SCREENING OF HUMORAL IMMUNE RESPONSES IN RODENTS EXPOSED TO STRYCHNOS POTATORUM

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ABSTRACT

Medicinal plants and their production has received increased attention in recent years because of the recognition that they are nutritious foods with health-stimulating properties and medicinal effects. 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. Laboratory breed mice of either sex (2 months of age; weighing 25-30g*)* were used to evaluate the immunomodulatory activity of different organic extracts of the tender fruits of *S. potatorum*. The ethanol and water extract showed the highest antibody titer (7 $\log_2^2$) followed by hexane and butanol extract (5 $\log_2^2$). B cell production of control and treated animals were estimated by rosette forming assay and recorded. Blood cell differential counts of control and treated animals were estimated by standard methodology and recorded.

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

INTRODUCTION

Immunity means the ability to resist infection and the implementation of the vaccination programmes to reduce the incidents of epidemics. But while we post that we no longer have to deal with the plaque or the measles, we ignore the magnitude of other health problems related to immune dysfunction such as raise in incidents in allergy, cancers of the immune system and autoimmune disease. Inflammation is the complex biological response of various tissues to harmful stimuli including pathogens, irritants or damaged cells. This is initial step to eradicate stimuli of injury and help to healing of tissue. Immunosuppression implies mainly to reduce resistance against the infectious organism (Haniffa *et al.,* 2014 and Puri 2003). The research on medicinal plants and their production has received increased attention in recent years because of the recognition that they are nutritious foods with health-

stimulating properties and medicinal effects. Various experiments are used to prove the Immunity of the traditional medicine and its safety (Chang, 1996; Davydov and Krikorian, 2000).

The modulation of immune response has been studied using varieties of natural and synthetic agents. Saponin containing *Quillaja saponaria Molina* (Rosaceae) were used to increase the antibody (IgG) level in mice but failed to stimulate the production of IgE antibodies (Kensil *et al*., 1991). The aqueous fractions of the 50% ethanol extract of *Nyctanthes arbor-tristis* Linn. The development of humoral and delayed type hypersensitivity of the sheep red blood cell are used to prove the specific and non specific immunity of the mice (Puri *et al*., 1994).

The modern system of medicine had always been enthusiastic to evoke non-specific defense mechanisms of human physiology, which led to the discovery of active immunization using microbial preparation to enhance the host defense against infection. Recently, the same enthusiasm has taken an important leap towards exploring a novel group of substances from natural resources that modulate the immune response of living systems to protect human beings from disease (Gutali *et al.,* 2002). Immunomodulation cause immunostimulation of cells or production of their metabolic inducers or by inhibiting the immunity limiting factors (Agarwal and Singh, 1999).

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. Laboratory breed mice of either sex (2 months of age; weighing 25-30g*)* were used to evaluate the immunomodulatory activity of different organic extracts of the tender fruits of *S. potatorum*. The mouse was housed in a polyvinyl cage littered with paddy husk under a standard condition of temperature (27°C), 12h/12h light /dark cycles and fed with a balanced pellet diet (Lipton, India Ltd) and tap water *ad libitum.*

The weighted quantity of the extract was dissolved in sterilized distilled water, and the concentration of 100 mg/kg/day was prepared. The plant extract was dissolved in water and fed to the mice along with drinking water using a special feeding bottle. Alloxan (Khandelwal Laboratories, India) was used as a standard immuno-suppressant drug. Tolbutamide (Envin-bioceuticals, Shorapur, India) was used as a standard immune potentiating drug.

Cellular antigens such as sheep erythrocytes were obtained from fresh blood of sheep sacrificed in the local slaughterhouse. Sheep blood was collected into Alservier's solution and stored. Sheep red blood cells (SRBC) was prepared by washing sheep blood in Alsevier's solution thrice by· centrifuging at 3000 rpm for 10 min. A packed volume of SRBC is resuspended to get a concentration of 0.1 ml containing 1 x1 0^8 cells for immunization and challenge. 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).*

Humoral mediated immunity

Screening of antibody: From the normal and antigens injected mice, serum samples were taken, the antibody levels were estimated. Quantitation of serum antibodies was carried out by antibody titer plate technique containing respective antigens. 25 µl of physiological saline was added into all wells of a microtitre plate, and then 25 µl of antiserum added into the first well of a microtitre plate, the antiserum was serially diluted in the well of the first row till the 11^{th} well of the microtitre plate leaving the 12^{th} well as a positive control. Then 25 µl of 1%

test antigen in saline was 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°C. The highest dilution of serum samples which shows detectable agglutination was recorded and expressed in log 2 of the serum.

B cell E rosette Assay: Blood is collected from treated and control mice as previous by mentioned using heparin pretreated vials B cell count in the blood are carried out by the following method.5-10ml of blood was collected and it was introduced into sterile conical flask/ beaker containing (4-5) sterile glass beads. It was then continuously swirled until no sounds were heard from the beads. This indicates that all the fibrins have adhered to the beads. This blood was considered as defibrinated blood.

This defibrinated blood was taken and diluted with an equal volume of physiological saline 3 ml of the lymphoprep solution was taken in a centrifuge-like. The tube was kept in slanting position and 9ml of diluted blood was slowly added along the side of the centrifuge tube using Pasteur pipette care was taken so that the FICON layer of the lymphoprep solution present in the centrifuge tube was not disturbed the content of the centrifuge tube was then centrifuged at 1600 rpm for 20min. the interphase (containing lymphocytes) was removed using a pipette. The cells were washed with 1 ml saline and excess FICON was removed.

After centrifugation the sample was washed with 1 ml of saline after that the saline and supernatant was removed by using filter paper; the pellet was then resuspended in 300ml of RPM1 1640 medium. 12-14 cm of drinking straw was cut. One end of the straw was slantly cut and sealed by slightly heating the tip in a flame. Nylon wool fibers were finely teased using a pair of forceps and the teased fibres were packed (loosely) into the straw. Adding 5 ml of physiological saline washed the packed nylon wool column. A small opening was made at the sealed end of the straw to drain the physiological saline. After washing with physiological saline the nylon wool was then filled with 3 ml of RPMI 1640 medium in a horizontal position. The nylon wool column was kept in the incubator for 30 min at 37°C in a horizontal position. This process activates the nylon wool column.

Resuspended lymphocytes were loaded into the activated nylon wool column. Then the column was held vertically above an eppendorf tube, now hot saline (at 60°C) was slowly dripped into the column. The hot saline passing out of the column was collected in the eppendrof tube, which containsT lymphocytes. After hot saline elution, cold saline was added

to separate B lymphocytes. The column is gently squeezed to release the adhered B cells (repeat twice) the cold saline dripping in another eppendorf tube. 0.2 ml of the saline containing B lymphocyte (from the Eppendorf tube containing B cell) was taken in a separate 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 for 5 min at 4°C. 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 B cell under the microscope (20x/40x) for rosettes. No. of B cell rosettes formed were observed among hundred lymphocytes observed was tabulated.

Differential count: The blood cells were stained with Leishman's strain to show the following appearance to differentiate the blood cells and also to confirm the sample stained with coomassie brilliant blue.

RESULTS AND DISCUSSION

When the antigens entered into the host, the host immune system immediately recognized the antigen and activate to the clonal proliferation of plasma cells to produce antibodies to react with antigen quantification of antibody titer in the experimental groups of mice after the administration of plant drugs will reflect on the immunomodulatory effect of the plant drugs. The ethanol and water extract showed the highest antibody titer (7 $\log_2^2$) followed by hexane and butanol extract (5 $\log_2^2$). In the plant immunomodulatory efficiency compared with the standard immunosuppressive and immunoenhansing drugs (Table No: 1).

Several modulations of immune responses to alleviate the disease have been an interest for many years and the concept of "*Rasayana in Ayurveda is spaced on related principles*" (Sharma *et al.,* 1994). Several Indian medicinal plants have been exploited to enhanced antibody-mediated immune responses (Paul *et al.*, 2002; Ranjith Singh *et al.,* 2004). Similar findings were obtained in *S. potatorum* on antibody-mediated responses. In cyclophosphamide treated groups the antibody titer level decreased by 80 %. In mice administered with *S. potatorum,* the antibody titer levels increased to 20, 10, 40, 20 and 10 percentages of hexane, butanol, ethanol, chloroform, and water extracts and immunoenhancing drug respectively. The plant extract combined with an immunosuppressive agent (alloxan) showed a moderate level of decrement compared to alloxan administrated

groups. At the same time the plant extract combined with immunoenhancing (tolbutamide) showed the predominant antibody titer compared to all other types of treatments and control.

B cell production of control and treated animals were estimated by rosette forming assay and recorded in Table 2. The result showed significant changes in all kind of treated animals, when compared to control of five kinds of treatment, the increment in 'B' lymphocyte number was much pronounced in plant extract and immunoenhancing drug combination followed by immunoenhancing drug and plant pronounced in immunosuppressive drug followed by immunosuppressive drug combined with plant extract (Table 2). The increment in 'B' cell count was because of impact of plant drug on the synthesis, proliferation, and activation of 'B' cells was noticed, decrease in 'B' cell count in animal administered with immunosuppressive agent. Similar results were reported by Banerjee *et al.,* (2001) in mice administered with plant extracts. Excess oxygen free radical production increased rapid peroxidation, damaged to membrane DNA fragmentation and apoptosis due to immunosuppressive drug suppresses the functioning of the immune system (Trebeden Negre *et al.,* 2003 and Dhasarathan *et al.,* 2014).

Blood cell differential counts of control and treated animals were estimated by standard methodology and recorded in Table 3. The result showed significant changes in all kind of treated animals, when compared to the control of five kinds of treatment, the increment in blood cell counts 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.

SUMMARY AND CONCLUSION

Any damage to physiological mechanism and to cells of the body could reduce cytokines production. Cytokines are soluble monomorphic (glucose) protein that is released by living cells. Most cytokines are produced by a large variety of cell types. This growth factor cytokines play an important role in activating B cells, T cells, macrophages and various other cells that participate in the immune response. Cytokines [IL-1, IL-2, interferon r (IFN-r)] are immune mediators and they also act on the neuroendocrine system, which in turn carries out immune regulatory function. For *eg*.hormone glucocorticoid deficiency loads to an immune disorder like Hashi Moto like autoimmune thyroiditis and lupus erythronotus. All the plant

substances tested were found to modify the different species of white blood cells and peripheral lymphocytes. Hence the cytological changes in these cells were one of the reasons for low/high production of cytokine and this had affected their role in immunoregulation. Reduction in phagocytic monocytes and immunity-producing neutrophil counts in WBC reported in the study further questions the immunity. Enhancement of immune response by plant based drugs motivates to find out the active principle and mechanism for further study.

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Table 1 Estimation of antibody titre in control and treated mice

Group	Antibody Titre (log_2^2)		
	I week	*II week*	*III week*
Group – 1	4	5	5
Group – II	5	6	7
Group – III	4	5	6
Group -IV	4	4	5.5
Group -V	5	6	7
Group – VI	4	5	6
Group – VII	4	5	5.5
Group – VIII	4	4	5
Group – IX	4	5	6
Group –X	4	5	6
Group – XI	5	4	5
Group - XII	4	4	4
Group – XIII	4	5	6
Group – XIV	4	5	6
Group – XV	5	7	9
Group – XVI	4	5	6
Group – XVII	4	5	5
Group –XVIII	4	3	1
Group - XIX	5	6	8

Table 2 Enumeration of B cells using rosette-forming assay in treated mice

Group	Number of B cells rosette formed in 100 lymphocytes observed		
	I week	*II week*	*III week*
Group - 1	24	24	24
Group – II	26	26	27
Group – III	23	24	25
Group -IV	23	24	26
Group -V	25	25	26
Group – VI	26	26	27
Group – VII	25	25	27
Group – VIII	22	24	26
Group – IX	22	23	25
Group –X	24	24	26
Group – XI	23	24	25
Group - XII	22	23	25
Group – XIII	24	26	28

Group – XIV	25	27	30
Group – XV	25	27	29
Group – XVI	24	25	27
Group – XVII	24	25	28
Group –XVIII	22	19	17
Group - XIX	25	27	29

Table 3 Enumeration of differential blood cells in control and treated mice

Group	Differential counts				
	Monocyte	Lymphocyte	Basophil	Neutrophil	Eosinophil
Group – 1	4	62	3	2	0
Group – II	8	65	4	4	0
Group – III	5	62	3	3	0
Group -IV	5	63	3	4	0
Group -V	6	62	3	3	0
Group – VI	5	62	3	3	1
Group – VII	5	62	3	2	0
Group – VIII	5	52	2	3	2
Group – IX	5	53	2	2	2
Group –X	5	56	1	3	1
Group – XI	5	54	2	2	2
Group - XII	5	54	2	2	1
Group – XIII	4	61	3	4	0
Group – XIV	5	64	4	4	0
Group – XV	5	68	5	5	0
Group – XVI	4	66	3	3	0
Group – XVII	4	64	3	2	0
Group –XVIII	4	45	2	5	2
Group - XIX	5	63	5	2	0