immune response was measured by hypersensitivity test (foot pad thickness was measured after 24 hrs of challenge with SRBC). After primary immunization, control and Septilin<sup>(R)</sup> treated animals showed significant increase in cell-mediated immune (CMI) response ( $P < 0.01$ ) and marginally significant ( $P < 0.05$ ) increase with Septilin<sup>(R)</sup> plus prednisolone. However,

values were insignificant ( $P > 0.05$ ) in prednisolone treated animals when compared with initial values (Table II).

Secondary immunisation, showed highly significant ( $P < 0.001$ ) increase in cell mediated immunity (CMI) in Group II, significant ( $P < 0.01$ ) increase in Group I and IV and marginally significant ( $P < 0.05$ ) increase in Group III when compared to initial values (Table II).

After primary immunization, IgM antibody titre (without 2ME) was significantly increase ( $P < 0.001$ ) in Septilin<sup>(R)</sup> treated group when compared to control,

TABLE I: Body and spleen weights of control and drug treated mice after primary and secondary immunization.

Group	Primary immunization			Secondary immunization		
	Body wt(gm) Initial	Body wt(gm) Final	Spleen/body wt ratio $\times 10^{-3}$	Body wt(gm) Initial	Body wt(gm) Final	Spleen/body wt ratio $\times 10^{-3}$
I Control	21.5 $\pm$ 5.3	23.34 $\pm$ 5.60**	5.49 $\pm$ 2.1	28.5 $\pm$ 1.3	31.5 $\pm$ 3.0***	5.9 $\pm$ 0.16
II Herbal	20.7 $\pm$ 3.0	24.4 $\pm$ 7.60**	6.11 $\pm$ 1.6	23.7 $\pm$ 4.0	28.5 $\pm$ 3.0***	6.4 $\pm$ 0.19*
III Prednisolone	24.0 $\pm$ 2.3	22.3 $\pm$ 3.4*	4.45 $\pm$ 1.2*	23.4 $\pm$ 3.1	21.4 $\pm$ 2.9***	4.8 $\pm$ 0.51 <sup>b</sup>
IV Herbal + Prednisolone	21.9 $\pm$ 5.2	23.2 $\pm$ 5.1*	5.07 $\pm$ 2.0	24.0 $\pm$ 4.8	26.8 $\pm$ 4.5***	5.3 $\pm$ 0.42

Values are Mean  $\pm$  S.D. of 7 animals in each group. \*\*\*P < 0.001 as compared to initial values.  
\*P < 0.05; \*\*P < 0.01, \*P > 0.05 vs initial values. \*P < 0.001 as compared to group I, III and IV.  
<sup>a</sup>P < 0.001 as compared to group I, II and IV. <sup>b</sup>P < 0.001 as compared to group I and II.

TABLE II: Measurement of footpad thickness in control and drug treated mice after primary and secondary immunization.

Group	Primary immunization		Secondary immunization	
	Footpad thickness (mm)		Footpad thickness (mm)	
	Initial	Final	Initial	Final
I Control	10.3 $\pm$ 1.90	12.8 $\pm$ 1.9**	12.3 $\pm$ 1.3	13.0 $\pm$ 1.3**
II Herbal	10.9 $\pm$ 0.80	13.2 $\pm$ 1.2**	11.2 $\pm$ 1.8	13.6 $\pm$ 1.6***
III Prednisolone	11.5 $\pm$ 1.52	12.9 $\pm$ 1.7*	9.1 $\pm$ 2.5	9.6 $\pm$ 2.4*
IV Herbal + Prednisolone	11.9 $\pm$ 1.98	12.8 $\pm$ 1.1*	12.4 $\pm$ 2.8	13.2 $\pm$ 2.8**

Values are Mean  $\pm$  S.D. of 7 animals in each group.  
\*P < 0.05, \*\*P < 0.001, \*\*\*P < 0.001, \*P > 0.05 vs initial values.



TABLE III: Effect of Septilin and prednisolone on primary and secondary haemagglutination titres in mice.

Group	Primary immunization -log <sup>2</sup> titre		Secondary immunization -log <sup>2</sup> titre	
	Without 2ME	With 2ME	Without 2ME	With 2ME
I Control	4.8 ± 0.37	3.0 ± 0.57	7.1 ± 0.69	5.8 ± 0.89
II Herbal	6.2 ± 0.75***	3.4 ± 0.53	9.8 ± 1.4***	8.5 ± 1.6***
III Prednisolone	4.5 ± 0.53***	2.5 ± 0.53**	4.7 ± 0.8***	3.5 ± 0.53***
IV Herbal + Prednisolone	5.7 ± 0.40***	3.7 ± 0.48	8.4 ± 0.78†	6.8 ± 0.69†

Values are Mean ± S.D. of 7 animals in each group.

\*\*\*P < 0.001, Group II and IV vs Group I.

+++P < 0.001 Group III vs Group II and IV.

++P < 0.01 Group III vs Group II and IV.

†P < 0.001 Group IV vs Group II.

prednisolone treated group showed significant decrease in both IgM (without 2-ME) and IgG (with 2-ME) antibody titre due to its immunosuppressive effect (Table III).

However, secondary antibody titre showed significant increase (P < 0.001) in both IgM and IgG in Septilin<sup>(R)</sup> treated group, suggesting significant potentiating action of Septilin<sup>(R)</sup> on humoral immune response.

Bhasin (7) has also demonstrated a marked increase in serum IgG levels, following treatment with Septilin<sup>(R)</sup>. It has also been reported that phagocytic activity of polymorphonuclear cells is also stimulated by Septilin<sup>(R)</sup> and further studies are in progress to determine the type of phagocytic stimulation (oxygen dependent or independent) by Septilin<sup>(R)</sup>. It has already

been reported that septilin stimulates phagocytosis to inhibit the growth of bacteria (8).

Above results suggest, that septilin enhances both primary and secondary immune response in mice immunized with SRBC and counteracts IgG and IgM suppression induced by prednisolone when administered orally with immunosuppressive drug (prednisolone). Though the present study can not answer questions as to the mechanism of immune protection offered by Septilin<sup>(R)</sup>, it could probably be due to increased number of activated macrophages by Septilin<sup>(R)</sup> treatment and further studies to examine this possibility are required. Thus, Septilin<sup>(R)</sup> may prove to be a safe and suitable long term treatment in immunosuppressed high risk patients.

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