

IMMUNOMODULATORY ACTIVITY OF BRIDELIA RETUSA

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Summary

Bridelia retusa belonging to the family of Euphorbiaceae, commonly known as Kasai or Gondai is a traditional perennial plant. The plant is used in the treatment of rheumatism, gynaecological, strengthens immune system. It is also used in the treatment of vata, lumbago and hemiplegia and also fodder for animals. The plant possesses tannins, triterpene ketone, decanoic acid octadecyl ester, stigmasterol, dehydrostigmasterol, gallic acid and ellagic acid. Proteins were isolate from leaf extract. Thus in the present study the immunomodulatory effect of *Bridelia retusa* was studied in experimental rats. *Bridelia retusa* was administered orally at a dose of 200 mg/kg to healthy rats divided into three groups consisting of six animals each. The assessment of immunomodulatory activity was carried out by testing the humoral (antibody titre) and cellular (foot pad swelling) immune responses to allergic challenge by sheep RBCs and by neutrophil adhesion test. On oral administration of the extract a significant increase neutrophil adhesion in and delayed type hypersensitivity response whereas the humoral response to sheep RBCs was unaffected. Thus *Bridelia retusa* significantly potentiated the cellular immunity by facilitating the foot pad thickness responses to the sheep RBCs in sensitized rats. With a dose of 200 mg/kg the delayed type hypersensitivity response (mean \pm SEM). The responses were statistically significant when they were compared with the control. The study stated that *Bridelia retusa* shows a significant stimulation of the cell mediated immunity and no effect on the humoral immunity.

Key words: *Bridelia retusa*, Immunomodulatory Activity, Humoral Activity.

Introduction

Bridelia retusa, (Euphorbiaceae), commonly known as Ekdania (1) which is used in our Traditional System of Medicine. It is a tall tree with blackish brown, irregularly fissured bark; leaves are elliptic oblong, obtuse, entire and tomentose beneath. Flowers creamy –white, in terminal panicles of erect (2,3). It is used in the treatment of rheumatism and gynaecological problems. The root and bark are valuable astringent. Used in vata, lumbago and hemiplegia and

also as a fodder (4,5). Bark contains tannins, triterpene ketone, decanoic acid octadecyl ester, stigmasterol, dehydrostigmasterol. Fruits contain β -sitosterol, gallic acid and ellagic acid. Leaves contain crude proteins (6). Thus in the present investigation the successive extraction of *Bridelia retusa* leaves and roots were screened for immunomodulatory properties using standard operating procedures.

Material and Methods

Collection of plant material: The plant (Leaves) were collected from the road side hills of Satapura District, Shirpur, Maharashtra in the month of February, it was identified and authenticated by Dr. D.A. Patil. A voucher specimen (No1) has been deposited in the Department for further reference.

Preparation of extracts and Standards: The plant parts (Leaves) were washed, shade dried and extracted with methanol for 36 h by hot continuous method in a soxhlet extraction unit. The mixture was filtered and evaporated to dryness. The dark green and brownish semisolid mass was obtained for leaf and root respectively. Both were stored in a well closed air tight light resistant container. The extracts of leaf and roots were subjected to preliminary phytochemical screening (7) which indicated the presence of alkaloids, glycosides, flavonoids, tannins, phenolic substances and saponins.

Animal: Adult male Wistar rats, weighing 150-200 gms, were used to study the immunomodulatory activity. The animals (six per cage) were maintained under standard laboratory conditions (light period of 12 h/day and a temperature $25 \pm 2^\circ\text{C}$) with access to a standard commercial diet and water *ad libitum*. The experiment was carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the Institutional Animals Ethics Committee had approved all the procedures. Experiment studies were undertaken according to their rules and regulations (8).

Antigen: Sheep Red Blood Cells were collected in Alsever's solution, washed three times in large volumes(30 ml) of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.05×10^9 cells/ml for immunization and challenge.

Immunomodulatory Activity

Neutrophil adhesion test (9)

On the 14th day drug treatment, blood samples were collected (before challenge) by puncturing the retro orbital plexus into heparanized vials and analyzed for total leucocyte count (TLC) and differential leucocyte count (DLC) by fixing blood smears and staining with Field stain I and II-Leishman,s stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37°C . The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percentage neutrophil adhesion was calculated as shown below

$$\text{Neutrophil adhesion(\%)} = \frac{\text{NI}_u - \text{NI}_t}{\text{NI}_u} \times 100$$

Where NI_u = Neutrophil index of untreated blood samples

NI_t = Neutrophil index of treated blood sample.

TABLE 1: EFFECT OF EXTRACT OF *Bridelia retusa* ON NEUTROPHIL ADHESION IN RATS

Group	Neutrophil index		Neutrophil Adhesion (%)
	UB	FTB	
Control	30.80 ± 0.58	24,2 ± 0.468	
Extract (100 mg)	35.8 ± 0.345	26.2 ± 0.365	35.06 ± 0.56
Extract (200 mg)	40.45 ± 0.34	30.12 ± 0.45	39.70 ± 0.66 ***

The values are mean ± SEM of 6 rats in each group. One way ANOVA followed by Dunnett's test, ***p, 0.001 Vs Group I, Ub= untreated blood: FTB= Fibre treated Blood.

Haemagglutinating antibody (HA) titre (10)

Rats of group II and III were pretreated With CB for 14 days and each rat was immunized with 0.05 X 10⁹ SRBC/rat by i.p. route including control rats. The day of immunization was referred to as day 0. The animals were treated with CB for 14 more days and blood samples were collected from each rat on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBC (0.025 X 10⁹ cells). The micro titre plates were incubated at room temperature for 2 hours and examined visually for agglutination. The highest number of dilution of serum showing haem agglutination has been expressed as HA titre.

Delayed Type Hypersensitivity (DTH) Response (10)

Six animals per group (Control and treated) were immunized on day 0 by i.p. administration of 0.05 X 10⁹ SRBC/rat and challenged by subcutaneous administration of 0.025 X 10⁹ SRBC/ml in to right hind foot pad on day 14. The extract of CB was administered orally from day 14 until day 13. DTH responses were measured at 24 h after SRBC challenged on day 14 and expressed as mean percent increase in paw volume (plethysmometrically)

TABLE 3: EFFECT OF EXTRACT OF *Bridelia retusa* ON DTH RESPONSE TO ANTOGENIC CHALLENGE BY SHEEP RED BLOOD CELLS IN RATS

Group	DTH response (%) at 24 hrs	DTH response (%) at 48 hrs
Control	74 ± 1.51	65 ± 0.68
Extract (100 mg)	52 ± 1.89	45 ± 1.56
Extract (200 mg)	64 ± 1.4 **	56 ± 1.09 **

The values are mean ± SEM of 6 rats in each group. One way ANOVA followed by Dunnett's test, **p,0.001 Vs Group I

Statistical Analysis

The data was analyzed using one way analysis of variance (ANOVA) followed by Dunnett's Test. P values, 0.001 were considered as significant.

Estimation of total phenolics: The total phenolic contents of the extract was determined with Folin-Ciocalteu reagent according to Slinard & Singleton (11) and slightly modified. The stock solution of extract 1mg/ml in water was prepared. From the stock solution, 5 ml was transferred to a 25 ml volumetric flask and made up with distilled water. Out of this 5 ml of sample and 2 ml of standard was taken in 25-ml volumetric flask, to this 10 ml of distilled water, and 2ml of phenol reagent (20%v/v) was added, and then the volume was made up with 29% sodium bicarbonate. The mixture was kept in the dark for 20 min. and the absorbance was read at 760 nm. The total phenolic content was calculated as gallic acid and expressed as percent of gallic acid detected. Standard used was gallic acid.

Results and Discussion

It has been observed that the immunomodulatory agents obtained from the plant and animal source serves as the best for immune responsiveness of any organism against a pathogen by activating the system. In the present investigation BR (*Bribdelia retusa*) when administered orally, significant increased in the adhesion of the neutrophils to the nylon treated fibers which interrelated the process of margination of cells in blood vessels. It was also found to be highly significant when compared with the control group. The HA titre showed very mild increase with BR administration. The DTH response directly correlated the cell mediated immunity and was found to be significant. Thus in this process it was observed that T-lymphocytes were sensitized when they were challenged by an antigen which thereby converted to lymphoblast's and secreted the lymphokines and attracted the scavenging cells to the specific site of reaction. The increase in the response indicated by BR had a stimulating effect on the lymphocytes. Thus it can be also be correlated with the free radical oxidative stress which has a major role in the pathogenesis of a wide range of clinical disorders resulting from different natural antioxidant defences So it can be concluded that aqueous extract of leaf of BR was found to be highly stimulating agent for both the responses. The standards exhibited IC 50 values 76.66 ± 1.52 and 57.00 ± 0.77 $\mu\text{g/mL}$ respectively.

Conclusion

Thus from the above activity it was observed that *Bribdelia retusa* showed a significant activity against Delayed type hypersensitivity and neutrophil adhesion models. Thus the activity observed can be evaluated by the isolation of active constituents from the extract and the most potent one can be carried further for exploration

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