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Immunomodulatory activity of *Ocimum sanctum* and its influence on cyclophosphamide induced immunosupression

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The development of antimicrobials has revolutionized the treatment of bacterial diseases. In recent years there is an increasing interest in the search for potential drugs, especially of plant origin, that are capable of modifying immune responses with comparatively low side effects. These immunorestorative drugs are used to improve general health in normal and diseased condition by the stimulation of immune system (Singh 1990) without side effect (Praveen Kumar *et* al. 1999).

Ocimum sanctum is a popularly known traditional herb possessing various medicinal values. In the present study immunomodulatory activity of hydro-alcoholic extract of leaves of *Ocimum sanctum* was evaluated in healthy and immunosupressed rats.

Hydro-alcoholic extracts (50%) of *Ocimum sanctum* was prepared (*Indian Pharmacopoeia* 1985). The extract was obtained by evaporating in rotatory vacuum evaporator at 65°C and 600 mm Hg negative pressure and the per cent yield 5.8 (w/w) was recorded. The study was conducted on 48 adult albino rats (150–200 g) of either sex procured from experimental animal house of the college. The rats were maintained in polypropylene cages and housed in the animal shed of the department under good management conditions. Animals were fed standard ration *ad lib.* and had free access to clean drinking water.

Aqueous solution (10%) of the prepared extract was given to rats orally with the help of mouth gag and oesophageal tube. However, cyclophosphamide was injected intraperitonially. The rats were randomly divided equally into following each containing 12 rats, of either sex.

Group A: Untreated control

Group B: Hydro-alcoholic leaf extract of *Ocimum sanctum* @ 100 mg/kg body weight *po* daily for 7days

Group C: Cyclophosphamide @ 50 mg/kg body weight intraperitonially once

Group D: Hydro-alcoholic leaf extract of *Ocimum sanctum*, along with cyclophosphamide (dosage regimen vide supra)

The normal and cyclophosphamide-treated rats concurrently received hydro-alcoholic extracts of *Ocimum sanctum*. The blood samples were collected on eighth day in rats under chloroform anaesthesia, directly from the heart in anticoagulant tubes (EDTA) @ 1 mg/ml blood for haematological estimations. Spleens from slaughtered rats were collected in freshly prepared ice-cold phosphate buffer saline, for performing lymphocyte stimulation test.

Haematological parameters, *viz.* total leukocyte count (TLC), differential leukocyte count (DLC), computation of absolute lymphocyte count were evaluated (Jain 1986) within 2 h of collection of sample. Protein profiles namely total serum proteins, serum albumin, serum globulins, computation of albumin globulins ratio were estimated (Oser 1971). Total serum immunoglobulins were estimated by zinc sulphate turbidity method (McEwan *et al.* 1969). Humoral and cell-mediated immune responses were assessed by lymphocyte stimulation test using the method proposed by Rai-el-Balhaa *et al.* (1985), with minor modifications. Statistical analysis by using one way ANOVA to test significance of means was done as per the method described by Snedecor and Cochran (1994).

The per cent yields (w/w) of 50% hydroalcoholic extraction of *Ocimum sanctum* was 5.80. The literature could not reveal any citation regarding 50% hydroalcoholic extraction of *Ocimum sanctum*. We observed significant rise in total leukocyte count (Table 1) following oral administration of hydroalcoholic extract of *Ocimum sanctum* (group B). In cyclophosphamide treated rats of group C, total leukocyte counts decreased, which might be due to myelosuppressive activity of cyclophosphamide (Praveen

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Kumar *et al.* 1994). However, Bains and Dhake (1992) reported leukocytosis in mice suffering from late wasting disease at 6–8 months age following cyclophosphamide inoculation at birth. The administration of extracts of *Ocimum sanctum* (group D) along with cyclophosphamide protected the cyclophosphamide induced leucopoenia. This finding of present investigation corroborates with the findings of Parveen Kumar *et al.* (1994), Brekhman II and Dardymov IV (1969), Sembulingum *et al.* (1999) and Ramnath *et al.* (2002).

Lymphocyte percentage significantly increased in the hydroalcoholic extract administered groups and remained low in the cyclophosphamide treated group in comparison to healthy control (group A) and immunosuppressed group (group C) of rats. The lymphopoenia observed in cyclophosphamide treated groups might be due to cyclophosphamide induced myelosuppression. Contrary to this Bains and Dhake (1992), reported lymphocytosis in mice suffering from late wasting disease at 6–8 months age following cyclophosphamide inoculation at birth. The hydroalcoholic extract of *Ocimum sanctum* possibly potentiates the lymphocyte proliferation and neutralizes the cyclophosphamide induced depression in lymphocyte count.

In the present study increase in absolute lymphocyte count was observed in rats of B group in comparison to group A, and in the rats of groups B and D when compared to immunosuppressed rats of group C. These findings suggest that extract of *Ocimum sanctum* might have caused lymphocytosis and cyclophosphamide lymphopoenia. The increase in total leukocyte, percent and absolute lymphocyte count observed in rats receiving extracts simulate with the findings of Mitra (2004) and Rao *et al.* (1994). The administration of extracts of *Ocimum sanctum* might have enhanced the immune response as evident by significant ($P<0.05$) rise in the total leukocyte, percent lymphocyte and absolute lymphocyte count in rats of group B and also overcame the cyclophosphamide induced leucopoenia in rats of group D. This is in complete corroboration with the observations of Praveen Kumar *et al.* (1994) and Ramnath *et al.* (2002).

The per cent neutrophil count was low in group B as compared to healthy control of group A, and in groups B and D as compared to immunosuppressed rats of group C. However, increase in per cent neutrophil was recorded in immunosupressed rats of group C in comparison to group A the administration of extract of *Ocimum sanctum* reduced per cent neutrophils. This finding corroborates with Bains and Dhake (1992). Contrary to the above findings Praveen Kumar *et al.* (1994) reported neutophilia in rasayan treated mice. The variations observed in per cent neutrophil count in rats of various groups during present study were possibly due to relative change in response because of changes recorded in per cent lymphocytes. Monocyte count significantly ($P<0.05$) decreased in all the treated groups.

Table 1. Alterations in haematological, protein profiles and lymphocyte stimulation in rats of different groups (mean±SEM, n=12)

Parameters	Groups			
	A	B	C	D
Hematological profile				
Total leukocyte	8.95^{a}	10.10	8.23	9.19^{a}
count (10^9/l)	±0.09	±0.23	±0.08	±0.08
Neutrophil	19.47	17.79	23.17	20.23
(%)	±0.17	±0.09	±0.25	±0.14
Lymphocyte	73.55	75.97	70.02	74.20
(%)	±0.25	±0.09	±0.29	±0.16
Monocyte	5.11	4.67^{b}	4.71^{b}	4.0
(%)	±0.20	±0.08	±0.17	±0.09
Eosinophil	$1.28^{c,,d,e}$	$1.13^{c,e,f}$	1.75	$1.17^{d,,f,}$
(%)	±0.12	±0.07	±0.15	±0.10
Basophil	$0.59^{g,h,i}$	$0.45^{g,j,k,}$	$0.35^{h,j}$	$0.40^{i,k,}$
(%)	±0.06	±0.08	±0.08	±0.05
Absolute lymphocyte	6.58^{l}	7.67	5.76	6.82^{l}
count (10^9/l)	±0.07	±0.18	±0.07	±0.07
Protein profile				
Total serum	57.15^{m}	60.07	52.47	55.66^{m}
Proteins (g/l)	±0.22	±1.29	±0.16	±0.10
Serum	38.10^{n}	33.99^{o}	36.97^{n}	34.07^{o}
albumin (g/l)	±0.03	±0.06	±0.69	±0.34
Serum	19.08	26.53	16.14	21.59
globulin (g/l)	±0.21	±1.16	±0.71	±0.28
Albumin:	1.99	1.32	2.35	1.58
globulin ratio	±0.02	±0.07	±0.14	±0.37
Total serum	51.50	61.98	45.60	59.51
immunoglobulins (g/l)	±1.15	±0.74	±0.43	±0.29
Lymphocyte stimulation test				
Control	0.29	0.41^{q}	0.12	0.39^{q}
	±0.01	±0.01	±0.01	±0.01
PHA	0.41^{p}	0.51^{r}	0.23	0.48^{t}
	±0.01	±0.01	±0.01	±0.01
LPS	0.40^{p}	$0.48^{r,s}$	0.19	$0.47^{s,t}$
	±0.01	±0.01	±0.01	±0.01

Means bearing common superscripts (a to t) did not differ significantly ($P<0.05$).

group A : Untreated control

group B : Rats treated with hydro-alcoholic leaf extract of *Ocimum sanctum* @ 100 mg/kg body weight *po* daily for 7 days

group C : Rats treated with cyclophosphamide @ 50 mg/kg body weight intraperitonially once

group D : Rats treated with hydro-alcoholic leaf extract of *Ocimum sanctum*, along with cyclophosphamide (dosage regimen vide supra).

Significant ($P<0.05$) elevation (Table 1) in per cent eosinophil count was observed in cyclophosphamide treated rats (group C) in comparison to healthy control (group A), and extracts administered rats (groups B and D). These observations pertaining to variations in monocytes and eosinophila could not be explained because of paucity of literature.

In present study, a significant ($P<0.05$) increase in total serum protein in rats of groups B in comparison to healthy control rats of group A, and in groups B and D in comparison to immunodeppressed rats of group C indicated that serum total proteins level elevated following administration of extract of *Ocimum sanctum* and declined following administration of cyclophosphamide. The cyclophosphamide induced reduction in serum total proteins in group C in present study simulates with the observations of Padalkar *et al.* (1991), Toma *et al.* (1997) and Saxena and Singh (2002). Significant ($P<0.05$) increase in serum total proteins in extract treated group B and normalizing activity in immunosuppressed groups D in present investigation corroborate with the findings of Mitra (2004).

A significant decrease in serum albumin and increase in serum globulin concentration in extract treated groups B and D was recorded. However, there was significant ($P<0.05$) decrease in serum globulins in cyclophosphamide treated group C. This finding is in corroboration with Mitra (2004).

In the present study the significant ($P<0.05$) decrease in albumin globulin ratio in extract treated groups simulates with the observations reported by Mitra (2004). The total serum immunoglobulin concentrations increased in groups B and D and decreased in cyclophosphamide treated group C. The administration of extract might have led to increase in total serum immunoglobulins in groups B and D.

The cyclophosphamide induced decrease in serum immunoglobulin concentration in rats of group C simulates with the findings of Padalkar *et al.* (1991), Toma *et al.* (1997) and Saxena and Singh (2002).

In the present study, lymphocyte proliferation occurred in control, PHA (T-cell) and LPS (B-cell) in rats of groups B and D as compared to healthy control (group A) and immunodepressed (group C) rats. In cyclophosphamide treated groups, inhibition of both cellular and humoral immunity was observed. The decrease in humoral immunity in cyclophosphamide treated group might have occurred due to hyper secretion of steroid (cortisol) from adrenal, this finding simulates with the findings of Toma *et al.* (1997). The lymphocyte proliferation was also observed in all the rats receiving extracts of *Ocimum sanctum* by the mitogen PHA and LPS and this T and B lymphocyte proliferation has resulted in cell mediated and humoral immunity. This finding is in complete corroboration with Chauhan (1999) and Nemmani *et al.* (2002).

SUMMARY

The present investigation communicates the immunoimodulatory effects of *Ocimum sanctum* on cyclophasphamide-induced immunosupression in rats. The findings revealed that *Ocimum sanctum* produced significant increase in total leukocyte count, per cent lymphocyte count, absolute lymphocyte count, total proteins, globulins and total immunoglobulin concentrations. However, neutrophils, monocytes, serum albumin and albumin: globulin ratio decreased in treated groups as compared to normal and immunosuppressed rats. Lymphocyte stimulation test revealed significant increase in lymphocyte proliferation in rats administered with extract of the plant.

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