IMMUNOMODULATORY EFFECTS OF BARRINGTONIA RACEMOSA ROXB. IN EXPERIMENTAL ANIMALS
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ABSTRACT
Barringtonia racemosa is used medicinally in treatment of diarrhoea, asthma, coughs, jaundice. It is also used as an analgesic and antipyretic. This plant has also significant anti-tumor activity. However, systematic evaluation of its immunomodulatory effects has not been reported. In present study isolation of Bartogenic acid was done from powdered fruits of B. racemosa and the activity of this isolated bartogenic acid was evaluated in in-vivo models of immunity. Bartogenic acid was isolated from Barringtonia racemosa and the isolated compound, Bartogenic acid was suspended in distilled water using Tween 80 (0.1 % v/v) and used for the studies SRBC induced haemagglutination titre in C57BL/6 mice and SRBC induced delayed type hypersensitivity reaction in C57BL/6 mice. In the SRBC induced haemagglutination titre in C57BL/6 mice, the bartogenic acid at the dose of 2 mg/kg p.o., 5 mg/kg p.o. and 10 mg/kg p.o. showed the antibody titer at dilutions of 1:96, 1:53 and 1:32 respectively. In the SRBC induced delayed type hypersensitivity reaction in C57BL/6 mice, the bartogenic acid showed concentration dependent inhibition in DTH response as compared to that of control group animals.
INTRODUCTION:

Millions of people around the world use traditional systems of medicine for developing immunity, resistance against infections/diseases, to prevent or alleviate the symptoms of the disease or cure it. The main factors that make natural products attractive candidates for human use include their ease of availability, cost effectiveness and presumed safety. [1]. In general, immunomodulators are biological response modifiers (BRM) that affect the immune response in either a positive or a negative fashion. The immunosuppressive therapy has become part of everyday medical practice, either to prevent allograft rejection in transplanted patients or in treating a variety of autoimmune diseases, asthma and allergy. With the exception of a monoclonal antibody, all the immunosuppressive drugs used in medicine are exogenous molecules, either microbial secondary metabolites (CsA, FK506, rapamycin, etc.) or synthetic substances (leflunomide, azathioprine, brequinar, etc.) [2].

*Barringtonia racemosa* (*B. racemosa*) (Barringtoniaceae) is a tall tree with one seeded, ovoid fruits, distributed in eastern and western seacoasts of India. The fruit pulp of *B. racemosa* had been in use as a fish poison[3]. Some recent reports claim that *B. racemosa* bark extract has significant antinociceptive property [4]. This effect has been proposed to be related to cytotoxic and immunomodulatory effects of this plant. However, systematic evaluation of different fractions of fruit extract of plant *B. racemosa* has not been reported. In present investigation, the extracts of fruit of *B. racemosa* have been evaluated for its immunomodulatory effects.

Bartogenic acid is a triterpene dicarboxylic acid. Its structure was established as 2α,3β,19α-trihydroxyolean-12-ene-24,28-dioic acid [5]. Bartogenic acid is mainly present in the bark and seeds of *Barringtonia racemosa* [6].

![Bartogenic Acid](image)

**Bartogenic Acid** (2α,3β,19α-trihydroxyolean-12-en-24,28-dioic acid)

In present work, isolation of Bartogenic acid was done from powdered fruits of *B. racemosa* followed by the confirmation of its structure by HPLC, IR and LC-MS using Bartogenic acid marker provided by
Department of Natural Products, IICT, Hyderabad. The activity of this isolated bartogenic acid was evaluated in in-vivo models of immunity.

2. Materials and Methods:

2.1 Animals:
Six to eight weeks old C57/BL6 mice were purchased from Advanced centre for treatment, education and research in cancer (ACTREC), Kharghar, Mumbai, India. The animals were maintained under standard laboratory conditions (Temperature 25 ± 2°C; Photoperiod of 12 hours). The commercial standard palletized feed and water were provided ad libitum. The study was approved by Institutional Animal Ethical Committee registered with Committee for the Purpose of Control and Supervision of Experiments on Animals, India (Registration No. 651/02/C/CPCSEA).

2.2 Drug preparation:
The isolated compound, Bartogenic acid was suspended in distilled water using Tween 80 (0.1 % v/v) when it was to be used for per oral administration.

2.3 Assessment of Humoral Immune Function

2.3.1 Haemagglutination titer (HT) assay:
HT assay was performed in mice according to the procedure by Bilal Bin-Hafeez (2003). The C57BL/6 mice were immunized with 1X10^9 SRBC (i.p.) and divided into four experimental groups each containing 7 mice. Three of these groups were administered with bartogenic acid at doses of 2, 5 and 10 mg/ kg/ day (p.o.) for 7 days post immunization. The control group received only vehicle at the same amount as required for administration of drug in others. On 8th day post immunization, blood samples were collected from individual animals through retro orbital plexus and serum was separated. A serial two fold dilution of serum was made in 50 µl of PBS (pH 7.2) in 96 well ‘V’ bottom microtiter plates. To all these wells, 50 µl of SRBC suspension was added. After proper mixing of contents of each well, the plates were incubated at 37°C for 2 hours and were visually observed for formation pellets at the bottoms. The dilutions after which pellet formation occurred were recorded as HA titer [8].

2.4 Assessment of Cellular Immune Function

2.4.1 Delayed Type of Hypersensitivity (DTH) Response:
DTH response was determined in mice by an earlier reported method [9]. The grouping of C57BL/6 mice, immunization with SRBCs and drug treatments were similar to that of HA titer assay. On the seventh day post immunization, all the mice were challenged with injection of 0.05 ml of 1X10^9 SRBC sub-planter region of right hind leg. The rise in paw volume was measured after 24 hours using a digital
plethysmometer (Ugo Basile 7140, Italy). The percentage rise in the paw volume was calculated in each mouse to determine intensity of DTH.

2.5 Statistical analysis:
The statistical analysis was done using One Way analysis of variance (ANOVA) followed by the Dunnett’s multiple comparison tests. Results with p <0.05 were considered statistically significant. Data has been expressed as mean ± S.E.M.

3. Results and Discussion:
3.1 Effect on Acquired Immune Response:
3.1.1 Effect of Bartogenic Acid on SRBC Induced Haemagglutination Titre in C57BL/6 Mice:
The formation of antibodies in mice was induced by intraperitoneal injection of sheep RBCs. The mice were administered with different doses of bartogenic acid after initial injection of SRBCs. On 8th day, blood samples were collected and serum was titrated with SRBC suspension to determine the titer. In this assay, the dilution at which there was sedimentation of RBCs and button formation occurred was taken as an antibody titer. In case of control group the average titer value was 1:234. Bartogenic acid at the dose of 2 mg/kg p.o., 5 mg/kg p.o. and 10 mg/kg p.o. showed the antibody titer at dilutions of 1:96, 1:53 and 1:32 respectively. At the doses of 2mg/kg (p<0.05) as well as 5mg/kg and 10 mg/kg (p<0.01) a statistically significant decrease in titer value was observed as compared to control group titer. Such decrease in the titer value indicates that bartogenic acid causes suppression of antibody formation against the injected SRBCs.

3.2 Effect of Bartogenic Acid on SRBC Induced Delayed Type Hypersensitivity Reaction in C57BL/6 Mice:
The treatment with different concentrations of bartogenic acid i.e. 2mg/kg, 5mg/kg, and10mg/kg was given for fifteen days after first injection of SRBCs. The DTH reaction was significantly inhibited at the doses of 5mg/kg and 10mg/kg (p<0.001) as compare to control.

To evaluate the effect of the Bartogenic acid on humoral response, its influence was tested on the SRBCs specific haemagglutination antibody titer assay in mice. In this model we studied the antibody mediated immunity. 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 [10].
Bartogenic acid showed significant inhibition in production of circulating antibody titer when tested on SRBC specific haemagglutination antibody titer assay in C57BL/6 mice. The reason behind suppressed antibody production may be the effect of Bartogenic acid directly on cells like macrophages and T & B lymphocytes which are essential for immune system activation. Bartogenic acid showed a dose dependent suppression of antibody production induced by SRBC in mice.

The mechanism behind the elevation or suppression of DTH during the CMI responses could be due to sensitized T-lymphocytes. When challenged by the antigen, they are converted to lymphoblasts and secrete a variety of molecules including proinflammatory lymphokines, attracting more scavenger cells to the site of reaction. The infiltrating cells are probably immobilized to promote defensive (inflammatory) reaction. A decrease in DTH response in present study indicates that the Bartogenic acid has a suppressive effect on lymphocytes and accessory cell types required for the expression of the DTH reaction [11]. Bartogenic acid showed a potent dose dependent inhibition on the SRBC induced DTH response.

References:

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