

Immunomodulatory Activity of Septilin, a Polyherbal Preparation

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*Septilin is a Polyherbal preparation, claimed to be effective in conditions such as chronic stubborn URTI, tonsillitis, cutaneous infections, dental infections and also prescribed as a health supplement. In view of this, the present experimental study was undertaken to evaluate the effect of Septilin on different arms of the immune system. The experimental animals (male albino rats and mice) were divided into three groups. Group I received distilled water; group II received Septilin in a dose of 1 g/kg (rats) or 1.5 g/kg (mice); group III received Septilin 2 g/kg (rats) or 3 g/kg (mice) orally for 28 days. They were evaluated for immunological function on day 29 by studying weight gain, resistance against *E. coli* sepsis, haemogram, phagocytic activity of PMN cells and reticuloendothelial system, delayed hypersensitivity to oxazolone and the plaque forming cell response of splenic lymphocytes to sheep erythrocytes.*

*Neither of the doses of Septilin altered weight gain absolute lymphocyte counts, or host resistance against *E. coli* sepsis. The higher dose of Septilin reduced phagocytic activity of the PMN cells/reticuloendothelial system, but both doses increased the percentage and absolute number of circulating neutrophils, stimulated humoral immunity and suppressed cellular immunity.*

Thus, Septilin has dual effects on the immune system, with lower doses showing greater stimulant and higher doses showing predominantly suppressant effects.

INTRODUCTION

Septilin (The Himalaya Drug Company, Bangalore, India) is a herbal preparation containing powders of *Balsamodendron mukul* and Shankha bhasma; Maharasnadi quath; and extracts of *Tinospora cordifolia*, *Rubia cordifolia*, *Emblica officinalis*, *Moringa pterygosperma* and *Glycyrrhiza glabra* (Table 1). It has been reported to possess antibacterial (Ross, 1984), anti-inflammatory (Kumar *et al.*, 1993) and wound healing properties (Udapa *et al.*, 1989). It is said to be helpful in treating Gram-positive as well as Gram-negative infections (Gadekar *et al.*, 1986; Sharma *et al.*, 1986). There are reports that Septilin is effective in chronic stubborn URTI (Bhasin, 1990), tonsillitis (Gadekar *et al.*, 1986), tropical eosinophilia (Prusty *et al.*, 1985), infective dermatoses (Sharma *et al.*, 1986) and dental infections.

		Constituent	Quantity
Powder		<i>Balsamodendron mukul</i>	162 mg
		Shankha bhasma	32 mg
Extract		Maharasnadi quath	65 mg
		<i>Tinospora cordifolia</i>	49 mg
		<i>Rubia cordifolia</i>	32 mg
		<i>Emblica officinalis</i>	16 mg
		<i>Moringa pterygosperma</i>	16 mg
		<i>Glycyrrhiza glabra</i>	6 mg

Septilin is claimed to build up resistance to infection (Bhasin, 1990; Prusty *et al.*, 1985) and is widely used as a health supplement. In view of the various claims about the efficacy of Septilin in the treatment of infections at different sites, the present experimental study was designed to evaluate the effect of Septilin on different arms of the immune system.

MATERIALS AND METHODS

The experiments were performed on male albino mice weighing 20-30 g, except for the carbon clearance test where male albino rats weighing 150-200 g were used. The experimental protocol was approved by the Institutional Ethical Committee. The experimental animals were acclimatized in the laboratory animal house for at least 1 week. The animals were provided standard animal feed (Chakan Oil Mills) and tap water *ad libitum*. They were randomly divided into three groups. Group I received pretreatment with distilled water and acted as the control group. In group II, mice received 1.5 g/kg and rats received 1 g/kg of Septilin, while in group III, mice received 3 g/kg and rats received 2 g/kg of Septilin in the form of an aqueous suspension orally, daily for 28 days. At the end of the pretreatment phase, the animals were subjected to immunological screening using the following experimental models: resistance to *E. coli* induced abdominal sepsis; haemogram; carbon clearance; polymorphonuclear function; delayed hypersensitivity to oxazolone (cellular immunity); plaque forming cell response of splenic lymphocytes to sheep erythrocytes (humoral immunity). A fresh set of animals was used for each test.

EXPERIMENTAL MODELS

Determination of host resistance against *E. coli* induced abdominal sepsis (Thatte *et al.*, 1987). This test was performed on male albino mice that had completed the drug pretreatment. On day 29 of the test, abdominal sepsis was induced in the test mice by challenging them, intraperitoneally, with 3×10^8 *E. coli* (hospital strain), suspended in phosphate buffered saline. The test mice were observed for 24 h and the percent mortality at 24 h after induction of sepsis was estimated. The survivors were observed further for 7 days.

Determination of haematological parameters At the end of the drug therapy blood was collected by cardiac puncture and the total and differential WBC counts were done.

Determination of phagocytic function: carbon clearance (Biozzi *et al.*, 1953). To evaluate the phagocytic activity of the reticulo-endothelial system *in-vivo*, a carbon clearance test was performed after completion of the drug pretreatment. On day 29, the treated rats received an intravenous injection of carbon suspension (1:50 dilution of Indian ink, Camel) in a dose of 0.5 mL/100 g body weight. Blood was withdrawn from the retro-orbital venous plexus before injection, immediately after injection and at 5 min intervals upto 20 min after injection of the carbon suspension. 0.05 mL of blood was lysed with 4 mL of 0.1% Na_2CO_3 and the optical density was measured spectrophotometrically at 650 nm wavelength. The results were expressed as the granuloplectic index, calculated by the formula

$$\frac{\text{Log}(OD_0) - \text{log}(OD_t)}{t}$$

Where OD_0 is the OD at 0 min and OD_t is the OD at t min.

***In vitro* phagocytic activity of polymorphonuclear cells** (Poornima, 1996). At the end of the drug treatment phase, 2 drops of blood were collected on a clean, dry glass slide and placed in a moist chamber to permit adherence of PMN cells, after which the clot was gently removed without disturbing the adherent PMN cells. This layer of PMNs was covered with a suspension of *C. albicans* (yeast cells) (10^6 candida/mL) and incubated for 1 h. The slide was then stained with Giemsa stain and the effect of Septilin on phagocytic activity was expressed as the percentage of cells showing phagocytosis and the average number of Candida per PMN.

Determination of T-lymphocyte function (Lakadawala *et al.*, 1988; Borell *et al.*, 1977; Florentin *et al.*, 1978). To determine the effect of the drugs on cell mediated immunity, the delayed hypersensitivity to oxazolone was assessed.

On day 20 of drug therapy, the mice were shaved from the mid-abdominal region. 0.1 mL of a 3% solution of oxazolone sensitizing agent in ethanol was applied to this region 24 h later. Drug therapy was continued and 7 days later (i.e. on day 28 of drug therapy), 0.01 mL of 3% oxazolone sensitizing agent was applied to the inner and outer aspect of the left ear after measuring the initial ear thickness with a micrometer screw gauge. Twenty four hours after the challenge, the thickness of the left ear was measured again. The increase in the ear thickness was taken as a measure of delayed hypersensitivity to oxazolone.

Determination of B-lymphocyte function (Jerne *et al.*, 1963; Dresser, 1986). To determine the effect of the drugs on humoral immunity, the plaque forming cell response of splenic lymphocytes to sheep erythrocytes was assessed. On day 23 of pretreatment, the mice were challenged with 1 mL of a suspension of sheep RBCs (10^7 RBC/mL) in saline, intraperitoneally. Drug therapy was continued until day 28. At the end of the pretreatment phase, the mice were killed, the spleen was removed and gently homogenized. The splenic lymphocytes were suspended in 10 mL of cold Hank's gelatin solution. The number of lymphocytes per spleen were counted and 0.05 mL of this suspension of splenic lymphocytes was incubated with 0.05 mL of 8% SRBC suspension in 0.5 mL of agarose on an immunodiffusion plate at 37°C for 2 h. Later, 1 mL of 20% fresh guinea-pig serum was surfaced on the immunodiffusion plate and incubation continued at 37°C for another 1 h. At the end of incubation, the number of plaques (clear zones) per spleen were calculated and taken as a measure of humoral immunity.

Statistical analysis The results obtained in each of the treatment groups were compared with those of the control group using unpaired *t*-test for all tests except for the host resistance against *E. coli* induced abdominal sepsis where the Chi-square test was applied.

RESULTS

Measurement of body weights before and after drug treatment showed that the change in weight was not significantly different from the control group (Table 2).

Similarly, neither of the doses of Septilin improved the host resistance against *E. coli* induced abdominal sepsis

Group	Weight gain (g)
Group I (Control)	2.2 ± 0.33
Group II (low dose)	1.1 ± 0.37
Group III (high dose)	2.5 ± 0.72
All values are mean ± SE.	

(mortality at 24 h: control 100%; low dose Septilin 83.3%; high dose Septilin 100%) (Table 3).

Both the doses of Septilin increased the percentage of neutrophils ($p<0.001$) as well as the absolute number of circulating neutrophils (low dose $p<0.001$; high dose $p<0.01$). Both the doses showed a trend towards leucocytosis, but it was not statistically significant (Table 4).

Group	Mortality at 24 h
Group I (Control)	12/12
Group II (low dose)	10/12
Group III (high dose)	12/12
The differences are not significant	

The high dose of Septilin reduced the phagocytic activity of PMN cells as evidenced by a decrease in the average number of *Candida* per PMN cell ($p<0.001$) and reduction in the percentage of PMN cells showing phagocytosis ($p<0.01$) while the lower dose of Septilin did not affect the PMN function (Table 5).

Group	Total WBC (cells/mm ³)	Lymphocyte counts		Neutrophil counts	
		%	Absolute	%	Absolute
Group I (Control)	10267 ± 1032	76.7 ± 2.8	8302 ± 899	17.5 ± 2.6	2022 ± 482
Group II (low dose)	14533 ± 1585	52.2 ± 1.5 ^b	7665 ± 1010	41.2 ± 1.5 ^b	5903 ± 530 ^b
Group III (high dose)	14866 ± 2231	55.3 ± 2.2 ^b	8109 ± 999	39.0 ± 2.2 ^b	5945 ± 1246 ^a
All values are mean ± SE. ^a 0.001< p <0.01; ^b p <0.001.					

Similarly, the granulopoietic index of the reticuloendothelial system was reduced by the high dose of Septilin ($p<0.01$) while the lower dose of Septilin did not affect it (Table 5).

Group	Phagocytic activity of PMN cells		Carbon clearance Granulopoietic index (min ⁻¹)
	Average number of <i>Candida</i> /PMN	PMN showing phagocytosis (%)	
Group I (Control)	3.87 ± 0.26	87.2 ± 2.3	0.043 ± 0.0048
Group II (low dose)	3.88 ± 0.13	90.0 ± 1.2	0.035 ± 0.0046
Group III (high dose)	2.96 ± 0.084 ^b	79.7 ± 1.3 ^a	0.023 ± 0.0036 ^a
All values are mean ± SE. ^a 0.001< p <0.01; ^b p <0.001.			

The number of lymphocytes per spleen was not altered by either dose of Septilin. However, the number of plaque forming cells per spleen, which is a measure of humoral immunity was increased by both the doses of Septilin (low dose $p<0.001$; high dose $p<0.05$) (Table 6).

Group	Lymphocytes/spleen (x 10 ⁷)	Plaques/10 ⁶ splenic lymphocytes	Plaques/spleen (x 10 ²)
Group I (Control)	9.40 ± 0.94	221.3 ± 19.51	198.7 ± 12.49
Group II (low dose)	9.76 ± 0.63	313.3 ± 28.38 ^a	299.7 ± 18.11 ^b
Group III (high dose)	9.49 ± 0.97	267.2 ± 20.89	245.0 ± 9.79 ^a
All values are mean ± SE. ^a 0.001< p <0.01; ^b p <0.001.			

Delayed hypersensitivity to oxazolone (cellular immunity) was reduced by both doses of Septilin (low dose $p<0.05$; high dose $p<0.01$) (Table 7).

DISCUSSION

In the present study six different animal models were used to monitor the influence of Septilin on different arms of the immune system. The results show that neither of the doses studied altered weight gain, lymphocyte counts or host resistance against acute abdominal sepsis.

Both the doses of Septilin increased the percentage of neutrophils as well as the absolute number of circulating neutrophils but the higher dose reduced the phagocytic activity of the circulating polymorphonuclear cells as well as the reticuloendothelial system. This is contradictory to effects of Septilin on phagocytic function reported by Rao *et al.*, (1994), which is probably because the doses used in our study are higher than the previously reported studies

The humoral immunity was enhanced by Septilin. The increase in humoral immunity is in accordance with the study of Bhasin (1990) where Septilin treatment increased serum IgG levels, and Sharma and Ray (1977) where Septilin increased the primary as well as secondary immune response to sheep RBCs.

The cellular immunity was suppressed by Septilin. Skin test response to an antigen is complex and involves many aspects of the immune response (Urbaniak *et al.*, 1986). The delayed hypersensitivity that was measured here has a few major components – sensitization, release of cytokines and inflammation. Kumar *et al.*, (1993) and Udapa *et al.*, (1989) have already reported the anti-inflammatory effect of Septilin. To what extent this anti-inflammatory effect could have contributed to the lesser increase in the ear thickness in response to oxazolone cannot be commented upon at this stage. In fact, there is a report where rohitukine, a compound isolated from the plant *D. binectariferum*, showed anti-inflammatory effects in models of acute inflammation but actually enhanced delayed hypersensitivity to oxazolone (Lakadawala *et al.*, 1988). Thus, it appears that the anti-inflammatory effect of Septilin may have contributed only negligibly to the reduction in delayed hypersensitivity to oxazolone and that the reduction in delayed hypersensitivity must be a result of the inhibitory effect of Septilin on cell mediated immunity.

In conclusion, Septilin has dual effects on the immune system as it stimulates some of the immune functions but suppresses others. Also, it is observed that the immunostimulant effects are more prominent with the lower dose, while the immunosuppressant activity was better documented with the higher dose of Septilin. The immunomodulatory activity of drugs is known to vary with the dose level and most of the immunosuppressants show immunostimulation at low dilutions (Patwardhan *et al.*, 1990; Van Dijk and Voermans, 1978). Abrams *et al.*, (1993) observed that administration of low dose of cyclophosphamide, an immunosuppressant, to volunteers with advanced malignancies enhanced the ‘lymphokine

Group	Ear thickness
Group I (Control)	5.25 ± 0.44
Group II (low dose)	3.42 ± 0.60 ^a
Group III (high dose)	2.75 ± 0.38 ^b
All values are mean ± SE. ^a 0.01 < p < 0.05; ^b 0.001 < p < 0.01.	

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). This suggests that immunostimulation may be a general feature of immunosuppressive drugs and a similar phenomenon may be responsible for the dual effects of Septilin, observed in the present study. Another consideration is the presence of extracts of several plants in the Polyherbal preparation Septilin, some of which may contribute to the immunostimulant and others to the immunosuppressant effects of Septilin on the immune system. Therefore, it is likely that different doses of Septilin could have different indications and this should be borne in mind while prescribing it.

Finally, we propose that still lower doses of Septilin should be studied to assess its effect on the immune system and individual components of the polyherbal preparation should be screened for immunomodulator activity.

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