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## RESEARCH ARTICLE

# EVALUATION OF IMMUNOMODULATORY ACTIVITY OF ETHANOL EXTRACT OF *ASYSTASIA TRAVANCORICA* BEDD (ACANTHACEAE)

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**ABSTRACT**

*Asystasia travancorica* Bedd an ethnomedicinal plant was studied for its immunomodulatory activity. Immunomodulatory activity of different doses of ethanol extract of *Asystasia travancorica* was evaluated in Swiss albino mice. Mice were treated with two doses (200 and 400mg/kg body weight) for 5 days. Body weight, relative organ weight, delayed type hypersensitivity (DTH) response and Haemagglutinin titre (HT) were studied in various groups of animals. The results obtained show a significant increase ($p<0.05$) in body weight and relative organ weight of spleen, liver and kidney at dose of 400mg/kg. The *Asystasia travancorica* extract eliciated a significant increase ($p<0.05$) in the DTH response at dose of 400mg/kg. In the HT test, the plant extract showed a stimulatory effect at all doses. The doses of 400mg/kg significantly ($p<0.05$) increases the WBC count, compared with the control group. Overall, *Asystasia travancorica* showed a stimulatory effect on both humoral and cellular immune functions in animal models.



## INTRODUCTION

The immune system is involved in the etiology as well as pathophysiologic mechanisms of many diseases. It can be modulated and this involves induction, expression, amplification or inhibition of any part or phase of the immune response (Alamgir and Uddin, 2010).

There exist two types of immunomodulators based on their modes of action; immunosuppressant which suppress the activity of the immune system and immunostimulators which stimulate its activity. There has been a growing interest in identifying and characterizing natural compounds with immunomodulatory activities. Medicinal plants are a rich source of substances which are claimed to induce paraimmunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions (Sainis *et al.*, 1997). Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades (Fong, 2002). A number of plants used in traditional medicine have been shown to possess immunostimulating activities acting as different levels of the immune system (Lee *et al.*, 2009; Mouokeu *et al.*, 2013; Odette *et al.*, 2013)

*Asystasia* includes approximately 70 species of perennial herbs and subshrubs from tropical Africa, India and Asia. *Asystasia* belongs to the family Acanthaceae. Paste of leaves and flowers of *Asystasia travancorica* mixed with honey is taken orally, twice a day, for three weeks for the treatment of rheumatism (Sutha *et al.*, 2010). The biological activities such as antiinflammatory, and anticancer were reported (Komalavalli *et al* 2014a,b). There was no reports on the ability of *A.travancorica* (AT) whole plant on immunomodulatory activity. Hence, this study was taken up to investigate the immunomodulatory activity of the whole plant in mice.

## MATERIALS AND METHODS

### *Plant material*

The whole plant of *Asystasia travancorica* Bedd was collected from Natural forests of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin for further reference.

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### *Animals*

Study was conducted in Swiss albino female mice (20 - 25 g). The animals were bred and maintained under standard laboratory conditions (temperature 25 ± 2°C and light period of 12 h). The rats were fed with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

### *Treatment protocol*

The plant extract was administered i.p. for 5 days at doses of 200 and 400 mg/Kg body weight. The dose volume was 0.2 ml. The control animal group received the same volume of normal saline and left untreated. The animals were divided into four groups (Groups I - IV). Each group comprised of a minimum of six animals. The control group (Group I) was given normal saline and the treatment groups were given the whole plant extract of *A.travancorica* at the doses of 200 mg/kg and 400 mg/kg body weight (Groups II and III) for five days, respectively. Group IV mice were given dexamethasone at 20mg/kg body weight. The animals were humanized 24 h after the last dose. Body weight gain (percentage) and relative weight of kidney, liver and spleen (organ weight/100 g of body weight) were determined for each animal.

### *Assessment of humoral immune functions*

Animals within the experimental groups were challenged with 0.2 mL of 10% sheep red blood cells (SRBC), i.p., on the 10th day of the initiation of experiment. The haemagglutinin titre was also studied in these animals.

### *Haemagglutinin titre assay*

Haemagglutinin titre (HT) assay was performed as per the procedure given by Bin- Hafeez *et al*. (2001). On the fifth day after immunization, blood was collected from the heart of each mouse for serum preparation. Serial two fold dilution of serum was made in PBS (pH 7.2) in 96 - well microtitre plates and mixed with 50 µ L of 1% SRBC suspension in PBS. After mixing, the plates were kept at room temperature for 2 h. The value of antibody titre was assigned to the highest serum dilution showing visible haemagglutination.

### *Delayed type hypersensitivity response*

The delayed type hypersensitivity (DTH) response was determined using the method of Raisuddin *et al*. (1991). On the day of termination of the treatment with plant extract, animals were immunized with $1x10^8$ SRBC, subcutaneously. On the fifth day of immunization, all the animals were again challenged with $1x10^9$ cells in the left hind footpad. The right footpad was injected with the same volume of normal saline, which served as the trauma control for non specific swelling. Increase in foot pad thickness was measured 24 h after the challenge by using a dial clipper.

### *Assessment of haematological and liver marker enzymes*

Red blood cell (RBC) count, haemoglobin (Hb) content and White blood cell (WBC) count were measured from freely following tail vein blood. Total billirubin was determined as described by Balistrei and Shaw (1987). Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalo transaminase (SGOT) and alkaline phosphatase were determined by the method of King and Armstrong (1934).

### *Statistical analysis*

All values were expressed as mean ± standard error of mean (S.E.M) and comparison between the groups were made by Analysis of Variance (ANOVA). The data were analysed using the statistical analysis system SPSS (SPSS Software for windows release 10.0; SPSS Inc., Chicago IL, USA).

## RESULTS

After treatment with two different doses (200 and 400 mg/Kg body weight) of whole plant ethanol extract of *A.travancorica* for 5 days, the Swiss albino female mice were evaluated for immunomodulatory activity. Body weight, relative organ weight, delayed type hypersensitivity (DTH) and haemaggtutinin titre (HT) were studied in all the treated animal groups.

### *Effect of plant extract on Body weight and Relative organ weight*

In the present study treatment with the whole plant ethanol extract of *A.travancorica* was effective in increasing the body weight and also the weight of spleen, liver and kidney (Table1).

**Table 1** Effect of whole plant ethanol extract of *Asystasia travancorica* (AT) on the body weight and relative organ weight

| Treatment Groups | Treatment type and Dosage | Body weight and relative weight of organs (mean± SE) in g | | | |
|---|---|---|---|---|---|
| | | Body weight | Spleen | Liver | Kidney |
| Group I | Saline (Normal control) | 23.56±1.38 | 0.39±0.11 | 3.98±0.26 | 1.28±0.04 |
| Group II | AT extract (200 mg/kg b. wt.) | 28.15±1.84 | 0.49±0.26ns | 4.63±0.16 | 1.39±0.07 |
| Group III | AT extract (400 mg/kg b. wt.) | 29.40±1.58* | 0.88±0.18** | 5.90±0.11* | 1.69±0.08* |
| Group IV | Dexamethasone (20 mg/kg b. wt.) | 24.80±1.45 | 0.64±0.73* | 4.78±0.18 | 1.48±0.05 |

Each Value is SEM of 6 individual observations: *Comparison between normal control and drug treated groups. Level of significance: * $p$ <0.05; ** $p$ <0.01.

***Effect of plant extract on humoral immunity parameters***

In the haemagglutinin titre (HT) (Table 2), doses 200 mg and 400 mg/kg showed titre value of 4.88 and 6.94 respectively, while the titre value of control was 2.73, thus showing a significant increase in the titre values with doses of 200 and 400 mg/kg in the treated groups ($p< 0.05$).

Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotheraphy (Wagner and Proksch, 1983). Immunostimulation in a drug-induced immunosuppression and immunosuppression in an experimental hyperreactivity model by the same preparation

**Table 2** Effect of whole plant ethanol extract of *Asystasia travancorica* (AT) on DTH response in comparison with dexamethasone and on the HT titre by using SRBC as the antigen in mice

| Treatment Groups | Treatment type and Dosage | Parameter | |
|---|---|---|---|
| | | Foot Pad Edema (mm) | HT titre |
| **Group I** | Saline (Normal control) | 0.34±0.018 | 2.73±0.053 |
| **Group II** | AT extract (200 mg/kg b. wt.) | 0.48±0.012* | 4.88±0.054* |
| **Group III** | AT extract (400 mg/kg b. wt.) | 0.69±0.026** | 6.94±0.018** |
| **Group IV** | Dexamethasone (20 mg/kg b. wt.) | 0.53±0.018* | 5.84±0.03* |

Each Value is SEM of 6 individual observations: *Comparison between normal control and drug treated groups. Level of significance: * $p$ <0.05; ** $p$ <0.01.

**Table 3** Effect of whole plant ethanol extract of *Asystasia travancorica* (AT) on the haematological parameters and serum liver marker enzymes

| Treatment Groups Type and Dosage | Haematological parameters | | | Biochemical (serum) parameters | | | |
|---|---|---|---|---|---|---|---|
| | Hb (g/dl) | RBC ($x10^6/mm^2$) | WBC ($x10^6/mm^2$) | T Bilirubin (mg/dl) | SGOT (U/L) | SGPT (U/L) | ALP (U/L) |
| **Group I** Saline (Normal Control) | 14.01±0.94 | 3.50±0.16 | 6.13±0.65 | 0.57±0.06 | 36.22±2.13 | 34.29±1.84 | 131.64±4.61 |
| **Group II** AT extract (200 mg/kg b. wt.) | 12.60±0.16 | 4.12±0.74 | 7.36±0.74 | 0.63±0.08 | 41.86±2.63 | 43.65±0.96 | 146.31±6.16 |
| **Group III** AT extract (400 mg/kg b. wt.) | 11.46±0.24* | 3.24±0.65 | 8.93±0.16 | 0.76±0.07* | 49.33±0.84* | 47.68±0.73 | 156.88±5.17* |
| **Group IV** Dexamethasone (20 mg/kg b. wt.) | 12.55±0.19 | 3.96±0.28 | 8.84±0.18 | 0.74±0.01 | 47.65±0.16 | 51.33±1.62 | 184.27±2.67* |

Each Value is SEM of 6 individual observations: *Comparison between normal control and drug treated groups. Level of significance: * $p$ <0.05.

***Effect of plant extract on cell mediated immunity parameters***

The plant extract at dose of 400mg/kg elicited a significant ($p< 0.05$) increase in DTH response (Table 2), compared to the control animals. In this study, dexamethasone (Group IV) decreased DTH response, compared to the control group.

***Effect of plant extract on blood parameters and liver enzymes***

There was no significant elevation in the levels of SGOT, SGPT and ALP as a result of treatment with *A.travancorica* (Table 3). Total bilirubin content was slightly increased. No significant difference in blood parameters was recorded in various test groups. The doses of 200 and 400mg/kg increased the WBC count, compared with the control group.

## DISCUSSION

Modulation of the immune response through stimulation or suppression may help in maintaining a disease – free state.

can be said to be trace immunomodulation (Bamunnarachi and De Silva, 1989). The presence of immunostimulant compounds in higher plants has been extensively reviewed but only a limited amount of immunosuppressive products of plant origin have been reported.

The organ weight is an important indicator of the physiological and pathological state in humans and other animals. The relative weight of each organ of the immune system to the body weight of an individual is a commonly used index to reflect the developmental status of the organ (Dong *et al*., 2007). The increase in spleen weight observed in our experiments may be partly due to the stimulatory effect of the plant extract on the immune organ (Selgrade *et al*., 1982; Sharififar *et al*., 2009).

In the present study, ethanol extract of *A.travancorica* whole plant showed an overall stimulatory effect on the immune functions in mice. Stimulatory effect were observed on both humoral and cellular immunity. In HT test, the plant showed an increase response in all doses, but this increase was significantly only in dose 400 mg/kg. This activity could be due to the presence of flavonoids or saponins which augment

the humoral response, by stimulating the macrophages and B-lymphocytes subsets involved in antibody synthesis (Makare *et al*., 2001). It appears that 400 mg/kg is the optimum dose in mice in humoral immunity.

In the present investigation, SRBC-induced delayed type hypersensitivity was used to assess the effect of the fraction on cell-mediated immunity. Cell mediated immunity (CMI) involves effector mechanisms carried out by T-lymphocytes and their products (lymphokines) CMI responses critical to defence against infectious organisms, infections of foreign grafts, tumor immunity and delayed type hypersensitivity reactions (Miller *et al*., 1991). Therefore, increase in DTH reaction in mice response to T cell dependent antigen revealed the stimulatory effect of ethanol extract of *A.travancorica* on T cells. Thus, the immunostimulatory effect produced by ethanol extract of *A.travancorica* may be due to cell mediated and humoral antibody mediated activation of T and B cells.

The ethanol extract of *A.travancorica* enhanced the production of WBC and SGPT, SGOT and ALP. Results of the present study also revealed no significant difference in the other blood parameters. Findings of the present study establish that *A.travancorica* also have appreciable immunostimulatory activity.

The significant increase in the immunostimulatory activity of ethanol extract of *A.travancorica* could be attributed to the presence of flavonoids, alkaloids, tannins, saponins and phenolic compounds. Therefore, the plant holds promise for being used as an immunostimulating agent and an in-depth study on various fractions of the extract effective as immunomodulating entities from the plant is warranted to determine the most potent immunostimulating fraction from *A.travancorica.*

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