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SCREENING OF IMMUNOMODULATORY EFFICIENCY OF *LAURUS NOBILIS*

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ABSTRACT

Medicinal plants have an important role in our normal life. The plant immunostimulant activity was screened using animal modal. The efficiency of immunostimulant was analysed antibody titration, B and T cell counts and differential count method. From the results the antibody levels were significantly raised compared to pathogen treated animal. The T cell counts were increased due to the cellular level stimulation of plant extract in host. In the present investigation concluded that the plant extracts of *L. nobilis* have immunostimulant potential.

KEYWORDS: Medicinal plant, immunomodulation and *L.nobilis*.

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INTRODUCTION

Indian plants which are most effective and the most commonly studied in relation to diabetes and their complications are: *Allium cepa, Allium sativum, Aloe vera, Cajanus Cajan, Coccinia indica, Caesalpinia bonducella, Ficus bengalenesis, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzigium cumini, Tinospora cordifolia* and *Trigonella foenum graecum.* Among these Cohen (1998) and Collier (2001) have evaluated *M. charantia, Eugenia jambolana, Mucuna pruriens, T. cordifolia, T. foenum graecum, O. sanctum, P. marsupium, Murraya koeingii* and *Brassica juncea*. All plants have shown varying degree of hypoglycemic and anti-hyperglycemic activity (Sanda *et al.,* 2011). India has a rich tradition in the use of medicinal plant to develop drugs from plants. Now a day's herbal drug prescribed binding even when their biologically active compounds were unknown. Because of their effectiveness, medicinal side effects in clinical experiences and relatively low cost (Samy and Ignacimuthu, 2001). Hence in the present investigation screened immunomodulatory efficiency of *Laurus nobilis*.

MATERIALS AND METHODS

Laurus nobilis was chosen as a plant sample for screening antibacterial and immunomodulate activities. The leaves were collected from the local market Manavalanagar, Tiruvallur District, Tamil Nadu, India. Fresh samples of small healthy leaves were collected from plant in the early morning for investigation. The collected leave samples immediately transferred to the laboratory for shadow dry.

Preparation of plant sample

The plant material was dried in shadow place, after complete dry, the dried sample, powdered with the help of mixer grinder. The powdered sample used for preparation of extract. In the present study the plant extract prepared with different organic solvents (low polar to high polar). Hexane, butanol, ethanol, chloroform and aqueous solvents used for extract preparation by Pestle and Soxhlet method.

Immunological Assay

The immunological assay should be followed collecting blood in plant extracts injected mice.

Immunization of animals

For the experimental study, mice weighing 24 ± 0.2 gm (35 days old) were recruited from the acclimatized stock. Mice were grouped into several groups with six individuals each. These animals were housed in specially designed cage with provision for systematic supply of pellets and water. Animals were trained to take water and feed from the cage provided. Test bacterial antigens were given through intramuscular injection at optimum levels with primary and secondary doses, along with standard water and pellet feed given every day in *ad libitum*.

Immunization of animals with plant extracts

For the experimental study, mice weighing 24 ± 0.2 gm (35 days old) were recruited from the acclimatized stock (Fig 1). Mice were grouped into several groups with six individuals each. These animals were housed in specially designed cage with provision for systematic supply of pellets and water. Animals were trained to take water and feed from the cage provided. The test animal divided into 6 groups and 6 different plants extract were given orally to mice. Blood sample of stimulated plant extract mice were collected after 3rd weeks following antigen exposure by cardiac puncture after anaesthetizing mice with chloroform. The serum was separated for each group separately and kept at -20^{0}° C till analyses. Heparin was used in collecting whole blood.

Immunization and bleeding

These test animals were divided in 2 groups for stimulation of antigens and 1 group for non stimulation was used as control. For stimulation with antigen the mice were immunized with whole cell bacterial antigens, suspended in normal saline (0.15 M). Approximately primary and secondary

immunization should be given. Non stimulation group was not administrated with any antigen used as control. Blood sample of stimulated and non-stimulated mice were collected after 3rd weeks following antigen exposure by cardiac puncture after anaesthetizing mice with chloroform. The serum was separated for each group separately and kept at -200°C till analyses. Heparin was used in collecting whole blood.

Analysis of immune response

Host defenses that are mediated by antibody present in the plasma, lymph and tissue fluids, it protects against extra cellular bacteria and foreign molecules- transfer of antibodies confers this type of immunity on recipient. In the present study humoral immune response was analyzed by antibody titration, B cell e-rosette assay and isolation of antibody techniques.

Screening of antibody

From the normal and antigens injected mice, serum sample taken, the antibody levels were estimated. Quantitation of serum antibodies were carried out by antibody titre plate technique (Mitra *et al.,* 1999) containing respective antigens. 25µl of physiological saline was added into all wells of microtitre plate, and then 25µl of antiserum added into the first well of microtitre plate, the antiserum was serially diluted in the well of the first row till the 11th well of the microtitre plate leaving the 12th well as positive control. Then 25µl of 1% test antigen in saline were added to all the wells of the microtitre plate. The plate was hand shaken for the effective mixing of reagents and incubated for an hour at 37^0 C. The highest dilution of serum samples which shows detectable agglutination was recorded and expressed in log 2 of the serum.

e- rosette assay

Blood cells collected from test antigen and control mice using heparin pretreated vials. B-cell counts in the blood were carried out by nylon wool method. Three ml of the lymphoprep solution was taken in a centrifuge tube and over lay the blood and centrifuged at 1600 rpm for 20 minutes. The interphase containing lymphocytes were loaded into activated nylon wool column. Then the column was held vertically above an eppendorf tube, now hot saline was passed and T cells are eluted to eppendorf tube followed by this cold saline was poured and column was gently squeezed to release the adhered B-cells and repeated twice. The cold saline dripping out of the column was collected in another eppendorf tube. 0.2 ml of the saline containing B lymphocyte was taken in a separate eppendorf tube. To this 0.2 ml of 1% SRBC was added and then the mixture was centrifuged for 12 minutes at 1600 rpm. After centrifugation the samples were incubated in an ice box or refrigerator at 4^0 C for 5 minutes. After cold incubation the pellet in the eppendorf tube was resuspended by gentle flushing with a Pasteur pipette. Then a drop of it was taken in a clean dry slide to observe and enumerate B-cells under microscope (20x\40x). Number of B-cell rosettes formed was observed per hundred lymphocytes observed.

RESULTS AND DISCUSSIONS

Evaluation of immunomodulate effects

The LD_{50} value of *Laurus nobilis* plant materials was found to be 17.5 g / kg body weight. The plant materials of this plant did not cause any mortality when administered up to a dose of 5g / kg body weight. At this dose there were no gross behavioral changes. In normal experimental animals, the final body weight in control group was significantly increased than at the beginning of the experiment on the other hand, the administration of alloxan, extracts induced also a significant increase than the initial body weight.

Immunological Assay

T Cell Count

The T cells from plant extract injected mice showed in Table 1. Percentage of T cells counts were increased compared to control.

B cell count

The B cells from plant extract injected mice showed non significant observations. Tore *et al.,* (2003) studied erythrocyte rosette inhibition as an assay and it used to detect naturally occurring lymphocytotoxic antibodies in sera from patients with systemic lupus erythematosus (SLE). The incidence of lymphocytotoxic antibodies was 86% in all

systemic lupus erythematosus patients. Peter Hokland *et al.,* (1992) studied that mouse erythrocyte rosette formation with malignant human B-lymphocytes and the result showed of B cell was 5%. From the study the plant extract never act as carcinogenic, it acts as immunostimulant.

Antibody titration

The highest rank number of the serum was considered as an antibody titre.The haemagglution antibody titre values for the antigen was observed In *Staphylococcus aureus* antibody titre value is 6 $\log_2 2$, *Streptococcus pyogenes* is 7 $\log_2 2$, *Salmonella typhi* is 8 $\log_2 2$, *Bacillus substilis* is 4 $\log_2 2$ and *Shigella sonnei* is 7 $\log_2 2$. The antibody titre value is higher than normal mice. The effective solvent plant extracts injected into mice and that produce antiserum in mice and that antiserum react with the bacterial antigen by haemagglutination method. Here, the plant extract modulates the mice immune system to resist the pathogenic bacteria. Some of the plants such as Ethanol, Hexane and Ethanol Fraction 7 act as immunomodulator or immunostimulator. Sasikumar *et al*., (2003) studied antibody Titres against Canine Distemper Virus in Vaccinated and Unvaccinated Dogs. The results showed that the mean antibody titre in vaccinated dogs (114 ± 8.2) indicated immunity and was significantly higher when compared with unvaccinated animals (29 ± 8.8) or with dogs with unknown vaccination history (29 ± 5.0).

Differential count

The different cells present were counted with the help of a microscope. Various cells like neutrophils, eosinophils, basophils, monocytes, large lymphocytes and small lymphocytes were counted (Table 2).

Figure 1
Immunization of animals with plant extracts

Table 1
Enumeration of T-cell and B-cell using Erythrocyte rosette forming assay on mice

S.No	sample	Lymphocyte population		sub-set
		B-CELL	T-CELL	
1.	NORMAL	27.3%	58%	
2.	TREATED	27.3%	**72.7%**	

Table 2
Differential count of blood sample in plant extract administered animal

S.No	Sample	Percentage of differential cells				
		Neutrophil	Basophil	Monocyte	Large Lymphocyte	Small lymphocyte
1.	NORMAL	40%	10%	20%	10%	20%
2.	TREATED	27.3%	18.2%	18.2%	18.2%	18.2%

CONCLUSION

The plant extracts were showing immunostimulant activity in host system. The antibody levels were increased when treated with the plant extract showing the healing effect of the plant extracts in host system. The increment of the T cells was indicated the cellular level of immune response induced by plant extracts.

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