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STUDY OF IMMUNOMODULATORY ACTIVITY OF ETHANOLIC EXTRACT OF THESPESIA POPULNEA BARK IN WISTAR RATS

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ABSTRACT

In the present research immunomodulatory acitivity of Ethanolic extract of *Thespesia populnea* bark in wistar rats were studied. Initially drug was extracted with sufficient quantity of ethanol. The extract was tested for acute toxicity studies as per procedure given in OECD[61] guidelines. Albino Wistar rats, which were procured from central animal house of the Institute were used to carry out the experiment. Delayed type hypersensitivity(DTH) response, Humoral antibody (HA) titer, Total leukocyte count, Differential leukocyte count were determined. The test drug was compared with the standard drug Levimasole(50 mg/Kg). The ethanolic extract of *Thespesia populnea* bark in two different dose 200mg/kg and 400mg/kg were tested for their Immunomodulatory action, out of which the higher dose of 400mg/kg showed statistically significant Immunomodulatory activity.

KEYWORDS: *Thespesia populnea* bark, Delayed Type Hypersensitivity (DTH), Total Leukocyte Count, Levimasloe, Wistar rats.

INTRODUCTION

Immunology as a science probably began with the observations by Metchnikoff in 1882 that starfish when pierced by a foreign object (A rose thorn responded by coating it with cells (Latter identified as Phagocytes). Immunology – the study of the way in which the body defends itself against invading organisms or internal invaders (Tumors) has developed rapidly over the last 40 years, and particularly during the last 10 years with the advent of molecular techniques. It is now a rapidly moving field that contributing critical tools for research and diagnosing, and therapeutics for treatment of a wide range of human disease. Thus, it is an integral part of college like science course and medical studies.

The ubiquitous enemy

Both extra cellular and intercellular microbes can grow, reproduce and infect other individuals. They are many different species of microbes and larger organisms (such worms) which invade humans, some of which are relatively harmless and some even helpful (e.g. E.coli in our intestines). Many other causes diseases (human pathogens, and there is a constant battle invading microbes and immune system. Some microbes can even causemthe death of their hosts, although this should not be the property of the most successful microbes. The range of organisms that can infect humans.

The immune system

The human immune system has evolved over millions of years from both invertebrate and vertebrate organisms to develop sophisticated defense mechanisms highly specific for invading pathogens. Immune systems evolved to protect the host from microbes and their virulence factors. From invertebrates, humans have inherited the innate immune system, an ancient defense system that uses germ line- encoded protein to recognize pathogen. Cells of the innate immune system, such as macrophages and NK lymphocytes, recognize pathogen molecule that are highly on serve among many microbes (PAMPs) and use a diverse set of receptor molecules(PRRs).

The immune mechanism

The introduction of foreign substance (antigen)into the body provokes an immune reaction & for this it is essential that the body recognize it as “non self” Most antigens are first ingested & concentrated by the macrophages, & later passed to the nearly lymphocytes. The immune response is initiated by the interaction of the antigen with the receptors on the surface of the lymphocytes, and the response may be of true types.

Humoral immunity

In which circulating immunoglobulins(antibodies) are produce by the plasma cells, derived from lymphocytes

arising from the lymphoid tissue of the gut, known as B cells. Antibodies (Ab) are proteins known as immunoglobulins, produced by immature and mature B-cells (plasma cells) in response to each recognized exogenous foreign invader. In mammals there are five main isotypes of immunoglobulin: IgM (mostly stimulated by the primary response), IgG (for the memory response), IgA (in secretory fluids), IgE (cell-associated, often implicated in allergic reactions), and IgD (on membrane of B- lymphocytes, possibly important in recognition) **(Tizard, 1997).**

Inflammatory Response

The final consideration in discussion of defense mechanisms is how the nonspecific factors discussed above combine in what is termed the inflammatory response to combat an invasion by pathogens. If the microbe is able to activate and fix complement by the alternate pathway, the chemotactic complement-derived factors released to attract Leukocytes to the site, and anaphylatoxin also causes the degranulation of Tissue leukocyte to the site and anaphylatoxin also because the degranulation of tissue basophils called mast cells. These in turn release histamine and serotonin, which cause constrict of smooth muscles (e.g.in bronchioles and blood vessels) and increased capillary permeability, which promoted the passage of plasma and leukocytes into the affected tissue. The leukocytes pass through the junction between the capacity endothelial cells in response to the passage of plasma and leukocytes into the affected tissue.

MATERIALS AND METHODS

Preparation of *ethanolic* extract

The drug was extracted with sufficient quantity of ethanol; total 150 gm of drug was subjected to extraction. The powdered drug was boiled with distilled water. The drug and water was kept in the ratio of 1:5. Then it was filtered through a thin muslin cloth. The resultant extract was subjected to freeze and drying. The yield was 14 gm.

Acute toxicity studies

The TP extract was tested for acute toxicity studies as per procedure given in OECD [61] guidelines. Rats (n=6) were starved for overnight and fed orally with the extracts doses (50,100, 200, 400, 800 and 1600 mg/kg). Animals were observed for next 14 days for behavioral changes and mortality. The 200mg/Kg and 400mg/Kg dosages were selected for this study to carry forward.

Experimental animals

The experiment was carried out by using Albino Wistar rats, which were procured from central animal house of the Institute. The experimental protocol has been approved by institutional animal ethics committee, IISC Bangalore, Regd no:-52/1611/CPCSEA, Rats of Wistar strain weighing between 150 to 250grms. Were maintained under standard laboratory conditions. They were provided with a standard diet supplied by Pranav agro industries ltd India and water central animal house.

Experimental protocol

24 rats were divided into four groups of six animals each.

Group-I : Control

Group-II: *Thespesia populnea ethanol* extract was administered at a dose 200mg/kg/day by oral route for 14 days

Group-III: *Thespesia populnea ethanolic* extract was administered at a dose of 400mg/kg/day by oral route for 14 days.

Group-IV: Standard-Levamisole was administered at a dose of 50mg/kg/day by oral route for 14 days.

Experimental set up

The animal model is required to study the following;

A. Delayed type hypersensitivity (DTH) response
B. Humoral antibody (HA) titer
C. Total leukocyte count
D. Differential leukocyte count

A. DETERMINATION OF DELAYED TYPE HYPERSENSITIVITY RESPONSE (DTH)

The fresh sheep blood was collected from Veterinary College Hebbal (KVAFSU). It was washed three times with normal saline via centrifugation. The suspension was adjusted to 1X 108.

The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing 1X 108cells (1.0 X 108 SRBC/ml) intra peritonially, on day 0, on Day 8, after immunization the thickness of the right hind footpad was measured using a Vernier caliper. The rats were then challenged by injection of 1 X 10 8sub SRBCs in the left hind footpad. The foot pad thickness was measured again after 24 hours of challenge. The difference between the pre-and post challenge foot pad thickness, expressed in mm was taken as a measure of the DTH response. The following formula to be used to measure the DTH response.

$$\frac{\text{(Left foot pad challenged with antigen-Right foot pad control)}}{\text{(Left foot pad challenged with antigen)}} * 100$$

B. HUMORAL ANTIBODY TITRE

The animal were immunized by injecting 0.1 ml of SRB suspension containing 1X108 cells, intraperitinally on day 0. Blood samples were collected in micro centrifuge tubes from individual animals of all the groups by retro orbital vein puncture on day 10. The blood samples were centrifuged and the serum separated. Antibody levels were determined by the hemagglutination technique

Method for Serial dilution

This was performed by using 96 wells (12x8) U bottomed titre plate. The wells were marked from I to XII. In the first (I) and last well (XII) 25 micro liter of serum collected from treated animals was added and inactivated at 56 degree Celsius for 30 minutes. Afterwards to all the wells except well number XII, 25 microliter of PBS was added.25 microliter was taken from first well and added to 2nd well again 25 microliter from second well was taken and added to third well and continued the same procedure up to well number XI. After this 25 microliter of sample from well number XI was discarded. Finally 25 microliter of 1% SRBC was added to all the wells and was kept at room temperature for two hours.

Observation: The button formation was observed. The well which is previous to the well showing button formation is considered as Antibody titer.

Table: Button Formation With Well No.

1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024

C. TOTAL LEUKOCYTE COUNT

The white cells are counted in four corners of 1 square millimeter ruled area on both sides. The white cells are recognized by the retractile appearance and by the slight color given to them by the stain contained in the diluting fluid. The cells touching the left side and upper side of boundary line are not counted.

CALCULATIONS

The area of the smallest square =1/16mm3square
Volume of smallest square =1/160mm3
Total number of square counted =16×4=64
Total number of cells counted = X64/160mm3 of diluted blood contains =X cells

D. DIFFERENTIAL LEUKOCYTE COUNTS

A thin blood film was made on a clean, dry glass slide. It was dried fixed and stained to differentiate the different types of leukocytes. Hundred leukocytes were counted and percentage of different leukocytes was calculated.

RESULTS

The effect of test extract and standard drugs on the DTH response, Antibody Titre, Total Leukocyte count, Differential Leukocyte count in wistar rats using SRBCs as antigen, administration of *ethanolic* extract of *Thespesia populnea* at the dose of 200mg/Kg and 400mg/Kg and Levamisole 50mg/Kg treatments which were given orally for 14 days showed significant increase in paw edema compared to control group. The standard drug Levamisole showed the maximum increase in paw edema volume, Antibody Titre, Total Leukocyte and Differential Leukocyte Count compared to all groups. The Results are shown in Table below:

Table 1: Effect of Test and Standard Drug on the DTH Response in the Wister Rats.

Group	Treatment	Dose	DTH response (mm) mean paw edema ±SEM(n=6)
I	Control	-	2.527 ± 0.156
II	Test extract-I	*Thespesia populnea* - 200mg/kg	3.087 ± 0.196*
III	Test extract-II	*Thespesia populnea* - 400mg/kg	3.52± 0.103***
IV	Standard	Levamisole-50mg/kg	4.6±0.107***

Table 2: Effect of Test and Standard Drug on Antibody Titre Response in the Wister Rats.

Group	Treatment	Dose	Antibody Titre mean ± sem
I	Control		12.83±1.579
II	Test extract-I	*Thespesia populnea* -200mg/kg	248.66±9.218***
III	Test Extract-II	*Thespesia populnea* -400mg/kg	340.33±14.502***
IV	Standard	Levamisole	458±12.310***

Table 3: Effect of Test and Standard Drug on Total Leukocyte Count in the Wister Rats.

Group	Treatment	Dose	Mean total leukocyte count± sem
I	Control	-	14.98×10^3/Cu.mm±0.055
II	Test Extract-I	*Thespesia populnea*-200mg/kg	15.13×10^3/Cu.mm±.165
III	Test Extract-II	*Thespesia populnea*-400mg/kg	16.82×10^3/Cu.mm±0.157***
IV	Standard	Levamisole - 50mg/kg	17.6×10^3/Cu.mm±0.117***

Table 4: Effect of Test and Standard Drug on Differential Leukocyte Count in the Wister Rats.

Group	Treatment	Dose	Mean % of lymphocyte	Mean % of eosinophils	Mean % of neutrophils
I	Control		31.17 ± 0.792	2.24±0.285	45.167±0.601
II	Test extract-I	*Thespesia populnea*- 200mg/kg	32.66 ± 0.823	2.37±0.278	52.67±1.563
III	Test Extract-II	*Thespesia populnea*-400mg/kg	34.66 ±0.809*	2.93±0.321	65.5±1.947***
IV	Standard	Levamisole - 50mg/kg	37.5 ±1.607***	3.1±0.106	73.5±2.141***

DISCUSSION

The bark of *Thespepsia Populnea* bark which are use for immunomodulatory activity.

An immunomodulator is a substance that modulates immune system that include both innate and adaptive arms of the immune responses. These substance possess the pharmacological properties like immunostimulant tonis, antiaging, antistress and antirheumatic, antibacterial activity etc. Some plant are consider to promote positive health and maintain organic resistance against infection by establishing body equilibrium against infection by establishing body equilibrium.

The results of present study revealed that the *ethano* extract of bark of *Thespesia populnea*. Generally showed immune stimulatory effect on the humoral immune function and cell mediated immunity in wistar rats. No mortality and behavioral changes were observed in the treated groups up to 400mg/kg bodyweight.

Thespesia populnea produced a significant increase in DTH reaction i,e. foot pad reaction as shown above results. This increase in DTH reaction in rats in response to cell dependent antigen releived the stimulatory effect on T cell. The present finding shows significant for the development of alternative, inexpensive and perhaps safer stratergies for the treatment of disease. A detailed study is also required on structure determination of the compounds from bioactive fractions in order to find the structure activity significant for the development of alternative, inexpensive and perhaps safer stratergies for the treatment of disease. A detailed study is also required on structure determination. of the compounds from bioactive fractions in order to find the structure activity relationship.

CONCLUSION

This study was done to carry out the immunomodulatory activity of ethanolic extract of *Thespesia populnea* bark in wister rats. To conduct this experiment the dried bark were powedered and extracted with distilled water and was frozen dried. Two different dose i.e. 200 mg/kg and 400mg/kg of *Thespesia populnea* extract were tested for immunomodulatory activity. The higher dose 400mg/kg showed significantly immunomodulatory activity. This were proved from the different parameters measured.

1. Delayed type hypersensitivity response: In this parameter both lower dose and higher dose of the test showed significant result in increase in paw edema when compared with control. The standard drug Levamisole showed the maximum increase in paw volume.

2. Humoral antibody titre: In this parameter both the dose of 200 mg/kg and 400 mg/kg of *Thespesia populnea* produced significant result, standard drug Levamisole at a dose of 50mg/kg also produced significant increase in the titre value.

3. Total leukocyte count: In this parameter the lower dose of *Thespesia populnea* 200 mg/Kg showed no significant increase and higher dose of the ethanolic extract of *Thespesia populnea* 400mg/Kg showed a highly significant increase in the mean total leukocyte count, as compared to control. The results were highly significant for the standard drug levamisole.

4. Differential leukocyte count: The results revealed for lower dose of *Thespesia populnea* showed no significant increase in mean percentage of lymphocytes, eosinophils and neutrophils increase in values as compared to control. The results obtained from the animals that received higher dose of *ethanolic* extract revealed the fact there was a highly significant increase in the mean percentage of lymphocytes and significant increase in the mean percentage of neutrophils respectively when compared to control. The effect of this extract were comparable to the standard drug levamisole.

All the data represents the Immunostimulatory activity of *ethanolic* extract of *Thespesia populnea bark.* It has potential to stimulate the humoral immune response and cell mediated immune response and it may be a potential to stimulate the humoral immune response and cell-mediated immune response and it may be a potential candidate in several immune-suppressed clinical condition.

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