Research Article

A Research on Immunomodulatory Potential of Methanolic Extract of *Saraca indica* Bark, an *In-vivo* Approach

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ABSTRACT

***Objective*:** Evaluation of immunomodulatory activity of methanolic extract of *Saraca indica* bark by using wistar albino rats.

Methods: Different methods were used for this purpose, starting from collection of *Saraca indica* bark, then preparation of alcoholic extract of dried bark and ended with evaluating different parameters to determine the immunomodulatory activity of test drug.

Results: The results of this study showed stimulatory effect on the humoral antibody titer, delayed type hypersensitivity (DTH) response, total serum protein and also showed significant increase in total leukocyte count (TLC) values.

Conclusions: Methanolic extract of bark of *Saraca indica* (Dosages – 250mg/kg and 500mg/kg (P.O), showed moderate immunomodulatory activity, as compared to the standard drug.

Keywords: Immunomodulatory, *Saraca indica*, Methanolic extract, Delayed Type Hypersensitivity (DTH), Humoral antibody (HA) titer and Total Leukocyte Count (TLC).

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INTRODUCTION

The immune system is the body's defence mechanism for preventing or restraining infection. Without this defence, the body would be more susceptible to the attacks from bacteria, viruses; parasites etc. [1] The immune system contains a complex network of cells, organs, proteins, and tissues all over the body. It is designed to protect the host from invading pathogens and to eliminate disease. A fully functional immune system can distinguish healthy tissue from unwanted substances. If it detects an unwanted substance, it will mount an immune response - a complex attack to protect the body from invaders. It also recognizes and removes dead and faulty cells.[2]Mainly three kinds of immunity are found in humans. They are innate immunity, adaptive immunity and passive immunity. Innate immunity is that kind of immunity, which we are born with like skin-external barrier of our body. Adaptive immunity is that kind of immunity that we acquire over the time against specific pathogens. And the last one is passive immunity that is temporary and comes from another person like a new born receives antibodies from the mother through the placenta before delivery.[2,3]

And now, the substances which can modify the immune system's response towards the threats in a good and effective way are called as Immunomodulators. They act either by increasing the immune response (Immuno stimulants) or by decreasing it (Immunosuppressants). [4]

Traditional Indian systems of medicines like Siddha and Ayurveda have suggested means to increase the body's natural resistance to disease. A number of Indian medicinal plants and various 'Rasayanas' have been claimed to possess immunomodulatory activity. [5,6] Herbal drugs possess immunomodulatory property and generally act by stimulating both specific and non-specific immunity. [7]

According to the World Health Organization (WHO), about three-quarters of the total world population relies upon herbal remedies for the health care. In fact, herbs and/or plants are the oldest friends of mankind. They not only provided food and shelter but also served to cure different diseases.[8] Herbs having immunomodulatory properties are relatively recent concept in phyto medicine. These plants provide alternative approach to conventional chemotherapy for a variety of illness.[9] Natural products provide an excellent material for the discovery and development of novel immunomodulatory compounds. Herbal medicine, sometimes called traditional or natural medicine, has always existed in one way or another in different cultures and civilizations, such as Ayurvedic (India), Egyptian, Western, Chinese, Kampo (Japan) and Greco-Arab or Unani- Tibb (south Asia). [10]

MATERIALS AND METHODS

1. Procurement of Plant Material

For this study, the bark of *Saraca indica* was collected from ENVIS Centre of medicinal plant Yelahanka Bangalore, Karnataka. The plant material was taxonomically identified

and authenticated by Dr. K. Madhava Chetty, Plant Taxonomist (IAAT: 357), Assistant Professor, Dept. Of Botany, Sri Venkateswara University, Tirupathi – 517502, A. P, India (Voucher number – 0635). After collection bark was cleaned, washed and dried under shade at room temperature. Later the barks were grinded into powder and passed through mesh size no. 50. The powdered samples were stored in a closed sterile glass container to keep free from environmental contaminants.

2 Preparation of Plant Extract

Extraction was done with methyl alcohol using hot continuous extraction method.The powdered sample of *Saraca indica* bark (150gm) was extracted using 500ml. of methanol (80%) in Soxhlet apparatus for 48 hours, maintaining a constant temperature of about 45-50°C.The apparatus was intermittently shaken and the extracts was filtered and evaporated to dryness to obtain solid mass, which was stored for further use. The percentage yield of the extract was 8.9 %.[11]

3 Preliminary phytochemical screening of extract

Preliminary phytochemical analysis was carried out to check and identify the active constituents of the methanolic extract of *Saraca indica* bark such as alkaloids, carbohydrates, flavonoids, terpenes and steroids, saponins, and tannins by using test methods of Draggendorff's and Mayer's test, Molisch's and Fehling's test, lead acetate and magnesium ribbon test, Liebermann–Burchard test, foam formation test, ferric chloride test, and gelatin test, respectively (Trease and Evans, 1983).

4. Experimental Animals

Wistar albino rats (150-200 g) of either sex obtained from Animal House, Aditya Bangalore Institute of Pharmacy Education and Research, Yelahanka, Bengaluru, were used for the study. The animals were housed in cages at room temperature and moisture, under the naturally illuminated environment of a 12:12 h dark/light cycle. They were fed on a standard diet and had free access to water. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee on 4/12/2021 and it was approved by the same before beginning the experiment (Protocol Proposal no. 68/161/CPCSEA).

5. Acute toxicity study

Acute toxicity test was done on plant extracts after the animals had fasted overnight while only taking water. The weight of each mouse was recorded before administering the extract. Randomly the animals were divided into control and treatment groups, each group consisting of six mice. The control group received only the vehicle (1% Tween 80) and each treatment group received orally the 80% methanol extracts of *Saraca indica* dose of 1000, 2000, and 5000 mg/kg orally. Animals were kept under close observation for explicit toxicities and behavioral changes like restlessness, tremor, diarrhoea, sluggishness, loss of weight, and paralysis at regular intervals for the first four hours after administering the extract, and then they were observed daily for two weeks for any change in general behavior and other physical activities. Food was available after four hrs of administration of the extracts. [12]

6. Experimental Design

Rats were randomly allocated into 5 groups (n=6). Group I which served as negative control received normal saline (10 mL/kg), group II is positive control, group III served as standard received Levamisole (50 mg/kg,i.p) and group IV & V received (250 and 500 mg/kg orally) of the *Saraca indica* bark extract for 14 days. Animals of group (II, III, IV & V) were treated with an immunosuppressant (cyclophosphamide) at doses of 200 mg/kg bwt on day 0 of the study by subcutaneous injection. Blood samples were collected on the 14th day of experiment by retro orbital puncture and haematological parameters were studied for RBC, Hb %, Platelets, total WBC counts and differential leucocytes counts (DLC). Statistical analysis data were expressed as mean ± SEM and differences between the groups were statistically determined by analysis of variance followed by Dunnet's test.

7. Parameters to Determine Immunomodulatory Activity

- Delayed type hypersensitivity (DTH) response
- Humoral antibody (HA) titer
- Total leukocyte count
- Determination of total serum protein

Delayed type hypersensitivity (DTH) response

For the evaluation of delayed type of hypersensitivity (DTH) test animals were divided in to five groups, having six animals in each. Delayed type hypersensitivity was induced in rats using Sheep Red Blood Cells (SRBCs). On 10th day 0.1ml of SRBC solution was injected subcutaneously in to the right footpad. After 24, 48, 72, 96 hrs, thickness of footpad was measured by plethysmometer.

The difference in the thickness of the right hind paw and the left hind paw was then used as a measure of DTH reaction and was expressed as a mean percent increment in thickness/edema.

$$\frac{\text{[Left foot pad challenged with antigen−Right foot pad control]}}{\text{Left foot pad challenged with antigen}} \times 100$$

Humoral Antibody Titer

The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing 1×10^8 cells, intraperitoneally, on day 0 day. Blood samples were collected in micro centrifuge tubes from individual animals of all the groups by retro orbital vein puncture on 14th day. Blood samples were centrifuged and the serum separated. Antibody levels were determined by the hemagglutination technique.

Total Leukocyte Count (TLC)

Rats were anesthetized and blood was collected from retro-orbital plexus on the last day of the experiment. A 1-in-20 dilution of blood was prepared by the addition of 0.02 mL of whole blood to 0.38 mL of 2 % acetic acid. The suspension formed was mixed for two to three minutes to ensure complete RBC lyses than WBC were counted using Neubauer haemocytometer. The counting of WBC was performed in duplicate and the final cell count for each group of rats were expressed as the number of white blood cells per millilitre and calculated.

Total Serum Protein

Total Serum Protein was measured exactly 4 cc. of 10 per cent sodium hydroxide in a 10 ml standard flask and add 0.1 cc. of fresh serum with a Folin micropipette. Rinse out the pipette three times with sodium hydroxide solution. Mix by rotating and add 0.5 cc. of 1 per cent copper sulphate. Shake vigorously five to six times. Allow to stand for 25 minutes and absorbance read in a U.V Spectrophotometer at 540 nm.

Statistical Analysis

All the values are expressed as mean ± standard error of the mean (S.E.M) for groups of six animals, each data was analyzed by One way Analysis of Variance (ANOVA) and compared by using Tukey's Kramer multiple comparison test. P<0.05 was considered as significant.

RESULTS

1. Phytochemical screening

The main chemical constituents found in *Saraca indica* are alkaloids, carbohydrates, glycosides, phenols, phytosterols, thiols, gums, mucilage, flavonoids, terpenes, steroids, proteins, tannins, resins.

2. Acute oral toxicity study

Acute toxicity study revealed that the bark extract of *Saraca indica* caused no mortality in both doses (2 g/kg) within the first 24 h as well as for the following 14 days. Physical and behavioral observations of the experimental rats also indicated no visible signs of overt toxicity like lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhoea and convulsion. This suggests that $LD_{50}$of the extract is greater than 2 g/kg.[13]

3. Immunomodulatory Activity

Delayed Type Hypersensitivity Response: The effect of test extract and standard drugs on the DTH response in wistar rats using SRBCs as antigen, administration of methanolic extract of *Saraca indica* bark at the dose of 250mg/kg and 500mg/Kg and Levamisole 50mg/kg with Cyclophosphamide 200mg/kg after 24, 48, 72, 96 hrs showed significant increase in paw edema compared to control group.

Table 1: Effect of methanolic extract of *Saraca indica* bark on delayed type of hypersensitivity response

SL. NO.	Groups	PAW VOLUME (mm)			
		24hr	48hr	72hr	96hr
1	Group I (Negative control)	1.42±0.026	0.71±0.018	0.40±0.012	**0.15±0.011**
2	Group II (CYP)	1.45±0.013	0.82±0.016	0.47±0.018	0.17±0.009
3	Group III (Lev 50 mg/kg)	1.57±0.009	1.01±0.028	0.64±0.020	0.31±0.015
4	Group IV (SEE250 mg/kg)	1.49±0.010	0.87±0.017	0.50±0.014	0.19±0.020
5	Group IV (SEE500 mg/kg)	1.53±0.015	0.92±0.018	0.56±0.011	0.22±0.013

Values represent mean ± Standard Deviation (SD)

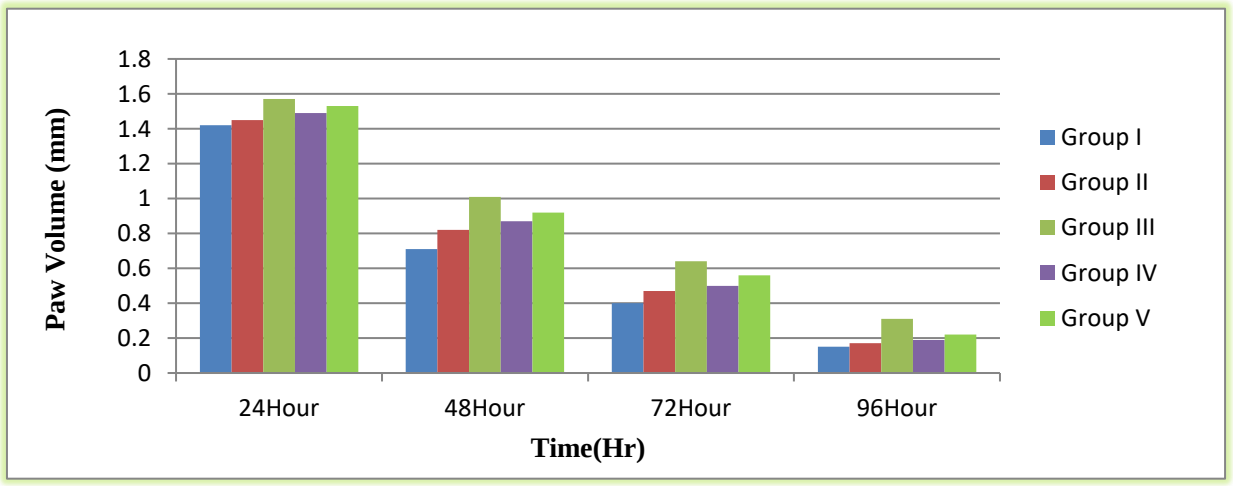

Figure 1: Delayed Type Hypersensitivity Response

Humoral Antibody Titer

Administration of methanolic extract of at the dose of (250 & 500 mg/kg) and Levamisole 50mg/Kg with Cyclophosphamide 200mg/Kg which were given orally and via I.P. route showed highly significant increase in antibody titre values compared to control group. The results are shown in table 2.

Table 2: Effect of crude methanolic extract of *Saraca indica* bark on Humoral Antibody titer

SL. NO.	Group	Humoral Antibody Titre
1	Group I (Negative control)	12±1.0211
2	Group II (CYP)	278±1.3842
2	Group III (Lev 50 mg/kg)	462± 2.6862
3	Group IV (SEE 250 mg/kg)	338.47±2.0402
4	Group IV (SEE 500 mg/kg)	413±1.5011

n=6, humoral antibody titre value mean ± Standard Error Mean (SEM)

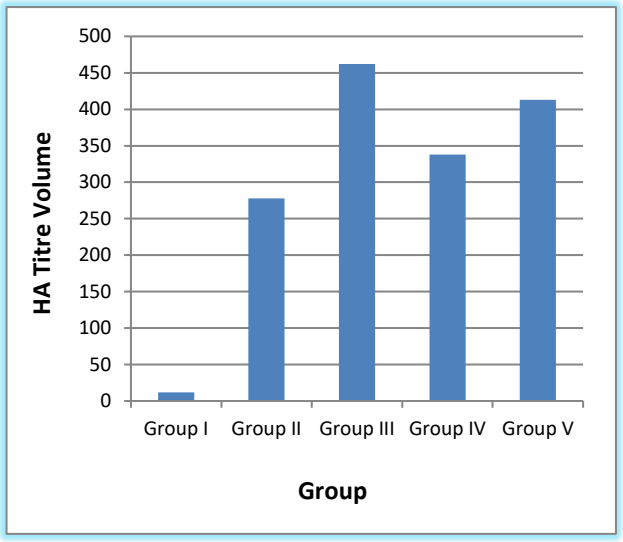

Figure 2: Humoral Antibody Titer

Table 3: Effect of crude methanolic extract of *Saraca indica* bark on Total Leukocyte count

SL. NO.	Group	Mean leukocyte count
1	Group I (Negative control)	5.01×10^3 cu.mm± 0.2640
2	Group II (CYP)	6.45×10^3 cu.mm± 0.0210
3	Group III (Lev 50 mg/kg)	15.01×10^3 cu.mm± 0.1381
4	Group IV (SEE 250 mg/kg)	6.93×10^3 cu.mm± 0.2461
5	Group IV (SEE 500 mg/kg)	9.56×10^3 cu.mm±0.3101

n=6. Mean Leukocyte count ± SEM

Total Leukocyte Count

The effect of test extract and standard drugs on Total Leukocytes in wistar rats, administration of methanolic extract of bark of *Saraca indica* at the dose of (250,500 mg/kg) and Levamisole 50mg/Kg + Cyclophosphamide 200mg/Kg which were given orally and via IP route. The low dose of extract (250mg/kg) show effect on TLC count compared to control group, whereas the 500mg/Kg and standard drug Levamisole 50mg/Kg with Cyclophosphamide 200mg/Kg showed significant increase in total leukocytes count values compared to control group. The results are shown in table 3.

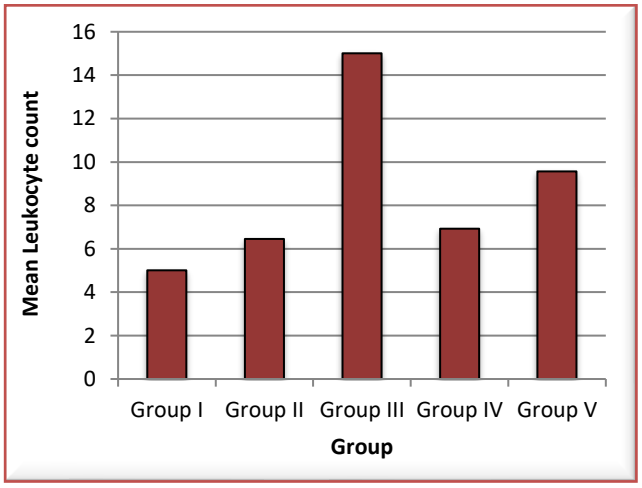

Figure 3: Total Leukocyte Count

Determination of Total Serum Protein

The effect of test extract and standard drugs on total serum protein in wistar rats, administration of ethanolic extract of *Saraca indica* at the dose of (250,500 mg/kg) orally and Levamisole 50mg/kg + Cyclophosphamide 200mg/kg treatments which were given intraperitoneally. The low dose of extract (250 mg/kg) and large dose (500 mg/kg) and standard drug Levamisole 50mg/Kg + Cyclophosphamide 200mg/kg showed significant increase in total serum values compared to control group. The results are shown in table 4.

Table 4: Effect of crude methanolic extract of *Saraca indica* bark on Total Serum Protein

Sl. No.	Group	Total serum protein (g/100 ml)
1	Group I (Negative control)	8±0.1202
2	Group II (CYP)	8.7±0.1420
3	Group III (Lev 50 mg/kg)	15.1±0.011
4	Group IV (SEE 250 mg/kg)	9.5±0.953
5	Group IV (SEE 500 mg/kg)	11.6±0.1013

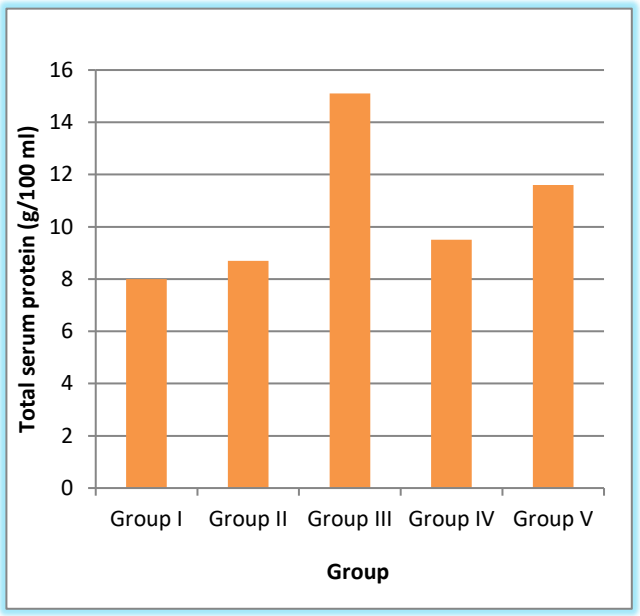

Figure 4: Total Serum Protein

DISCUSSION

In this study we found that methanolic extract of *Saraca indica* possess immunomodulatory activity in experimental models of cellular and humoral immunity. The extract was found to be effective at high dose (500 mg/ kg, p.o), whereas, lpw dose (250 mg/kg, p.o) was moderately effective in modulating immune system. The study was carried out using four different methods, each of which provides information about effect on different components of the immune system. The variety of plant products can modulate immune reaction either by stimulation or suppression and may assist as a supportive therapy along with conventional drugs in immune compromised patients.[13] Immunomodulators have therefore been used globally to control disease conditions. The study explored the immunomodulatory activity of the methanolic bark extract of *Saraca indica* by evaluating its effect on DTH reactions, hemagglutination antibody titers, white blood count and total serum protein. It is therefore used to assess the skin response following intradermal inoculation of the antigen which is dependent on antigen specific memory T-cells and the observed results were due to the recruitment of mononuclear cells and neutrophils. Activation of the T cells leads to the release of lymphokines which causes the activation and accumulation of macrophages, increases vascular permeability, induces vasodilatation and produces inflammation. It also produces a boost in phagocytic activity and increases the concentration of lytic enzymes for more effective killing of microorganisms. This results in the net increase in the thickness of the foot pad in previously immunized animals. This increment in footpad thickness of the Wistar albino rats that were subjected to extracts in this study could be attributed to the ability of the extract to activate lympohcytes and their accessory cell types leading to enhancement in the production of antibodies in the previously immunosuppressed animals thereby increasing cell-mediated immunity.

Humoral antibody titer assay is one of the key parameters used to assess the humoral immune response of the animal. As the antigen is expected to induce the production of antiserum against it, in the present study sheep red blood cells were used to elucidate the production of antibody against RBC. In an individual where immune system is primed antibody against a particular antigen is expected to be at higher titer. Accordingly in the present study a very high humoral antibody titer was recorded for group IV and group V individual which received the lowest and highest concentration (250 mg/kg and 500 mg/kg) of test drug. [14] The study showed that up to 500mg/kg of the crude drug could enhance the humoral immune response.

One of the earliest immune responses can be seen and measured by studying the hematological parameters of an animal. Accordingly, parameters like total leukocyte count for control group as well as group which received various concentrations of drug. Blood cells are the first cells to be responding to invading non-self-materials.[15] An immunomodulatory effect of any immune substance would first see as a change in leukocyte count. In the present study group III, which received standard drug showed highest leukocyte count of 15.01×10^3 cu.mm showing the initial triggering of blood cell to mount a potent immune response. The results showing standard drug concentration are better to elicit good immune response than test concentrations (250 mg/kg, 500mg/kg) of drug administered. The results are further strengthened with highest percentage of neutrophil being circulated in the group.

Serum protein is one of the earliest indicators of normal serum chemistry of an individual. A change in serum protein concentration and albumin ratio would hint us about the altered immune response status of the individual. Accordingly in the present study serum protein level is found to be similar in case of control and in group II animals. But in higher concentration (500 mg/kg) of the drug and in the lower concentration 250 mg/kg of drug groups showed increase in serum protein showing that higher immune response might have contributed to the serum protein in terms of different molecules such as immunoglobulin and other humoral factors. The serum protein increased in case of levamisole 50 mg/kg in combination with cyclophosphamide 200mg/kg administered by I.P. route.[16]

CONCLSION

Recently phytopharmaceutical research received much attention to develop safe and effective lead compounds with potential immunomodulatory activity. The present study was planned to evaluate the immunomodulatory activity of methanolic extract of bark of *Saraca indica.* Based on the findings from the study, the methanolic leaf extract of *Saraca indica* increased both the cell-mediated and humoral immune responses in rats. This could be attributed to the different macronutrients, micronutrients and phytochemicals present in the plant. More phytoconstituents can be isolated from the active fractions employing variety of solvent systems which can further be

investigated for their effect on immune system in experimental animals employing more detailed and specific tests like cell proliferations, natural killer cell assays etc. This type of approach will get us closer to the mechanism of actions of *Saraca indica* bark which can further be correlated with other medicinal properties of these barks.

As per the results of this study, it can be concluded that methanolic extract of bark of *Saraca indica* showed moderate immunomodulatory activity compared with standard drug.

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