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# ANALYSIS OF IMMUNOLOGICAL EFFICIENCY OF *TAMARINDUS INDICA* USING FISH MODEL

**Thangaraj A[1*]., Suganthi R[2] and Dhasarathan P[3]**

[1] Department of Plant Biology & Plant Biotechnology, Dr. Ambedkar Govt. Arts College, Vyasarpadi, Chennai-37
[2]Department of Biotechnology, St. Peter's University, Avadi, Chennai – 600 054
[3]Department of Biotechnology, Prathyusha Engineering College, Tiruvallur – 602025

**A R T I C L E I N F O**

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**A B S T R A C T**

Medicinal plants have an important role in normal life. The plants immunostimulant activity was screened using animal model. The efficiency of immunostimulant was analyzed antibody titration, B and T cell counts and lymphocyte migration assay method. When the result is normal experimental animal, the plant extract increased T cell counts compared to B cell counts and antibody level. From the result suggested the chosen plant ext5rach have potential to develop immunomodulatory effect in host.

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## INTRODUCTION

Indian plants are most effective and most commonly studied in relation to diabetes and their complications are *Allium cepa*, *Allium satium, Aloe vera*, *ociumum sanctum, Trigonella foenum.* Among these Sujatha *et al* (2010) have evaluated *M.charantia*, *Eugenia jambolana*, *Brassica juncea*. Medicinal Plants are having many secondary metabolites; they are potential source for formulation of drugs (Khatune *et al*., 2005). Plant based medicines are initially used in the form of tinctures, teas, poultices, powders, and other herbal formulations (Samuelsson, 2004).The plant based medicines are widely used because they are very low cost compared to synthetic drugs (Iwu *et al*., 1999). The effects of plants inhibit growth of micro-organisms and are important for human health (Erdogrul, 2002). Of the reasons in the development of resistance to chemotherapeutic agent is due to abuse of these drugs (Reuters, 2005).Hence in the present investigation screened immunomodulatory efficiency of *Tamarindus indica*.

Identifications of drug from medicinal plants have traditionally been lengthier and more complicated than other research methods. Therefore, many pharmaceutical companies have eliminated or scaled down their natural product research (Koehn and Carter, 2005). *T. indicia* was also reported to possess antimicrobial activity (Ahmed *et al.*, 2006). Reports related to antimicrobial activity of this plant using various organic solvents have not been thoroughly studied. Based on the thorough scrutiny of scientific literatures, no scientific information on bioactivity of *T. indica* was found. This study, thus presents the influence of various extracts of *T. indica* against bacterial pathogens.

## MATERIALS AND METHODS

### *Immunization of animals*

For the experimental study, fish weighing 24 ± 0.2 gm (35 days old) were recruited from the acclimatized stock. Fish 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. Test bacterial antigens were given through intramuscular injection at optimum levels with primary and secondary doses, along with standard pellet feed given every day in *ad libitum*

### *Immunization of animals with plant extracts*

For the experimental study, fish weighing 24 ± 0.2 gm (35 days old) were recruited from the acclimatized stock. Fish 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

**Corresponding author:* **Thangaraj A**
Dept. of Plant Biology & Plant Biotechnology, Dr. Ambedkar Govt.Arts College, Vyasarpadi, Chennai-37

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 fish. Blood sample of stimulated plant extract fish were collected after 3rd weeks following antigen exposure by cardiac puncture after anaesthetizing fish 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.

***Immunological Assay***

The immunological assay should be followed collecting blood in both antigens injected and plant extracts injected fish. The effective plants extract solvent in the antibacterial activity was injected into fish.

***Screening of antibody***

From the normal and antigens injected fish, serum sample taken, the antibody levels were estimated. Quantitation of serum antibodies were carried out by antibody titre plate technique 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 titre of the serum.

***B and T cell erythrocyte rosette assay***

Blood cells collected from test antigen and control fish using heparin pretreated vials. T-cell counts in the blood were carried out up to loading of lymphocyte in nylon wool column. Resuspended 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.

***Lymphocyte Migration assay***

Agar plates were prepared with 1.5% agarose. It was overlaid with a layer of antigen, here BSA was used. Serum was taken in a capillary tube, up to a marked level. This tube was placed vertically above the antigen in such a way that a contact was established between the Ag and the serum. Increase or decrease of serum level in the tube was noted.

## RESULT AND DISCUSSION

The $LC_{50}$ value of *T. indica* 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 antigen, extracts induced also a significant increase than the initial body weight.

The highest rank number of the serum was considered as an antibody titre. The haemagglution antibody titre values for the antigen was observed and shown in the table 1. In normal animal antibody titre value was 3 $\log_2 2$, Plant extract treated animal was 5 $\log_2 2$ and Plant extract and BSA treated animal was 7 $\log_2 2$. The antibody titre value is higher than normal fish. The effective solvent plant extracts injected into fish and that produce antiserum in fish and that antiserum react with the bacterial antigen by haemagglutination method. Here, the plant extract modulates the fish immune system to resist the pathogenic bacteria. Some of the plant such as Ethanol, Hexane and Ethanol Fraction 7 act as immunomodulator or immunostimulator. Several Indian medicinal plants have been exploited to enhance antibody mediated immune responses (Ranjith Singh *et al*., 2004 and Nafisa Hassan Ali *et al*., 2008). Several modulations of immune responses to alleviate the disease have been in interest for many years and the concept of "Rasayana in Ayurveda is based on related principles" (Sharma *et al*., 1994).

**Table 1** Analysis of antibody titre value of normal and *T.indica* treated fish

| S.NO | TEST SAMPLE | ANTIBODY TITRE $\log_2 2$ |
|---|---|---|
| 1 | Normal | 3 $\log_2 2$ |
| 2 | P.E. | 5 $\log_2 2$ |
| 3 | P.E. + B.S.A | 4 $\log_2 2$ |

The B and T cells counts from antigen injected fish and plant extract injected fish were observed and recorded in table 2.

**Table 2** Enumeration of T-cell and B-cell using Erythrocyte rosette forming assay on fish

| S.NO | SAMPLE | LYMPHOCYTE SUB-SET POPULATION | |
|---|---|---|---|
| | | B-CELL | T-CELL |
| 1 | NORMAL | 32% | 68% |
| 2 | TREATED | 27.3% | **72.7%** |

The Percentage of B cell was not shown any significant effect. T cells were increased (from 68% to 72.7%) in *T. indica* extracts treated animal. The increment in 'T' cell count may be due to the impact of plant drug on the synthesis, proliferation and activation of 'T' cells in treated animals. Similar results were observed by Paulsi and Dhasarathan (2011) in mice administered with plant extracts. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, especially when host defence mechanism has to be activated when the conditions of impaired immune response or when a selective immunosuppresion is despaired in situations like auto

immune disorders. There is a great potential for discovery of more specific immunomodulators which mimic antagonize the biological effect (Goldsby *et al.*, 2002 and Sujatha *et al 2010*).

Agar plates were prepared with 1.5% agarose. It was overlaid with a layer of antigen, here BSA was used. Serum was taken in a capillary tube, up to a marked level. This tube was placed vertically above the antigen in such a way that a contact was established between the Ag and the serum. Increase or decrease of serum level in the tube was noted (Table 3).

**Table 3** Estimation of lymphocyte migration
On normal and *T.indica* treated fish

| S.NO | SAMPLE | Lymphocyte migration | |
|---|---|---|---|
| | | INITIAL ( $x10^{-2}$ m) | FINAL ( $x10^{-2}$ m) |
| 1 | NORMAL | 2.0 | 1.9 |
| **2** | TREATED | 2.0 | 1.7 |

## CONCLUSION

Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, especially when host defence mechanism has to be activated under the conditions of impaired immune response or when a selective immunosuppression is desired in situations like autoimmune disorders. There is great potential for the discovery of more specific immunomodulators which mimic or antagonize the biological effects of cytokines and interleukins, and the refinement of assays for these mediators will create specific and sensitive screens. Natural remedies should be revisited as important sources of novel ligands capable of targeting specific cellular receptors.

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