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Immunomodulatory activity of alcoholic extract of *Terminalia belerica* Linn. in mice

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ABSTRACT

The objective of the present study was to investigate the immunomodulatory activity of Terminalia belerica on cellular and humoral immunity. Oral administration of the ethanolic extract of bark of Terminalia belerica, at the doses of 100mg/kg in mice, dose-dependently potentiated the delayed-type hypersensitivity reaction induced by sheep red blood cells (SRBC) . It significantly enhanced the production of circulating antibody titre in mice in response to SRBC.

Keywords: Cyclophosphamide, Ethanolic extract, Immunomodulatory activity *Terminalia belerica.*

INTRODUCTION

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence both immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world. Traditional Indian system of medicines like Siddha and Ayurveda have suggested means to increase the body's natural resistance to disease. A number of Indian medicinal plants and various 'rasayanas' have been claimed to possess immunomodulatory activity[1,2].

Terminalia belerica (TB) Roxb. belonging to the family-Combretaceae, commonly known as myrobalan, is a deciduous tree found throughout the Indian forests and plains. The tree is about 30-40 m. in height and 2-3 m. in girth. The stem is straight and the leaves are broadly elliptic clustered near the end of the branches. The flowers are simple, solitary in axillary spikes. The fruit is ovoid 1-2 c.m. in diameter drupe of grey to dark brown in colour. Fruit extract used as astringent, antiseptic, rejuvenative, brain tonic, expectorant and laxative. It is used in coughs and sore throat. Its pulp used in dysentery, diarrhoea and liver disorders. It is also useful in leprosy, fever and hair care [3-5].

Fruit contain about 20-40% of tannin, phyllemblin, β-sitosterol, anthraquinones, fixed oil ,mannitol, glucose, fructose and rhamnose[5,6].

MATERIALS AND METHODS

2.1. Animals

Swiss albino mice, (School of Life Sciences, DAVV, Indore) weighing between 20 and 25 g of either sex were used to evaluate the immunomodulatory activity of alcoholic extract of bark of *Terminalia belerica* Linn. Animals were housed under standard conditions of temperature (25 °C), 12 h/12 h light/dark cycles and fed with standard pellet diet (Godrej food) and *ad libitum*. All the protocols were approved by University Animal Ethics Committee, of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

2.1 Plant material

Plant material- Fruit of *Terminalia belerica* obtained from Yucca enterprises,Mumbai, were authenticated and identified by Dr.A.B.Sheerwani. (Retd. Prof. and Head), Deptt. of Botany, Holkar Science College, Indore. A voucher specimen has been deposited in our laboratory for further reference.

2.2 Extraction

Preparation of extract- Powdered fruit were soxhlet-extracted with 70% ethanol. The ethanolic extract was evaporated in vacuo and residue(yield:33%w/w).Ethanolic extract was subjected to tests of Kokate[7] . The phytochemical screening revealed the presence of tannins, glycoside, and phytosterol.

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2.4 Acute toxicity

Acute toxicity study was carried out according to Miller and Tainter methods in albino mice of either sex (wt.20-25gm.) were used[8]. An approximate LD50 can be initially determined as a pilot study by a so called 'staircase method' using a small number of animals (2 each dose) and increasing the doses of the drug. Five doses can be chosen for determination of LD50 starting from no death to 100% mortality. In our study for estimation of LD50 of *Terminalia belerica*, 5 doses were given orally to 5 groups of rats, 10 in each group. The animals were observed for first 2 hours and then at 6th and 24th hour for any toxic symptoms. After 24 hours, the number of deceased rats was counted in each group and percentage of mortality calculated.

The LD50 dose of ethanolic extract of bark of *Terminalia belerica* in mice was found 1000 mg/kg.1/10 of LD50 dose 100mg/kg used as therapeutic dose.

2.5 Drugs

Ethanolic extract of bark of *Terminalia belerica* was suspended in 1% sodium carboxy methyl cellulose to prepare suitable dosage forms (100mg/kg p.o.).

The control animals were given an equivalent volume of the sodium carboxy methylcellulose vehicle. Cyclophosphamide (Khandelwal Laboratories, Mumbai) was used as a standard immunosuppressant agent and vitamin-E (Evion-merck) was used as standard drug (150 mg/kg).

Antigen: Fresh blood was collected from sheep's sacrificed in the local slaughter house. Sheep red blood cells (SRBCs) were washed three times in normalsaline and adjusted to a concentration of 0.1 ml containing 1×108 cells for immunization and challenge.

2.6 Humoral antibody response to SRBC[9].

Mice of either sex were divided into four groups of six each. *Terminalia belerica* ethanolic extract (100 mg/kg, p.o.) was administered on day 0 and continued till the day of the experiment. Cyclophosphamide (50 mg/kg, p.o.) was administered 2 days before the experiment. On day 7, the mice were immunised with 0.1 ml of $1x10^8$ SRBC, i.p. Blood samples were collected from the orbital plexuses of individual animals on day 14 and the antibody titres were determined. Briefly, an aliquot (25 ml) of two fold diluted sera in saline was challenged with 25 ml of 0.1% v/v SRBC suspension in microtitreplates. The plates were incubated at 37^0C for 1 h and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre. The mean ranks of different groups were statistically compared (Table-I).

2.7 Cellular immune response[10]

To study the cellular immune response the edema was induced in the right paw of mice by injecting SRBC($0.025x10^9$ cells) in the subplanner region on 20^{th} day, the increase in paw volume in 48 h i. e. on 22^{nd} day was assessed by plethysmometer. The mean percentage increase in foot pad volume was considered as delayed type hypersensitivity and as an index of cell mediated immunity. The volume of the left hind paw, injected similarly with phosphate-buffered saline served as a control (Table-II).

2.8 Statistical analysis

All the data are expressed as mean±SEM and analyzed by ANOVA followed by Dunnett's *t* -test (n=6).

Table I- Effect of ethanolic extract of *Terminalia belerica* and vitamin E on humoral responses to sheep RBC.

Groups	Mean antibody titre
Control	6.1±0.64
Immunosuppressant (Cyclophosphamide,50mg/kg)	4.8±0.51*
Treated with ethanolic extract 100mg/kg p.o.	7.4±0.28*
Standard (vitamin E/150mg/kg)	8.11±0.82*

n=6, Data are presented as mean ±SEM (ANOVA followed by Dunnett's test)
**P≤0.01 when compared with control.*

Table II- Effect of ethanolic extract of *Terminalia belerica* and vitamin E on cell mediated immune responses to sheep RBC.

Groups	Mean increase in paw volume
Control	28.42±2.24
Immunosuppressant (Cyclophosphamide)	14.64±1.38*
Treated with ethanolic extract 100mg/kg p.o.	20.45±2.88*
Standard (vitamin E,150mg/kg)	17.22±2.12*

n=6, Data are presented as mean ±SEM (ANOVA followed by Dunnett's test)
**P≤0.01 when compared with control.*

RESULTS AND DISCUSSION

The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic cells. To evaluate the effect of *Terminalia belerica* on humoral response, its influence was tested on sheep erythrocytespecific haemagglutination antibody titre in mice. Cyclophosphamide at a dose of 50 mg/kg, p.o., showed significant inhibition in antibody titre response, while ethanolic extract of *Terminalia belerica* was found to significantly enhance the production of circulating antibody titre. This indicates the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis.

Cyclophosphamide induced suppression of humoral as well as cell mediated response were significantly attenuated by daily oral treatment with alcoholic extract of *Terminalia belerica*. Vitamin E treated group exhibited similar attenuation of the suspension in immune responces *Terminalia belerica* ethanolic extract at the dose of 100mg/kg was found to suppress delayed type hypersensitivity reaction induced by SRBCs in mice.

CONCLUSION

The ethanolic extract of *Terminalia belerica* produces stimulatory effect on the humoral and cell mediated immune response in the experimental animals and suggest its therapeutic usefulness in disorder of immunological origin.

Thus it can be concluded that flavonoids and phytosterols compounds present contribute to the effect of *Terminalia belerica* on the humoral and cell mediated immune response in the animal experiments in the present study. Further studies to elucidate the exact immunostimulatory mechanism of ethanolic extract of *Terminalia belerica* are in progress.

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