EVALUATION OF IMMUNOMODULATORY ACTIVITY OF METHANOLIC EXTRACT *OF BRIDELIA RETUSA (L.)* BARK

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ABSTRACT

The immunomodulatory effect of methanolic extract of the bark of *Bridelia retusa* was investigated in mice and rats. The assessment of immunomodulatory activity was carried out by various hematological and serological tests. In this study, different doses (100 mg/ml/day, 200mg/ml/day and 400mg/ml/day) of the methanolic extract of the plant showed significant activity and increased phagocytic response and both cellular and humoral antibody response. It is concluded that the test extract posses promising immuno stimulant properties.

KEY WORDS : *Bridelia retusa*, immunomodulatory, phagocytic response, delay type hyper sensitivity.

1.INTRODUCTION

The key component of healthiness is the real defensive strength of our body "THE HUMAN IMMUNE SYSTEM"(Hidden Killers). Immunity is a state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion and is related to the functions of the immune system(Wikipedia). The immune system has been called the "Pacemaker" of life(Balekar,2006).The immunology is the probably one of the most rapidly developing areas of biomedical research and has great promises with regard to prevention and treatment of wide range of disorders. In addition infectious diseases are now primarily considered immunological disorders while neoplastic diseases and organ transplantation and several auto immune diseases may involve in an immunosuppressive state(Ziauddin,1996). Modulation of immune response to elevate the disease has been of interest for many years of the concept of *Rasayana* in *Ayurveda* is based on related principles(Ansari). The function and efficacy of the immune system may be influenced by many exogenous factors like food and pharmaceuticals, physical, psychological stress and hormones etc. resulting in either immunostimulation or immunosuppression(Neelam Makare,2001).*Bridelia retusa(F: Euphorbiacea)* is a tree of moderate size growing in Sri Lanka. Roots and stem bark of this plant used in indigenous system of medicine for the treatment of rheumatism and as an astringent(Lalitha Jayasinghe,2003)**.** *Bridelia retusa* is used by the local people of Goa for meeting their primary health care(Paul,Singh,1996)**.** It is considered a valuable astringent. Mixed with gingelly oil (from seeds of *Sesamum orientale – Pedaliaceae*), it is used to relieve insect stings or as a liniment to relieve rheumatism. The stem bark is boiled and used as a vapour bath following childbirth for its reputed restorative and tonic action. In Ayurveda the plant is considered pungent, bitter and healing, and useful for relieving pain in lumbago and sciatica, the bark is used for removing urinary concretions(John).

2.MATERIALS AND METHODS

The bark of *Bridelia retusa* was collected from Warangal, Andhra Pradesh, which was authenticated by Dr.V.S.Raju, Toxanamist, Department of Botony, Kakatiya University, Warangal. Voucher specimen has been preserved in the herbarium of University college of Pharmaceutical Sciences, Kakatiya University.

Preparation of the extract

The coarsely powdered of shade dried plant material extracted with methanol by maceration method, after a week, it was filtered and concentrated under reduced pressure brown flakes were obtained, the so obtained methanolic extract of the plant. The concentrated extract was then kept in a desiccators to

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remove traces of solvent and moisture and stored properly until used. Preliminary screening of crude methonolic extract for the presence of various plant constituents.

Acute toxicity Study

Acute toxicity study was carried out according to the procedure given in the literature (Gopalakrishna, 2006;Ghosh, 1984;Turner, 1965). Toxicity study revealed that, methanolic extract did not produce any adverse effects and mortality for next 72 hrs. Since it was found to be non-toxic, the doses for further studies were fixed as 100, 200 and 400 mg/ kg b.wt.

Standard drug

Standard sample (Levamisole) was prepared and administered at a dose of 50mg/kg body weight to mice and rats.

Animals

Swiss albino mice and wistar rats of either sex weighing 20-30gms and 180-250 gms each respectively, were used as experimental models. The animals were obtained from mahaveer agency, Hyderabad, maintained in the UCPSc animal house under standard conditions of temperature (23 ± 1ÚC), 12h/12h night/dark cycles fed with standard diet and water. The mice and rats were divided into five groups, each group consisting of six animals. Individual body weights were recorded and suitably marked for the purpose of identification. All the animals were kept on same diet.

Antigenic material

Sheep erythrocytes (SRBC) were separated from sheep blood, collected in Alsever's solution, separation of SRBCs (Fresh sheep blood was added to Alsever's solution in 1:1 ratio and mixed well to prevent coagulation of blood) by centrifugation. The erythrocytes were washed three times in large volume of pyrogen free sterile normal saline, to make it free of serum and also salts of Alsever's solution and adjusted to a concentration of $2x10^7$ cells for immunization and challenge with normal saline. This preparation was stored in refrigerator at a temperature of 0-5^0C until used.

Experiment protocols

Five groups of mice were taken. Group I treated with gum acacia (orally) solution, group II was given 50mg/kg/day of levamisole as a standard drug, group III, IV & V treated with different doses (100mg/kg, 200mg/kg and 400mg/kg) of methanolic plant extract (orally) for 7 days. The measurement of phagocytic response by Carbon Clearance test(Ghule,2006), antigen-antibody titer by haemo agglutination test(Neelam Makare,2001;Ghule,2006) and delay type hyper sensitivity reaction by hind paw edema test(Neelam Makare,2001).

A)Carbon clearance (Phagocytic response) Test

On 8th day each mice was injected intravenously with 0.2 ml of (1.6% w/v suspension of carbon particles, size 20–25 µm stabilized in gelatin 30 min) through tail vein. Blood samples were collected (in 0.15% w/v disodium ededate solution) from retro orbital plexus at intervals of 0, 5, 10, 15min after injection. A 25µl sample was mixed with 0.1% Sodium carbonate solution (2ml) and % transmittance was determined spectrophotometrically at 660 nm. The rate of carbon clearance (phagocytic index, *K*) is calculated from the formula,

$$K = \frac{\log OD_1 - \log OD}{t_2 - t_1}$$

OD_1, OD_2 –optical densities

t_1, t_2-time at different intervals

B) Delayed type hypersensitivity (DTH)

Delayed type hypersensitivity response to SRBC is induced in rats. Five Groups of six rats were immunized by injecting 20µl of 5 X $l0^9$ SRBC/ml through I.P. day 0 (Primary challenge) after measuring the normal foot pad volume of each rat on plethismo meter. The rats were then challenged by S.C. into the right foot pad on day +5. Foot thickness was measured before challenge and again +24 hrs after the challenge (secondary challenge). The difference between the pre and post-challenge foot thickness, expressed in ml is taken as a measure of DTH. The extracts are fed orally (100 mg/kg, 200mg/kg and 400mg/kg) on days - 1, 0, + 1, + 2, + 3, +4, +5.

C) Humoral Anti Body Response to SRBC

The blood samples were collected (before secondary challenge) in micro centrifuge tubes from individual animal by retro orbital pluxes on 7th day. The samples were centrifuged and serum was obtained. Anti body levels were determined by the haemagglutination technique, equal volume of individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 25µl volume of normal saline, in 'U' bottomed micro titration 96 well plates

were added 25 µl of freshly prepared 1% suspension of SRBC in saline after mixing, the plates were incubated at 37^{0}C for 2hrs and examined visually for agglutination the reciprocal of highest dilution of the test serum causing visible haemagglutination was taken as the anti body titer.

3.RESULTS AND DISCUSSION

The yield of the methanolic extract of *Bridelia retusa* bark was found to be 17.9% w/w and preliminary phytochemical investigation revealed the presence of Phenolic compounds, steroidal compounds and glycosides.

BRM exhibited a significant (P<0.001) dose dependent increase in the clearance rate of carbon by the cells of RES (Table-1).

The phagocytic response of the standard drug Levamisole (50mg/kg) was statistically very very significant (P<0.001). However, the effect of the extract at dose 400mg/Kg p.o (P<0.001) was found to be comparable with that of the standard used, Levamisole (50mg/Kg p.o).

Table-1 Effect of methanolic extract of bark of *Bridelia retusa* on phagocytic response

Treatment	Dose (mg/kg. bwt) p.o for 7 days	Phagocytic response (Mean±SD)
Control (2% gum acacia)	-	0.00675 ± 0.0016
Standard (Levamisole)	50	0.01520 ± 0.0027***
Extract	100	0.00695 ± 0.0016
Extract	200	0.00876 ± 0.0010
Extract	400	0.01210 ± 0.0015***

n=6 per group. Comparision of I with II, III, IV and V
*** P < 0.001 Very very significant
** P < 0.01 Very significant
*P < 0.05 significant

Table-2 Effect of methanolic extract of bark of *Bridelia retusa* on HA titer and DTH response

Group	Treatment	Dose (mg/kg.bwt) p.o for 7 days	DTH Response (Mean±SD)	HA Titer (Mean±SD)
I	Control (2% gum acacia)	-	4.17 ± 1.08	4.667 ± 1.633
II	Standard (Levamisole)	50	12.81± 1.26**	13.00 ± 4.382**
III	Extract	100	4.81±1.12	5.667 ± 2.066
IV	Extract	200	8.89± 1.83	8.067 ± 3.933
V	Extract	400	12.16±1.03*	10.67 ± 4.131*

n=6 per group. Comparision of I with II, III, IV and V
*** P < 0.001 Very very significant
** P < 0.01 Very significant
*P < 0.05 significant

Administration of plant extracts BRM, was produced significant increase in humoral antibody response (P<0.05) at 400 mg/ kg only, as evident by haemagglutination at that dilution.

The results obtained (Table-2) indicated that animals treated with lower doses i.e. 100, 200 mg/kg didn't show significant increase in paw edema. The optimum dose of the extracts (BRM) showing statistically significant increase (P < 0.01, P < 0.001) was 400mg/kg.

Administration of Levamisole (50mg/Kg, P.O) resulted in significant increase (P<0.05, P<0.01) in humoral antibody titer compared with the animals of group I (control). After challenge on day 7 with SRBC significant increase (P < 0.01, P < 0.001) than BRM (P < 0.05) in the paw edema (DTH) was observed on +24 hrs.

Levamisole (50mg/kg.P.O) treatment appears to be more potent (P<0.01, P<0.001) then BRM (P<0.05, P<0.01) in increasing HA titer and in producing DTH response. However, the effect of the extract of BRM at dose 400 mg/ kg. p.o on HA titre (P<0.01, P<0.05) and DTH response (P<0.05) were found to be comparable with that of the standard used (Levamisole 50mg/kg.P.O).

Statistical Analysis

The data were analyzed by using one way analysis of variance (ANOVA) followed by Newman – Keul multiple comparison test. P values < 0.05 were considered significant.

4.CONCLUSION

BRM was found to have a significant immunostimmulatory activity (400mg/kg) on both the specific and non-specific immune mechanisms. These results are encouraging enough to persue further studies on the fractions of the extract and phytoconstituents of the bioactive fractions, leading to the development of new herbal drugs for immunomodulatory activity.

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