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Research Article

IMMUNOMODULATORY ACTIVITY OF METHANOLIC EXTRACT OF *OCIMUM AMERICANUM* SEEDS

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ABSTRACT

The study was conducted to investigate the immunomodulatory activity of methanolic extract of *Ocimum americanum* L. Seeds (OME) in mice and rats at doses of 100, 200, 300, 400 and 500 mg/kg orally. Immunomodulatory activities on humoral and cellular immunity were studied by carbon clearance method in mice and haemagglutination (HA) titre, delayed type hypersensitivity (DTH) reaction in rats. OME enhanced the carbon clearance, HA titre and DTH reaction in a dose dependent manner. OME also significantly increased the white blood cells and lymphocytes count. The effects were compared to the standard drug Levamisole. The results suggest that the methanolic extract of *Ocimum americanum* L. seeds possesses promising immunomodulatory activity.

Keywords: *Ocimum americanum* L. carbon clearance, delayed type hypersensitivity.

INTRODUCTION

In recent times, focus on plant research has been intensified all over the world and has been collected to show immense potential of medicinal plants used in various traditional systems[1].

The term immunomodulatory means regulation of the immune system by suppression and stimulation of the cells and organs of the immune system[2, 3]. The immunomodulatory agents from plant origin which are claimed to induce para immunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement fLunctions[4]. Ocimum americanumL., (Syn. Ocimum africanum Lour., Ocimum canum Sims, Ocimum brachiatum Blume), common name hoary basil, rosary basil is a aromatic herb of the family Lamiaceae distributed in tropical Africa and tropical Asia[5]. The plant is carminative, diaphoretic and stimulant; used in cold, coughs, catarrh and bronchitis, Leaf juice is used for dysentery and as a mouth-wash for relieving toothache; poured into nostrils for migraine. Decoction of the leaf is used for checking nose bleeding and malarial fever. Leaf paste is used as a cure for parasitical skin diseases. Tea or infusion of the leaf is used in fever, indigestion and diarrhoea. Dried plant is burnt as mosquito repellant (Yusuf *et al.* 2009)[6]. Essential oil of the leaves and inflorescences possesses strong antifungal and antibacterial properties (Begum *et al*., 1999) [7].

In view of the importance of this herbal plant the present study was undertaken to investigate the immunomodulatory effects of the methanolic extract of *Ocimum americanum* L. seeds in mice and rats.

MATERIAL AND METHODS

Plant Material

The fully mature *Ocimum americanum* L. seeds were collected locally in Karimnagar. The plant was botanically identified and authenticated by Dr.Naqui, Plant Taxonomist (Reader in Botany, Govt. SRR Degree College, Karimnagar, Andhra Pradesh, India) and voucher specimen was deposited in the department herbarium.

Preparation of Plant Extract

The seeds were dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with methanol using cold percolation method. The percentage yield was 10.38% in methanol. The extract was stored at -70^{O}C until further use. Henceforth, the methanolic extract of *Ocimum americanum* L. seeds will be called as OME. The extract and standard drug were administered in the form of suspension in water with 1% Sodium carboxy methyl cellulose (SCMC) as suspending agent

Drugs/Chemicals

Levamisole was supplied by Khandelwal Laboratories, Mumbai, India. All other chemicals used for this study were of analytical grade.

Animals

Albino rats (175-200 g) and albino mice (18-25 g) were procured from Mahaveer Enterprises, Hyderabad, India were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25±2^{O}C and 50±15% relative humidity with a 12-h light/12-h dark cycle. Animals were fed with a commercial pellet diet (Rayans Biotechnologies Pvt Ltd., Hyderabad) and water ad libitum. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

PHARMACOLOGICAL EXPERIMENT

1. In vivo Carbon Clearance Method

The mice were divided into 7 groups. Group-I act as control group, Groups- II to VI treated with OME at different doses (100, 200, 300, 400 and 500 mg/kg b.wt) and Group- VII was treated with Levamisole-50 mg/kg orally. At the end of 7 days, the mice were injected with carbon ink suspension-0.1 ml/10 g (1.6% w/v in 1% gelatin, in saline) via tail vein[8]. Blood samples were drawn (in 0.15% w/v disodium EDTA solution, 50 µl) from the retro orbital plexus at intervals of 2 & 15 min after injection. A 25 µl sample was mixed with 0.1% sodium carbonate solution (2 ml) and the absorbance was measured at 660 nm. The carbon clearance was calculated using the following equation[8, 9].

Carbon clearance = (log OD1 – log OD2)/ (T2 – T1)

OD1 and OD2 are optical densities at T1 and T2 respectively.

T1 = 2 min, T2 = 15 min

Antigen

Fresh blood was collected from sheep sacrificed in local slaughter house in Alsever's solution. Sheep red blood cells (SRBC) were washed three times in normal saline and RBC of this suspension was adjusted to a concentration of 5 X 10^{9} cells per ml for immunization and challenge[10].

2. SRBC-induced Delayed Type Hypersensitivity Reaction (DTH response)

The rats were divided into 7 groups of 6 rats in each. DTH response was induced in rats using SRBC as an antigen according to Doherty method[11]. Group-I act as control group, Groups- II to VI treated with OME at different doses and Group-VII was treated with Levamisole-50 mg/kg orally. OME at different doses (100, 200, 300, 400 and 500 mg/kg b.wt) were administered on day 0 and continued till the day of challenge. The rats were primed with 0.1 ml of SRBC suspension containing 5 X 10^{9} cells i.p. on day 8 and challenged on day 13 with 0.05 ml of 5 X 10^{9} SRBC on the right hind foot pad. The contra lateral paw received equal volumes of saline. The thickness of the foot pad was measured at 24 h after the challenge using vernier calipers. The difference in the thickness of the right hind paw and the left hind paw was used as a measure of DTH.

3. Humoral Antibody Response (HA response)

The rats were divided into 7 groups of 6 in each. Group-I act as control group, Groups-II to VI treated with OME at different doses and Group-VII was treated with Levamisole-50 mg/kg orally. OME was administered at different doses (100, 200, 300, 400 and 500 mg/kg body weight) on day 0 and continued till the day of the experiment. On day 8, the rats were immunized with 10% suspension of SRBC, i.p. Blood samples were collected from the retro orbital plexus of individual animals on day 13 and their antibody titre from serum was determined according to Puri *etal*.[12]. Briefly, an aliquot (25µl) of 2-fold diluted sera in saline was challenged with 25µl of 0.1%v/v SRBC suspension in micro titre plates. The plates were incubated at 37^{o}C for 1 h and then examined for

haemagglutination and expressed as HA titre.

Haematological Profile
After 8 days of administration of the extract, blood was collected via retro orbital plexus of each rat. Various haematological parameters such as white blood cells, neutrophills, lymphocytes, monocytes, eosinophills and basophills were estimated. White blood cells were estimated by haemocytometer and remaining parameters by Leishman's stain method[13].

Statistical Analysis
Data were expressed as mean ± SEM. Data were analyzed by using Analysis of variance and Dunnett's't' test. The difference was considered to be significant at $p<0.05$ level.

RESULTS

In vivo Carbon Clearance Method
The methanolic extract of *Ocimum americanum* L. seeds (OME) produced a significant increase in carbon clearance from the blood in a dose-dependent manner (Refer table 1) in mice. The maximum carbon clearance was observed with the 500 mg/kg of OME. The OME effects were compared with the standard drug, Levamisole-50 mg/kg.

DTH Response
OME produced a significant dose related increase in DTH reaction in rats (Refer table 1). The edema reached a peak at 24 h, the percent edema being 10% for the control group, after which it subsided. The maximum effect was observed with the 500 mg/kg of OME. The effects were compared with standard.

HA Response
OME produced a significant increase in humoral antibody titres in a dose-dependent manner (Refer table 1) in rats. The maximum effect was observed with the 500 mg/kg of OME. The OME effects were compared with the standard drug.

Haematological Profile
OME produced a significant increase in total WBC, neutrophils and lymphocytes count in dose-dependent manner (Refer table 2). Insignificant changes were observed in monocytes, eosinophils and basophils count. The maximum effect was observed with the 500 mg/kg of OME. The OME effects were compared with the standard drug.

DISCUSSION

OME was found to stimulate the phagocytic activity of the macrophages as evidenced by an increase in the rat of carbon clearance. The DTH directly correlates with cell-mediated immunity, was found to be highest at the maximum dose of SME (500 mg/kg). OME was tested on SRBC haemagglutination antibody titre in rats. OME was found to be significantly enhanced the circulating antibody titre when compared to untreated immunized rats. This indicates the enhanced responsiveness of T and B lymphocyte subsets involved in antibody synthesis. In the present study, SEM showed an enhancement in total WBC, neutrophils and lymphocytes counts indicating their effect on haematopoiesis. From the results observed in the current investigation, it may be concluded that the methanolic extract of *Ocimum americanum* L. seeds possesses immunomodulatory activity by stimulating both cell mediated and humoral immune responses. This study warrants the investigation to isolate and identify the active principles and to elucidate the exact mechanism of action.

Table 1: Effect of methanol extract of *Ocimum americanum* L. seeds on carbon clearance, DTH response and HA titre

Treatment	Carbon clearance	DTH response (% paw thickness)	HA titre
Control	0.070 ± 0.014	10.74 ± 0.91	6.33 ± 0.73
OME – 100 mg/kg	0.124 ± 0.018*	10.85 ± 0.75	6.64 ± 0.64
OME – 200 mg/kg	0.134 ± 0.016*	12.20 ± 1.11*	8.27 ± 0.66*
OME – 300 mg/kg	0.150 ± 0.018*	15.13 ± 1.12*	9.14 ± 0.77*
OME – 400 mg/kg	0.160 ± 0.020*	15.92 ± 1.94*	9.16 ± 0.78*
OME – 500 mg/kg	0.164 ± 0.020*	16.13 ± 1.83*	9.18 ± 0.80*
Levamisole-50 mg/kg	0.160 ± 0.018*	16.02 ± 1.86*	9.12 ± 0.82*

OME: Methanolic extract of *Ocimum americanum* L. seeds
* Significance at $p<0.05$ (Compared to control)

Table 2: Effect of methanol extract of *Ocimum americanum* L. seeds on haematological parameters in rats

Treatment	Total WBC (10^3/mm^3)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Control	6.91 ± 0.40	31.18 ± 0.90	68.02 ± 2.10	3.02 ± 0.20	3.02 ± 0.23	0 ± 0
OME – 100 mg/kg	9.98 ± 0.54*	40.95 ± 0.19	70.02 ± 1.90	3.18 ± 0.22	3.34 ± 0.45	0 ± 0
OME – 200 mg/kg	11.12 ± 0.48*	41.42 ± 0.72*	76.02 ± 1.90*	3.26 ± 0.24	3.22 ± 0.54	0 ± 0
OME – 300 mg/kg	11.93 ± 0.29*	42.18 ± 0.72*	77.02 ± 1.60*	3.32 ± 0.34	3.18 ± 0.32	0 ± 0
OME – 400 mg/kg	13.02 ± 0.36*	43.02 ± 0.87*	78.77 ± 2.50*	3.25 ± 0.33	3.15 ± 0.37	0 ± 0
OME – 500 mg/kg	13.81 ± 0.37*	44.35 ± 0.78*	79.02 ± 1.40*	3.18 ± 0.32	3.02 ± 0.27	0 ± 0
Levamisole-50 mg/kg	13.02 ± 0.99*	44.02 ± 0.14*	80.77 ± 2.80*	3.32 ± 0.33	3.16 ± 0.31	0 ± 0

OME: Methanolic extract of *Ocimum americanum* L. seeds
* Significance at p<0.05 (Compared to control)

REFERENCES

1. Dahanukar SA, Kulakarni RA and Rege NN. Pharmacology of medicinal plants and natural products. Ind J Pharmacol. 2000;32:S81-S118.
2. Bafna AR and Mishra SH. Immunomodulatory activity of methanolic extract of roots of Cissamplelos pareira Linn. Pharmacy Department. The MS University of Baroda, India.
3. Oladunmoye S. Immunomodulatory effects of ethanolic extract of Tridaprocumbens aeruginosa (NC1B950). Trends in Medicinal Research. 2006;1(2):122-126.
4. Sainis KB, Sumariwalla PE, Goel A, Chintalwar GJ, Sipahimalani AJ and Banerji A. In: Upadhayay SN, eds. Immunomodulatory properties of stem extracts of Tinospora cordifolia: Cell targets and active principles. In immunomodulation, 3rd ed. New Delhi, India. Narosa Publication House, 1997;95-97.
5. http://www.mpbd.info/plants/ocimum-americanum.php
6. www.banglajol.info/index.php/BJB/article/download/7303/5515
7. www.mpbd.info/plants/ocimum-americanum.php
8. Dash S, Nath LE, Bhise S, Kar P and Bhattacharya S. Stimulation of immune function by the alcoholic root extract of immune function by the alcoholic root extract of Heracleum napalense. DDon. Ind J Pharmacol. 2006; 38(5):336-348.
9. Jayathirtha MG and Mishra SH. Preliminary immunomodulatory activities of methanol extract of Lelipta alba and Centella asitica. Phytomedicine 2004;11:361-365.
10. Bafna AR and Mishra SH. Immunomodulatory activity of methanol extract of roots of Cissampelos pareira Linn. Ars. Pharm 2005;46(3):253-262.
11. Doherty NS. Selective effects of immunosuppressive agents against the delayed hypersensitivity response and humoral response to sheep blood cells in mice. Agents Actions. 1981;11:237-242.
12. Puri A, Sexena RP, Sexena KC, Srinivasan M and Tandon JS. Immunostimulant activity of Nyctanthes arbortristis L. J Ethnopharmacol. 1994;42:31-37.
13. Raphael TJ and Kuttan G. Effect of naturally occurring triterpenoids glycyrhizic acid, ursolic acid, oleanolic acid and nomilin on the immune system. Phytomedicine. 2003;10:483-489.