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ANALYSIS OF IMMUNE RESPONSES IN MICE EXPOSED TO ACACIA NILOTICA

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ABSTRACT

In the present study the immune response level and various immunological properties in experimental mice was assessed after administering various extracts of *A. nilotica* and immunosuppressive drugs. The ethanol extract of *A. nilotica* showed highest antibody titre (7 $\log_2^2$) followed by hexane and chloroform extract (6 $\log_2^2$) and butanol and water extract (5.5 $\log_2^2$). The increment in 'B' lymphocyte number was much pronounced in mice by the administration of *A. nilotica* in combination with immunoenhansive drug than the lone effect of immunoenhansive drug. B cell decrement was pronounced in mice treated with immunosuppressive drug while a moderate decrement was noticed due to *A. nilotica* in combination with immunosuppressive drug. The increment in blood cell counts was much pronounced in mice by the administration of *A. nilotica* in combination with immunoenhansive drug than the lone effect of immunoenhansive drug. 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.

KEYWORDS: Mice, immunomodulation, T-cell, B-cell and A.nilotica

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INTRODUCTION

Modern immunology aims to modify the immune system to prevent or control diseases using immunomodulatory tools. Immunomodulation through natural or synthetic substance may be considered as an alternative for the prevention and cure of infections[1]. Medicinal plants are rich sources of substances which are claimed to induce paraimmunity, the nonspecific immunomodulation of essentially granulocyte macrophage, natural killer cells and complement functions[2]. Because of the concerns about the side effects of conventional medicine the use of natural products as alternatives in healing and treatment of various diseases has been on the rise in the last few decades[3]. Interaction between foreign particle and immune cells was initiated by way of differentiation in immune cells in mice due to administration of plant extract[4]. Excessive application of antibiotic instead of controls the diseases, develop antibiotic resistant microbes and cause various side effects[5]. Secretary proteins present in plant act as an immunomodulator in immuno -suppressed animals and help to develop immunity[6]. The plant extract administered mice have immunoenhansive property due to plant extract proteins[7].Medicinal plants serve as therapeutic alternatives, safe choices, or some cases, as the only effective treatment. A large number of these plants and their isolated constituents have shown beneficial therapeutic values, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and immunomodulatory effects[8,9]. Some of the plants with established immunomodulatory activity are *Viscum album, Panax ginseng, Asparagus racemoscus, Azadirachta indica, Tinospora cordifolia, Polygala senega* and *Ocimum santum*[10]. Usefulness of the extracts of various plants against infections, inflammatory reactions has been extensively studied by Amritpal Singh *et al*., (2008)[11]. But their effectiveness in various other immunity reactions is still unexplored. In the present study the immune response level and various immunological properties in experimental mice was assessed after administering various extracts of *A. nilotica* and immunosuppressive drugs.

MATERIALS AND METHODS

In the present study, the immunomodulatory effect of the plant *A. nilotica* on the dynamics of 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 leaves of *A. nilotica*. Mouse were housed in polyvinyl cage littered with paddy husk under standard condition of temperature (27°C), 12h/12h light /dark cycles and fed with balanced pellet diet (Lipton, India Ltd) and tap water *ad libitum.* 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. Cyclophosphamide (Khandelwal Laboratories, India) was used as a standard immuno-suppressant drug. Proimmu (Envin-bioceuticals, Shorapur, India) was used as a standard immune potentiating drug. Cellular antigens such as sheep erythrocytes were obtained from the fresh blood of sheep sacrificed in the local slaughterhouse. Sheep red blood cells (SRBC) was prepared by washing sheep blood in phosphate buffer saline thrice by centrifuging at 3000 rpm for 10 minutes. Packed volume of SRBC is resuspended to get a concentration of 0.1 ml containing 1 $x10^8$ cells for immunization and challenge. A total of nineteen groups (each group containing six mice) of mice were experimented for immunological studies with an inclusion of control and immunised control. Drugs were administered to various groups of mice in the following manner. After treatment, the blood sample was drawn from each group of animals with a time interval of 7 days upto 21 days. {Group I- Control (sterile water), Group II- Immunised control, Group III- *A. nilotica* hexane extract treated group (dose levels of 100 mg/kg), Group IV- *A. nilotica* butanol extract treated group

(dose levels of 100 mg/kg), Group V- *A. nilotica* ethanol extract treated group (dose levels of 100 mg/kg), Group VI- *A. nilotica* chloroform extract treated group (dose levels of 100 mg/kg), Group VII- *A. nilotica* water extract treated group (dose levels of 100 mg/kg), Group VIII- *A. nilotica* hexane extract treated group (dose levels of 100 mg/kg) and cyclophosphamide (30mg/kg) treated group, Group IX- *A. nilotica* butanol extract treated group (dose levels of 100 mg/kg) and cyclophosphamide (30mg/kg) treated group, Group X- *A. nilotica* ethanol extract treated group (dose levels of 100 mg/kg) and cyclophosphamide (30mg/kg) treated group, Group XI - *A. nilotica* chloroform extract treated group (dose levels of 100 mg/kg) and cyclophosphamide (30mg/kg) treated group mg/kg), Group XII- *A. nilotica* water extract treated group (dose levels of 100 mg/kg) and cyclophosphamide (30mg/kg) treated group, Group XIII- *A. nilotica* hexane extract treated group (dose levels of 100 mg/kg) and Proimmu treated group (30 mg/kg), Group XIV- *A. nilotica* butanol extract treated group (dose levels of 100 mg/kg) and Proimmu treated group (30 mg/kg), Group XV- *A. nilotica* ethanol extract treated group (dose levels of 100 mg/kg) and Proimmu treated group (30 *mg/kg),* Group XVI- *A. nilotica* chloroform extract treated group (dose levels of 100 mg/kg) and Proimmu treated group (30 *mg/kg),* Group XVII- *A. nilotica* water extract treated group (dose levels of 100 mg/kg) and Proimmu treated group (30 mg/kg), Group XVIII - Cyclophosphamide alone treated group (30 mg/kg) ,Group XIX - Proimmu treated group (30 mg/kg).

Antibody titration[12]

From the normal and antigens injected mice, serum sample was taken and 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 negative control. Then 25µl of 1% test antigen 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 37^0 C. The highest dilution of serum samples which shows detectable agglutination was recorded and expressed in $\log_2 2$ of the serum antibody.

B and T cell E rosette assay

Blood cells collected from test animal and control mice using heparin pretreated vials. B and T-cell counts in the blood were carried out up to loading of lymphocyte in nylon wool column. Number of B and T cell rosettes formed were observed among hundred lymphocytes observed was tabulated.

Hematological investigation

To find out the impact of *A. nilotica* in haemopoietic organs, total and differential count of blood cells in mice was studied by following the method of Kannan *et al.,* (2007)[13].

RESULTS AND DISCUSSION

In the present study, quantification of antibody titre in the experimental groups of mice after the administration of plant drugs will reflect on the immunomodulatory effect of the plant drugs. The ethanol extract of *A. nilotica* showed highest antibody titre (7 $\log_2^2$) followed by hexane and chloroform extract (6 $\log_2^2$) and butanol and water extract (5.5 $\log_2^2$). Immunomodulatory efficiency of *A. nilotica* was compared with the standard immuno suppressive and immune enhancive drugs (Table.1).

Table 1
Estimation of antibody titre in mice maintained as control and Mice with various treatments.

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

Extract was found to increase the circulating antibody titre and antibody forming cells. In fact antibody forming cells were found to be stimulated much earlier 7th days then the maximum antibody titre obtained 14th day. However, an increased titre remained several days thereafter indicating that the immunological activity could be sustained for several days. Several Indian medicinal plants have been exploited to enhance antibody mediated immune responses[14]. Similar findings also obtained in *A.nilotica* on antibody mediated responses (Table 1). 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" [15]. B cell production of control and treated animals were estimated by rosette forming assay and recorded in Table 2. The result showed remarkable changes in all kinds of treated animals when compared to control. The increment in 'B' lymphocyte number was much pronounced in mice by the administration of *A. nilotica* in combination with immunoenhansive drug than the lone effect of immunoenhansive drug. B cell decrement was pronounced in mice treated with immunosuppressive drug while a moderate decrement was noticed due to *A. nilotica* in combination with immunosuppressive drug. The increment in 'B' cell count may be due to the impact of plant drug on the synthesis, proliferation and activation of 'B' cells in treated animals. Similar results were observed by Dhasarathan *et al*. (2010)[16] in mice administered with plant extracts. The decrease in 'B' cell count in animal administered with immunosuppressive agent might be due to excess oxygen free radical production, increased rapid peroxidation, damage to membrane DNA fragmentation and apotosis due to immunosuppressive drugs thereby suppressed the functioning of immune system [17].

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

Blood cell counts and differential blood cell counts of control and treated animals were estimated by standard methodology and recorded in table 3 and 4. The result showed notable changes in all kinds of treated animals, when compared to control of 17 kinds of treatment. The increment in blood cell counts was much pronounced in mice by the administration of *A. nilotica* in combination with immunoenhansive drug than the lone effect of immunoenhansive drug. The decrement in blood cell count much pronounced in mice treated with immunosuppressive drug while a moderate decrement was noticed due to *A. nilotica* in combination with immunosuppressive drug. Similar results were observed by Kannan *et al.,* (2007)[13] in mice administered with ethanol extract of *Nyctanthes arbortristis.*

Table 3
Enumeration of Blood cells in mice maintained as control and mice with various treatment.

Group	Blood cells (Cells/cu.mm)	
	RBC $x10^6$	WBC $x10^2$
Group – 1	3.15	7670
Group – II	3.42	7754
Group – III	3.14	7642
Group -IV	3.15	7648
Group -V	3.40	7752
Group – VI	3.13	7632
Group – VII	3.14	7640
Group – VIII	3.11	7340
Group – IX	3.11	7260
Group –X	3.12	7440
Group – XI	3.12	7420
Group - XII	3.13	7410
Group – XIII	3.40	7759
Group – XIV	3.43	7784
Group – XV	3.44	7854
Group – XVI	3.44	7764
Group – XVII	3.43	7754
Group –XVIII	3.01	6380
Group - XIX	3.56	7789

Table 4
Enumeration of differential blood cells in mice maintained as control and mice with various treatment.

Group	Differential counts (percentage)				
	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

T cell production of control and treated animals were estimated by rosette forming assay and recorded in Table 5. The result showed remarkable changes in all kind of treated animals when compared to control. The increment in 'T' lymphocyte number was much pronounced in mice by the administration of *A. nilotica* in combination with immunoenhansive drug than the lone effect of immunoenhansive drug. T cell decrement was pronounced in mice treated with immunosuppressive drug while a moderate decrement was noticed due to *A. nilotica* in combination with

immunosuppressive drug. 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)[18] in mice administered with plant extracts. The decrease in 'T' cell count in animal administered with immunosuppressive agent might be due to excess oxygen free radical production, increased rapid peroxidation, damage to membrane DNA fragmentation and apotosis due to immunosuppressive drugs thereby suppressed the functioning of immune system[17].

Table 5
Enumeration of T cells using rosette-forming assay in mice subjected with various treatment.

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

Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the 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.

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