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Immunomodulatory studies of herbal medicated feed in wistar rats

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Abstract
A large population of India is using herbal products for their healing, preventive, curative and many therapeutic properties along with immunomodulatory property. Clinical uses of immunostimulants are in treatments of diseases like immune compromise disorders such as AIDS, chronic infectious diseases and cancer. Several trials and studies have been done to recognize immunomodulatory agents to encounter the infection and enhance host immune mechanisms. *Asparagus racemosus, Withania somnifera Moringa oleifera* and *Ocimum sanctum*are medicinal plants having immunomodulatory activity. The present study was carried out by procuring 48 Wistar rats and was randomly divided into six groups consisting of 8 rats in each group. Herbal medicated feed prepared as additive over and above daily nutritional requirement of Wistar rats with addition of *Asparagus racemosus, Moringa oleifera, Ocimum sanctum, Withania somnifera* @ 2%, 3%, 3%, 3% respectively and combination medicated feed prepared using all these plants in 2%, 3%, 3%, 3% above same concentration ratio. Group I kept as control fed with normal feed and group II to VI fed with 2% *Asparagus racemosus,* 3% *Moringa oleifera, 3% Ocimum sanctum,* 3% *Withania somnifera* and combination feed respectively. HA and DNCB contact sensitivity test were performed to assess the immune response. One rat from each groupss were sacrificed at termination (42nd) day of experiment. Significant increase in HA titers and DNCB contact sensitivity was observed in all treatment groups and indicating increase in cell medicated immunity and humoral immunity.

Keywords: Medicated feed, immune response, cell mediated immunity, humoral immunity

Introduction
Immunity is a state of protection against pathogen induced injury with fast immune elimination of pathogenic invaders due to previous antigen contact or a specially acquired state of responsiveness (WHO). The immune system protects the body by neutralising, inactivating or eliminating potentially pathogenic invaders such as microorganisms (bacteria and viruses). Therefore, normal function of immune system or mechanism is important for natural self protection of an individual against infectious disease. Modulation of immune response through stimulation or suppression may help in maintaining a disease free state [1]. Agents possessing ability to normalize or modulate patho-physiology process, having stimulatory or suppressive effects are called immunomodulatory agents [2]. Immunomodulators are natural and synthetic substance, which cause the therapeutic benefits by altering the immune system. They may have ability to develop, replace or help to produce the desired immune response. Immunosuppression involved the decrease in resistance against infection, environmental and drug factors [3]. Medicinal uses of immunosuppressants are to minimize rejection of transplanted organ, tissues and to reduce the ocurrance of disease between graft and hostin bone marrow transplantation [4].

It is reported that human and animals are continuously exposed to different risk factors such as pathogenic agents or mycotoxins which reduce immune function and immunomodulators are suggested to improve the functioning immune system [5]. Immunotherapy is the treatment of disease by inducing, enhancing, or suppressing immune response using immunomodulators. Some immunotherapies activate the immune system, while others suppress depending on the situation. Today immunotherapy is sometimes given in combination with other conventional cancer treatments and sometimes for primary curative procedure [6]. Phytochemicals are non-nutritive plant chemicals that possess defensive and preventive action from different diseases and also have properties like an antioxidant or hormone. Plants play an important role in the maintaining health status and improving the immunological response against various diseases [7].

Recently the investigations have been focussed on the immunomodulatory properties of the plant materials. Some scientist are actively working on the immunotherapeutic potential of the Indian medicinal plants.

They have reported that some of these plants, such as *Tinospora cordifola, Asparagus racemosus* and *Withania somnifera,* provide protection against experimental infections in mice [8]. A variety of Indian medicinal plants have been identified as the potential sources of natural immunostimulants [9]. Hence several ayurvedic pharmaceuticals in India introduced the remedies based on medicinal herbs with immunostimulatory and general tonic properties. Herbs viz. Shatavari (*Asparagus racemosus*), Ashwagandha (*Withania somnifera*), Shewga (*Moringa oleifera*), Tulsi (*Ocimum sanctum*), are referred as immunomodulators. Apart from various therapeutic properties of plants, the researchers mostly emphasize on a variety of immunomodulators which could enhance the immune system as well as eventually combat the disease or infection by modulating immune responses. Nowadays immunomodulators have become very popular worldwide as people realize the importance of a healthy immune system in the maintenance of health and in the prevention and recovery from disease. Thus, the development of drugs for immunomodulatory activity from natural compounds has become an attractive area of research. Hence, the study was designed to assess the immunomodulatory effect of Shatavari (*Asparagus racemosus*), Ashwagandha (*Withania somnifera),* Shewga (*Moringa oleifera*), Tulsi (*Ocimum sanctum*) these medicinal plants on Wistar rats.

Materials and Methods

The study was carried out in Laboratory Animal House, Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, M.A.F.S.U., Maharashtra and rats were purchased from Wockhardt Research Center, Aurangabad (Maharashtra).

Studies were conducted on health adult female and male Wistar rats weighing more than 150g. All the rats were housed in standard polypropylene rats cages (Four rats / cage) at constant temperature 22 ° ±3 °C, humidity 30-70% and a 12 : 12 interval light/ dark cycle throughout the experiment period for 42 days by taking necessary precautions. During the experiment, they were maintained under standard laboratory hygienic condition, providing medicated feed and water *ad libitum.* The approval of the institutional animal ethical committee was obtained prior to commencement of the experiment. After 5 days of acclimatization the animals were assigned to experimental group. Medicated feed of 2% *Asparagus racemosus, 3% Withania somnifera, 3% Moringa oleifera, 3% Ocimum sanctum* and combination of all these plants in same concentration was given to rats respectively to the group II, III, IV, V and VI. Group I kept as control fed with normal feed. One rats from each group were randomly sacrifice at termination day experiment after collection the blood from retro-orbital sinus.

Cell mediated immunity (CMI)

Chemical contact sensitization of skin is regarded as a form of CMI. 2, 4, Dinitro chloro benzene (DNCB) was used for contact sensitization [10].

Procedure

An area of 3cm diameter was marked on the back (near thoracic region) of the rat. Hair around the region was clipped and 0.4ml of 2% solution of DNCB in acetone was applied drop by drop on the marked area for primary sensitization on day 0. The solution was allowed to evaporate quickly by blowing gently. The sensitizing dose was applied only once. After 14days of primary sensitization a challenging dose of 0.25ml of 2% solution was applied at the same site. The skin thickness was measured by using slide clippers before challenging dose and at 0, 24, and 48 hrs intervals after the challengingdose [11].

Hemagglutination test (HA)

The titers of the agglutination antibody in the serum were measured by Hemagglutination test using SRBC as antigen. Sheep red blood cells were used as antigen to test the humoral immune response. Sheep blood was collected in Alsever's solution, just one day before giving it to the rats and stored at 4^0C and washed thrice in pyrogen free sterile normal saline. 5% SRBC suspension was prepared and 0.5ml was given intraperitoneally on 0th and 14th day of the experiment. The blood was collected from all treated groups on 28th and 42nd day of experiment and serum was separated and stored at – 4^0 C for until use.

Procedure

To the microtitre plates, 50µl of normal saline was added to all the wells. A 50µl of serum was added to the first well and mixed. After mixing, 50µl from the first well was serially transferred to the succeeding wells, from the last well 50 µl was discarded. A 50 µl of 0.5% SRBC was added to all the wells. Contents were mixed well and incubated at room temperature till the negative control well showed button formation. A positive control was kept consisting of 50µl of serum and 50 µl of 0.5%. SRBC and a negative control consist of 50 µl of NS and 50 µl of 0.5% SRBC suspension. The highest dilution of hem agglutination was taken as an antibody titer of the serum samples and the results were expressed in log base.

Gross and Histopathology

A detailed postmortem examination was conducted on all the sacrificed rats and in all the experimental groups. The gross lesions were recorded and representative tissue pieces from spleen, thymus and lymph node were collected and preserved in 10% neutral buffered formalin for histopathological studies. Fixed tissues were processed by routine paraffin embedding technique. Sections of 5-6 microns thickness were cut and were stained with routine Haematoxylin and Eosin method (H&E) [12].

Results

DNCB dermal sensitivity test

DNCB dermal sensitivity test results were expressed in terms of increase in thickness of skin (mm) 0, 24 and 48 hours after application of DNCB. The mean values in group I to VI were 2.79, 2.71, 2.85, 3.19, 2.80 and 2.21 respectively and given Figure 1. There was observed non significant ($P<0.05$) increase in group III, IV and V than group I also non significant decrease ($P<0.05$) in group II and VI when compared to control groupI.

Hemagglutination titers (HA)

The titers were converted into base and the mean values of HA titer levels in groups I to VI were 1.81, 1.66, 2.11, 1.88, 1.88 and 1.58 respectively and given in Figure 2. There was observed a highly significant ($P<0.01$) increase in group III, IV and and V when compared to control group There was a significantly decrease in group II and VI compared to control group.

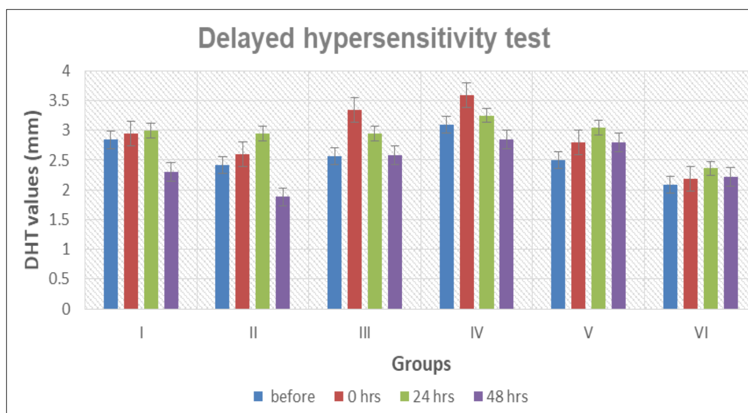
Gross and histopathological investigations

Gross pathology

The gross pathological examination of the organ such as Spleen, Thymus, Lymph node (mesenteric) was attempted in the experimental rats of control and treatment on 42nd day of study period. None of the organ shown any appreciable gross pathological change.

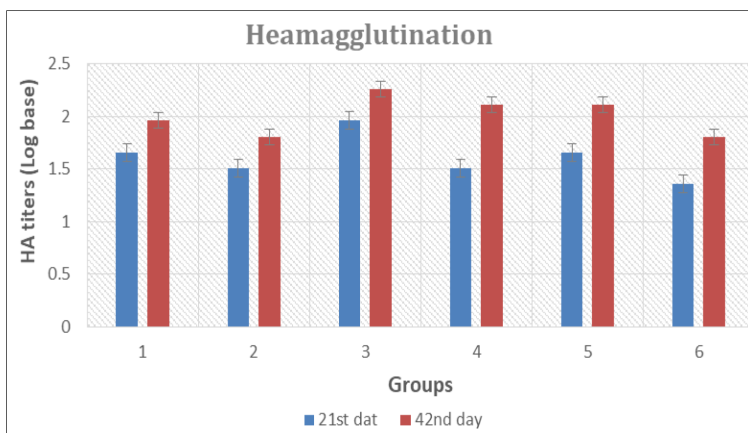
Histopathological alterations

On the day termination (42nd) of the study after gross pathological examination the tissue sample of spleen, thymus, and lymph node (mesenteric) were subjected for histopathological studies. The histo-architecture of spleen, thymus and lymph node (mesenteric) of representative experimental rat from control and treatment group of one each. None of the organ tissue could shown any noticeable histo-morphological change. The major lymphoid organ on histopathological assessment did not show any adverse effect on the histomorphology of targeted lymph organs.



The values are Mean ± S.E non significant ($P<0.05$)

Fig 1: Mean values DNCB test results in rats different in experimental groups



The values are Mean ± S.E significant ($P<0.01$)

Fig 2: Mean values of HA titers (Log base) in experimental rats of different groups

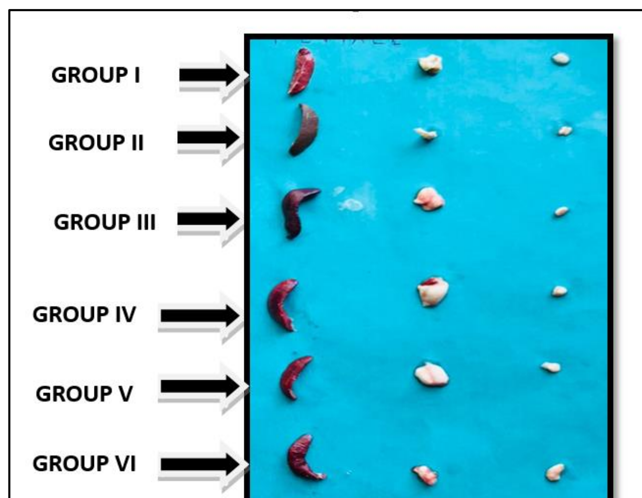
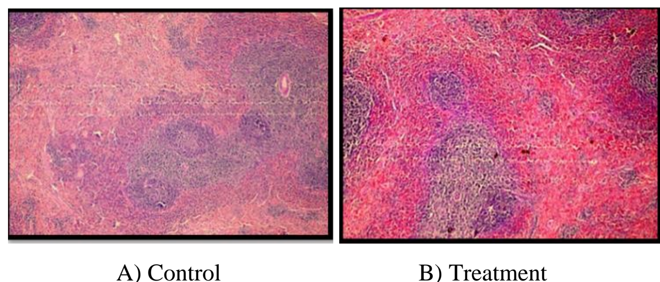
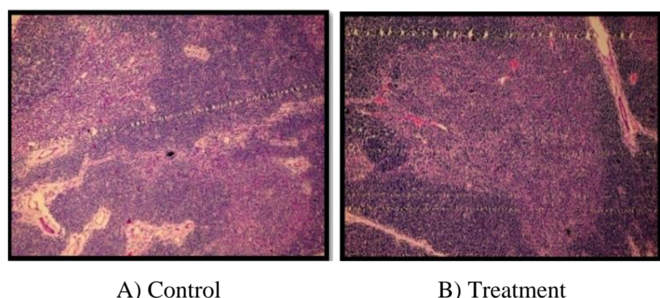


Fig 3: Gross examination of organ at the time of sacrifice spleen, thymus, lymph node of group I to VI respectively.



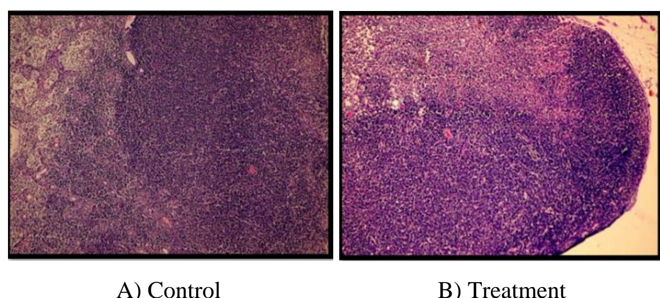
A) Control B) Treatment

Fig 4: Micro-photograph of Spleen with normal histoarchitecture from control and representative treatment group (H & E Stain, X100)



A) Control B) Treatment

Fig 5: Micro-photograph of Thymus with normal histoarchitecture from control and representative treatment group (H & E Stain, X100)



A) Control B) Treatment

Fig 6: Micro-photograph of Lymph node (mesenteric) with normal histoarchitecture from control and representative treatment group (H & E Stain, X100)

Discussion

Significant increase in DTH reaction was observed in III, IV and V groups when compared to control group except group II and VI indicative of increase in cell mediated immunity [13, 14]. Significant increased in the skin thickness which suggest that medicated feed might affect the regulation of T-lymphocytes and their product like lymphokinens. This regulation might be due the presence of important constituents in plants. Activated T-lymphocytes which subsequently release inflammatory mediators including histamine, cytokines and product of arachidonic acid metabolism. These inflammatory mediators increase vascular permeability as well as induce vasodilatation and macrophage accumulation which increase the thickness [15].

In present study significant increase in HA titers was observed in III, IV and V groups. Highest titer shown by group III [16, 17]. Increase in antibody production against SRBC (sheep red blood cells) that indicates *Withania somnifera, Moringa oleifera* and *Ocimum sanctum* had ability to enhance the number of antibody producing cell in spleen and modulate humoral immune response by acting at various level in immune mechanism such as antibody production. All its may be due to immunostimulatory activity of Ashwgandha, Shewga and Tulsi.

None of the organs (spleen, thymus and lymph node) showed any appreciable gross pathological changes. The major lymphoid organ on histopathological assessment did not show any adverse effect on the histomorphology of targeted lymph organs [18, 19, 20].

Conclusion

Considered together, all these observations further strengthen our conclusion that the use of medicated feed 3% *Withania somnifera* whole plant powder, 3% *Moringa oleifera* leaves powder and *Ocimum sanctum* leaves powder in diet no doubt has immunomodulatory effects. It may offer much efficacy against humoral and cell mediated immune responses. Hence the use of medicated feed for 42 days is most efficient and useful as immunomodulators than combination, 2% *asparagus racemosus* root powder medicated feed and normal feed rats for health benefits.

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