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Research Article

EFFICACY OF *OCIMUM BASILICUM* FOR IMMUNOMODULATORY ACTIVITY IN WISTAR ALBINO RAT

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ABSTRACT

Aim: Immunomodulatory effect of *O. basilicum* was studied.

Methods and Results: Low and high dose of O. basilicum was administrated in wister albino rat. Antibody titre was estimated by SRBC titre method. RBC, WBC, Haemoglobin count was recorded. The biochemical parameters also estimated in treated and control animals. It enhanced the antibody titre value. It improves the RBC and haemoglobin count. Biochemical results were good when compared to control.

Conclusion: *O. basilicum* showed immunomodulatory effect.

Keywords: *Ocimum basilicum,* Immouno modulator, SRBC, RBC, Haemoglobin.

INTRODUCTION

Medicinal plants have been used for centuries and have become part of complementary medicine world wide because of their potential health benefits. Some of their metabolites have been successfully used directly in the treatment and prevention of infectious diseases and cancer, or indirectly by stimulating the immune system [1]. Now a day large number of drugs in use are derived from plants, like morphine from *Papaver somniferum*, Aswagandha from *Withania somnifera*, Ephedrine from *Ephedra vulgaris*, Atrophine from *Atropa belladonna* etc. Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against much pathology [2]. The immune system is designed to protect the host from invading pathogens and to eliminate disease [3]. Immunomodulatory agents are used to either suppress or stimulate the immune responsiveness of an organism [4]. Plant extracts are potentially curative. Some of these extracts can boost the humoral [5] and cell mediated immunity [6], against virsuses [7], bacteria [8], fungi [9], protozoa [10] and cancer [11]. Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials.

Ocimum basilicum (Lamiaceae) basil or sweet basil, thiruneetruppachchai in Tamil. It grows to a height of 50 – 70 cm, leaves are oval and slightly toothed and flowers are white in colour. It is mostly used in cosmetics, liqueurs, medicines and perfumes. The leaves and its oil have insecticidal property. Nematicidal, fungistic, antimicrobial and anti inflammatory properties are also reported [12-14]. Wound healing property was studied by Salmah *et al*., [15].

In this present study, immunomodulatory effect of *O. basilicum* in rat was studied.

MATERIALS AND METHODS

Plant extract

Fresh plants were cleaned, dried at 37°C for 3 days and powered well. From this dried powdered extracts were prepared.

Experimental designs

Animals were divided into three groups, each having five rats and treated accordingly, Group I: control

Group II: Animals treated with aqueous extract of *O. basilicum* (100 mg/kg)

Group III: Animals treated with aqueous extract of *O. basilicum* (200mg/kg)

Antigen Preparation

Crystalline Bovine Serum Albumin fraction V (BSA) was used as non cellular antigen for the present investigation.

Soluble Bovine Serum Albumin (S - BSA)

S - BSA was prepared by overlaying the BSA powder in isotonic saline 1.0mg/ml of saline (0.15 N). It was allowed to dissolve without agitation and used as antigen.

Collection of Sheep red blood cells

SRBC were collected in Alserver's solution from animal husbandry without contamination. To avoid allogenic difference the Sheep red blood was used throughout the study.

Immunization

After 3days of exposure to the toxicant, rats were immunized with optimum dose of 0.5 ml of antigen. The antigen was injected through the intraperitoneal route using 3ml tuberculin syringe. Secondary immunization was also done with the same dose of antigen through the same route on the 15th day after primary immunization. Antigen administration and serial bleeding of animals were always done between 2 – 4pm to avoid circadian rhythmic variations on the immune response.

Blood Collection from Test Animal

Blood samples were collected from a tail vein by snipping the tip of the tail. The tip of the tail was cleaned with spirit and snipped with clean scissors. The blood was collected in EDTA rinsed vials for hematological studies and antigen-antibody titration.

Normal Serum and Antiserum Collection

The blood was collected from the control and test animals by snipping the caudal vein rinsed with 1% EDTA and kept at room temperature for 20 min. The serum was separated by spinning down the clot at 3000 rpm for 15- 20 min and then collected in sterilized storage vials. It was kept at 57ºC in a water bath for 30mts to inactivate complement and stored at 20ºC until use.

Antibody Titration

Passive Haemoagglutination Assay

Chromic chloride method

This assay was used to determine anti-BSA antibodies in the serum. Two fold dilutions of the antiserum (50 µl per well) were made with saline in 'U' bottom microtitre plate 50µl of 2% BSA coupled

SRBC in saline was added to each well. For effective mixing the microtitre plate was hand shaken and incubated for an overnight at 37 ºC. The highest dilution of the serum samples showed detectable macroscopic agglutination was recorded and expressed as Log 2 antibody titre of the serum.

Coupling of BSA to SRBC

The chromic chloride method for immunological purposes was followed by Goding (1976). In this present study, $CrCl_3$ used as a coupling agent for the coupling of BSA to SRBC. Fresh sheep erythrocytes were washed thrice by using phosphate buffered saline and stored at 4ºC. One volume of the chromic chloride solution was added to an equal volume of the protein antigen in 0.15M saline and then added to one volume of packed red cells immediately. Then it was mixed well and kept at room temperature for 4 min.The coupled red cells are then washed three times in 10-20 volumes of 0.11 M NaCl and resuspended in 0.15 M NaCl with 2 % BSA .

Haematological analysis

The fresh whole blood samples were used for the estimation of leucocyte, erythrocyte counts and haemoglobin.

Biochemical tests

Total plasma protein, albumin, globulin, alkaline phosphatase, SGOT, SGPT were analysed by Semi auto analyzer (Chem 400).

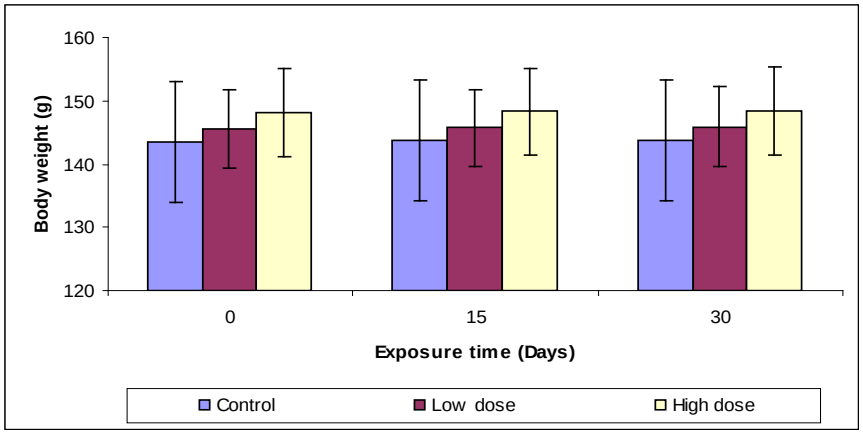


Fig. 1: Effect of *Ocimum Basilicum* on body weight of Wistar albino rats

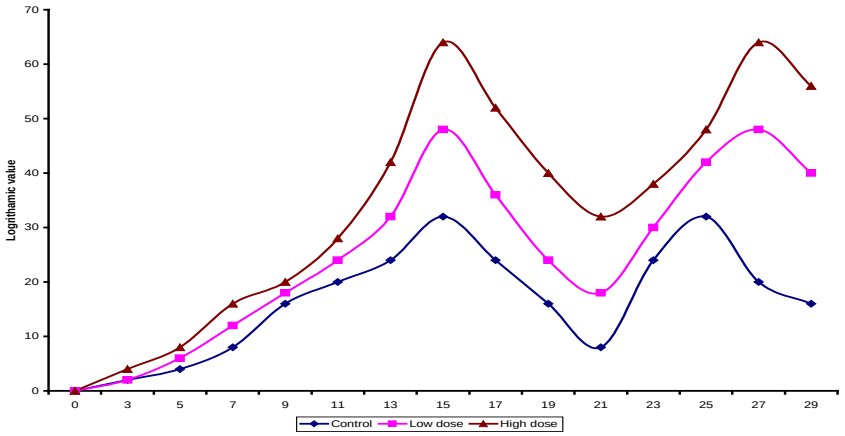


Fig. 2: Effects of *Ocimum basilicum* on humoral immune response to S-BSA exposed for 30 days

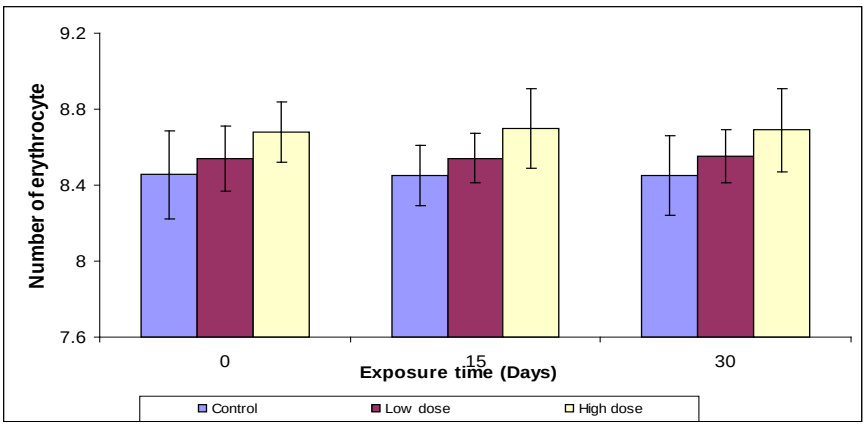


Fig. 3: Effect of *Ocimum bacilicum* on erythrocyte count of Wistar albino rats

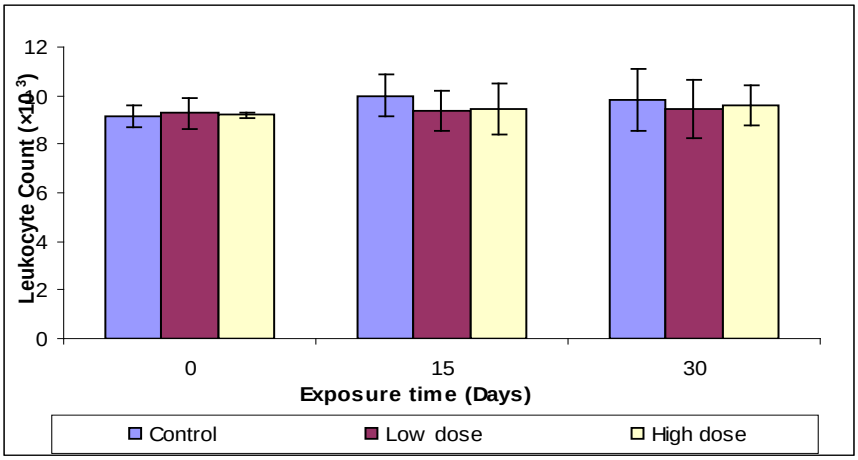
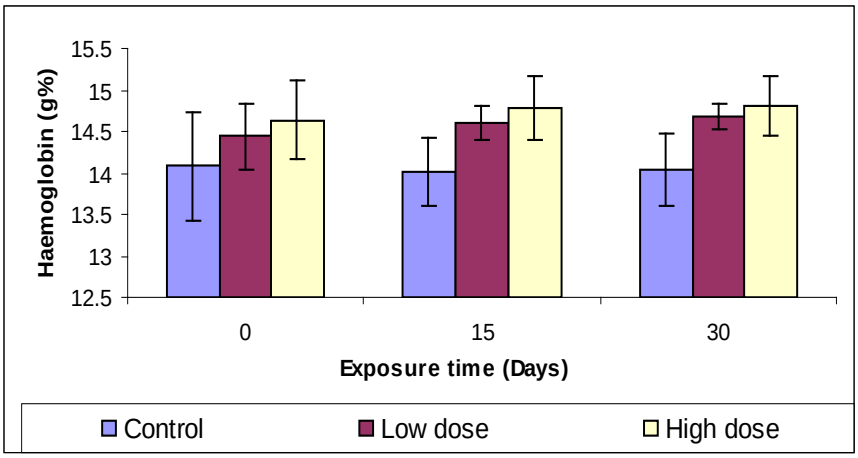


Fig. 4: Effect of O*cimum basilicum* on leukocyte count of Wistar albino rats



'Fig. 5: Effect of O*cimum basilicum* haemoglobin count of Wistar albino rats

Table 1: Effect of Ocimum basilicum on leucocyte count of Wistar albino rats

Exposure (Days)	Control	100 mg/kg	200 mg/kg
0	66.6 ± 5.94	58.80 ± 3.27	59.2 ± 3.35
15	68.4 ± 8.02	61.40 ± 3.58	61.80 ± 2.68
30	68.2 ± 6.65	63.2 ± 4.97	65.00 ± 3.67

Table 2: Effect of Ocimum basilicum on differential count of Wistar albino rats

Biochemical Parameter	Exposure (Days)	Control	100 mg/kg	200 mg/kg
	0	6.02 ± 0.38	6.12 ± 0.29	6.24 ± 0.54
Protein	15	6.24 ± 0.30	6.36 ± 0.25	6.26 ± 0.72
(g/dl)	30	6.5 ± 0.34	6.52 ±0.44	6.46 ± 0.78
	0	4.14 ± 0.30	4 ± 0.16	3.98 ± 0.16
Albumin	15	4.1 ± 0.30	4.12 ± 0.22	4.04 ± 0.05
(g/dl)	30	4.18 ± 0.16	4.16 ± 0.13	4.18 ± 0.08
	0	1.88 ± 0.54	1.98 ± 0.31	2.26 ± 0.55
Globulin	15	2.14 ± 0.36	2.24 ± 0.17	2.22 ± 0.73
(g/dl)	30	2.32 ± 0.42	2.36 ± 0.55	2.28 ± 0.75
	0	58.5 ± 11.00	58 ± 12.78	59.04 ± 7.80
SGOT	15	60.56 ± 10.72	58.38 ± 12.57	58.88 ± 7.51
(U/L)	30	62.36 ± 10.35	58.94 ± 12.69	59.8 ± 7.54
	0	23.06 ± 4.18	22.48 ± 2.84	23.42 ± 3.27
SGPT	15	23.2 ± 3.77	22.64 ± 2.45	23.48 ± 3.35
(U/L)	30	23.46 ± 3.67	22.92 ± 2.44	23.94 ± 3.06
	0	81.1 ± 11.67	81.64 ± 17.81	85.54 ± 12.29
ALP	15	82.64 ±15.60	84.1 ± 11.68	86.18 ± 11.56
(U/L)	30	83.08 ± 11.66	85.7 ± 10.64	88.50 ± 9.60

RESULTS

O. basilicum was evaluated for immunomodulatory effect. It showed increase in Body weight in *O. basilicum* treated animals than the control animal (Figure. 1). Administration of aqueous extract of (100 mg/kg) and 200 (mg/kg) *Ocimum basilicum* produced dose dependent significant increased in antibody titre value compared to control. The results were given in the Figure. 2.

Haematological changes

WBC count was increased in *O. basilicum* treated groups. RBC count was slightly increased but not in significant manner. The results were given in the Figure 3 and 4. Leucocyte count was given in Table. 1.

Biochemical analysis

The results showed that the increasing level of total protein in low and high dose *O. basilicum* treated animals from 0 day to 30th day. When compared to control, albumin level was not significantly changed for both low and high dose. Globulin level was increased from initial day to the end of the experimental period. SGOT, SGPT, ALP, were analysed. The results were given in the Table. 2.

DISCUSSION

Immuno modulatory effect of *O. basilicum* was studied in wistar albino rat. The immune system is a complex system, to protect the host from invading and to eliminate diseases. Immunomodulators are being used as an adjuvant in conditions of immunodeficiency in cancer and other immunodeficiency syndrome[16]. In this present study, *O. basilicum* showed increasing antibody production upto 15th day and reduced. Again it was increased after 21st day upto 25th day and reduced. The same result was proved by the methanol extract and aqueous suspension of *Ocimum sanctum* leaves by Mediratta *et al*., [17]. Both humoral and cell mediated immune response of *Ocimum sanctum* seed oil was proved by Mitra *et al*., [18]. He concluded that it may be due to the activity on GABA nergic pathways. Alcoholic and aqueous extracts of *Ocimum sanctum* the increase in haemagglutination titer in mice [19]. The same result was reported by Jeba *et al*., [20] in *O. sanctum*.

Immuno modulatory effect of *Cynodon dactylon* was studied by Santhi and Annapoorani [21]. Immuno modulatory effect of *Ocimum sanctum* and *valairasa chendhuram* was studied by Anuradha and Murugesan [22] against copper acetate induced toxicity on fish *Oreochromis mossambicus*. They found that *O.sanctum* and *Valairasa chendhuram* were efficient in enhancing the immune response and setting back the haematological parameters. The common carp (*Cyprinus carpio*) treated with herbal immunostimulant (*O. basilicum, Cinnamomum zeylanicum, Juglans regia, Mentha piperita*) enhanced bactericidal activity, serum lyzozyme, respiratory burst activity, WBC, RBC, haemoglobin, total serum protein, albumin, globulin [23]. In this present study increase in antibody titre value indicated *O. basilicum* potentiates humoral immunity. RBC and haemoglobin content was increased.

The herbal immuno modulator containing *O. sanctum*, *Phllanthus emblica*, *Withania somnifera* and Shilajit is very helpful in boosting the immune system and fighting against *Caecal coccidiosis* [24]. Lymphocyte proliferation of *O.basilicum, P. Americana, P.virginica and Rosa spp*., were studied by Gomez-Flores *et al*., [1]. He concluded that methanol and aqueous extract of *O. basilicum* showed 80 and 83% of lymphocyte proliferation, respectively. In this present study, lymphocyte count was gradually increased. It is may be due to the presence flavonoids and terpenoids [25-27]. reported that *O. basilicum* modulate both humoral and cell-mediated immune responses.

The present study suggests that the aqueous extract of *O. basilicum* stimulate the antibody production in rat. It enhances the production of RBC and haemoglobin.

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