ANALYSIS OF CELL MEDIATED IMMUNE RESPONSES IN RODENTS EXPOSED TO STRYCHNOS POTATORUM

Arunthathi, K., Vasanthan, M and K.C. Aarthy

Dept. of Microbiology, Thassim Beevi Abdul Kader College For Women, Kilakarai-623517.

Dept. of Biotechnology, Madha Engineering College, Kundrathur, Chennai-69, India

ABSTRACT

To enhance cell-mediated and humoral immune response many drugs are being developed every day. In this search, plant drugs are no exception for a long time. In the present study, the immunomodulatory effect of the plant S. potatorum, on the dynamics of the SRBC antibody response, was assessed in a mice (Swiss albino) model. Animal maintined in standard conditions and fed with a balanced pellet diet (Lipton, India Ltd) and tap water ad libitum. Mouses were divided into nineteen groups, each group containing six mice and separate control and immunized control groups were maintained. In the present study, the T-cell response (CM1) level in the form of DTH response in experimental mice was assessed after administering the selected medicinal plant extracts and immunosuppressive drugs.

Keywords: Immunity, *S. potatorum* and humoral immune response.

INTRODUCTION

Immune stimulation and immune suppression are maintained for the proper function of immune system. Some example of Immunostimulation and immunosuppression agents are natural adjuvants, synthetic agents, and antibody reagents, but usage of this agents has some limitation because sometimes it increased infection level and also affect the whole immune system. There are a number of plants that have been reported to have immunomodulatory activity (Puri 2003). The immune system defends the body against invasion by infectious agents in two ways. They are humoral immunity (HI) and cell-mediated immunity (CMI). Hence, the present study is designed to investigate the changes in the immune system in mice administered with plant extracts.

Cell-mediated immunity

If the symptoms for hypersensitive reactions are expressed after days of the antigenic challenge, it is called Delayed-type hypersensitivity reactions. In delayed-type hypersensitivity (DTH) some subpopulations of activated TH cells when encountering some types of antigen, they secrete cytokines that induce a localized inflammatory reaction. In DTH reaction the antigens are injected into animal , so that the animal produce specific Th-1 cell cells for the particular antigen it indicates the positive test reaction On injection of antigen, DTH response is diagnosed based on the development of a red, slightly swollen, firm lesion at the site of injection between 48 and 72 hours later. The skin lesions result from intense infiltration of cells to the site of injection during a DTH reaction; 80% - 90% of these cells are macrophages (Goldsby *et al*., 2003). Although the rodents may exhibit different pharmacokinetic properties than in humans, rodents still appear to be the most appropriate model for examining the immunotoxicity of non-species specific compounds (Dean *et al*., 1994).

To enhance cell-mediated and humoral immune response many drugs are being developed every day. In this search, plant drugs are no exception for a long time. Herbal products are extensively used and tested for antimicrobial activities and other studies. But their role in immunity-related studies needs more information. In the search for new immunomodulating agents over the past few years, many traditional herbal medicines have been evaluated (Ranjitsingh *et al.,* 2004 and Arunthathi et al., 2018). In this search for new plant treasure to enhance immunity, traditionally used medicinal plant *S. potatorum* was thoroughly studied in this work.

METHODOLOGY

In the present study, the immunomodulatory effect of the plant *S. potatorum*, on the dynamics of the SRBC antibody response, was assessed in a mice (Swiss albino) model. Animal maintined in standard conditions and fed with a balanced pellet diet (Lipton, India Ltd) and tap water *ad libitum*. The lab chamicals were purchased in standard company and animals were grouped for study. Mouses were divided into nineteen groups, each group containing six mice and separate control and immunized control groups were maintained. Drugs were given to various groups i.e. Group I- Control (sterile water), Group II-Immunised control, Group III - *S. potatorum* hexane extract treated group (*100 mg/kg),* Group IV - *S. potatorum* butanol extract treated group (*100 mg/kg),* Group V - *S. potatorum* ethanol extract treated group (*100 mg/kg),* Group VI- *S. potatorum* chloroform

extract treated group (*100 mg/kg),* Group VII - *S. potatorum* water extract treated group (*100 mg/kg),* Group VIII - *S. potatorum* hexane extract treated group (*100 mg/kg)* and alloxan *(30 mg/kg)* treated group Group IX - *S. potatorum* butanol extract treated group (*100 mg/kg)* and alloxan *(30 mg/kg)* treated group. Group X - *S. potatorum* ethanol extract treated group (Dose levels of *100 mg/kg)* and alloxan *(30 mg/kg)* treated group. Group XI- *S. potatorum* chloroform extract treated group (*100* and alloxan *(30 mg/kg)* treated group. Group XII - *S. potatorum* water extract treated group (*100 mg/kg)* and alloxan *(30 mg/kg)* treated group. Group XIII - *S. potatorum* hexane extract treated group (*100 mg/kg)* and tolbutamide treated group (30 *mg/kg).* Group XIV - *S. potatorum* butanol extract treated group (*100 mg/kg)* and tolbutamide treated group (30 *mg/kg).* Group XV - *S. potatorum* ethanol extract treated group (*100 mg/kg)* and tolbutamide treated group (30 *mg/kg).* Group XVI- *S. potatorum* chloroform extract treated group (*100 mg/kg)* and tolbutamide treated group (30 *mg/kg).* Group XVII - *S. potatorum* water extract treated group (*100 mg/kg)* and tolbutamide treated group (30 *mg/kg).* Group XVIII - Alloxan alone treated group (30 *mg/kg).* Group XIX - Tolbutamide treated group (30 *mg/kg).*

T cell E rosette assay: Blood is collected from control and treated mice as previous by mentioned using a heparin pretreated vials T cell count in the blood were carried out by the nylon wool column method. The hot saline passing out of the column was collected in the eppendorf tube, which contains T lymphocytes. 2.0ml of the saline containing T cell was taken in an eppendorf tube. To this 0.2ml of 1%, SRBC was added and then the mixture was centrifuged for 12 min at 1600 rpm. After centrifugation, the sample was incubated in an icebox or refrigerator (at 4°C) for 5 min. After cold incubation, the pellet in the eppendorf till was re-suspended by gentle flushing with a Pasteur pipette. Then a drop of it was taken in a clean dry slide, observed and enumerated T cell under the microscope (20x/40x) for rosettes. No of T Cell rosettes formed were observed among hundred lymphocytes and tabulated.

Delayed type hypersensitivity: Delayed type hypersensitivity was studied by standard method for mice. The experimented mice (control and 7 days antigen exposed mice) were sensitized. A positive response is conventionally assessed as one giving ≤ 5 in durations. Responses can be graded with 3-4 mm = +; 5-8mm = ++, 9-11= +++; 12mm or more = ++++.Mice were sensitized by subcutaneous injection in the intranasal region with 0.5 ml of Freund's adjuvant containing 500 mg of intradermal injection to sterile phosphate buffer with a Vernier caliper prior to challenge, i.e. 0^{th},

3rd and 24th hour post challenge, each with three readings. The increase in skin thickness (MST) of mice was obtained after deducting the skin thickness of the same oil before a challenge. Overall MST was obtained by taking the mean of individual mice with the group.

RESULTS AND DISCUSSION

T cell production of control and treated animals were estimated by rosette forming assay and recorded in Table 1. The result showed significant changes in all kind of treated animals when compared to a control of five kinds of treatment, the increment in 'T' lymphocyte number was much pronounced in plant extract and immune enhancing drug combination followed by immune enhancing drug and plant pronounced in immunosuppressive drug followed by immunosuppressive drug combined with plant extract.

The plant extract significantly alters the DTH responses in mice exposed to SRBC. At the 24 hours of injection maximum enhancement of DTH response to SRBC was absorbed with plant extract and immunoenhancing drugs. The immunosuppressive drug administered mice showed fewer DTH responses against SRBC (Table 2 and 3) from the results it is clear that the plant extract induced immunomodulating potential. On administration of plant extracts an enhanced and visible DTH response was observed. The suppressing DTH responses by larger close of plant extracts compared with the work of kannan (2008) who have reported a suppressor of DTH responses in mice only when large doses of plant drugs were used.

Delayed-type hypersensitivity (DTH) is an expression of cell-mediated immune response which has been used to assess immunomodulatory mechanism in animals. The DTH assay is a simple and inexpensive method to assess the immune response. Immunosuppressive chemicals elevate DTH response by eliminating the population of T-suppressor cells (Amrite *et al*., 2006 and Hemamalini and Nirmala, 2014). The DTH response to antigenic challenge (SRBC – sheep Red Blood Cells) provides a useful system for the identification of compounds with respective effects on the immune response (Vane and Bolding, 1995). In the present study, the T-cell response (CM1) level in the form of DTH response in experimental mice was assessed after administering the selected medicinal plant extracts and immunosuppressive drugs.

The effector cells that promote DTH reactions (T DTH cells) cause the activation of macrophages, infiltration of polymorphonuclear cells, increased vascular permeability and edema, thereby it induced T-cell mediated response also observed an elevation in DTH response in mice treated with the plant extract (Patwardhan, 2000). Mice treated with alloxan (30 mg/kg) had a suppressed footpad thickness indicating the suppressive act on cell-mediated immunity. According to Gutai *et al*., (2002) the DTH response is associated with T cells and sensitized T-cells release mediators to promote inflammatory processes. The possible mechanism behind DTH reactions includes activation of complements releasing of mediators by activated most cells; kinin reactive oxygen or nitrogen species by benzofuranone metabolites histamine and pro-inflammatory cytokines (Khatune *et al*., 2005).

SUMMARY AND CONCLUSION

The results of the present study provide unequivocal evidence of the immunostimulant activity of the active principles of *S. potatorum* since the active principles obtained from these plants were influenced through the non-binding interactions it may be well connected with receptors of the immune system and this had elevated immune response.

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Table 1. Enumeration of T cells using rosette-forming assay in treated mice

Group	Number of T cells rosette formed in 100 lymphocytes observed		
	I week	*II week*	*III week*
Group - 1	55	57	56
Group – II	56	59	60
Group – III	45	46	47
Group -IV	48	48	49
Group -V	49	50	52
Group – VI	45	44	47
Group – VII	45	44	47
Group – VIII	43	44	45
Group – IX	44	44	45
Group –X	43	44	45
Group – XI	43	44	45
Group - XII	43	44	44
Group – XIII	43	44	49
Group – XIV	44	45	49
Group – XV	44	45	52
Group – XVI	43	44	49
Group – XVII	43	45	49
Group –XVIII	43	40	37
Group - XIX	45	49	52

Table 2. Evaluation of DTH response of different plant extracts of *S. potatorum* for three weeks

Group	DTH response - Time intervals (Weeks)		
	I Week	II Week	III Week
Group – 1	++	++	+++
Group – II	+++	+++	+++
Group – III	++	++	+++
Group -IV	++	++	++
Group -V	++++	++++	++++
Group – VI	+++	++	+++
Group – VII	++	++	++
Group – VIII	++	++	++
Group – IX	++	+	+
Group –X	+++	+++	+++
Group – XI	++	++	++
Group - XII	++	+	+
Group – XIII	++	++	+++
Group – XIV	++	+++	+++
Group – XV	++++	++++	++++
Group – XVI	+++	+++	++++
Group – XVII	++	++	+++
Group –XVIII	+	+	+
Group - XIX	++++	++++	++++

++++ **Erythema with large blisters, +++Erythema with small blisters, ++ Erythema with oedema, +Erythema alone**

Table 3. Evaluation of DTH response using tuberculintest in mice exposed to different plant extracts of *S. potatorum* for three weeks

Group	DTH response - Time intervals (weeks)		
	12 hrs	**24 hrs**	**48 hrs**
Group – 1	0.08	0.11	0.10
Group – II	0.09	0.14	0.13
Group – III	0.09	0.10	0.12
Group -IV	0.08	0.11	0.13
Group -V	0.09	0.13	0.14
Group – VI	0.08	0.13	0.13
Group – VII	0.08	0.12	0.12
Group – VIII	0.08	0.10	0.07
Group – IX	0.08	0.11	0.08
Group –X	0.08	0.11	0.08
Group – XI	0.08	0.10	0.07
Group - XII	0.08	0.10	0.07
Group – XIII	0.09	0.11	0.13
Group – XIV	0.08	0.12	0.14
Group – XV	0.09	0.14	0.16
Group – XVI	0.08	0.14	0.15
Group – XVII	0.08	0.13	0.14
Group –XVIII	0.08	0.09	0.06
Group - XIX	0.09	0.15	0.18