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Original Article

EFFECTS OF FRUITS OF BARRINGTONIA RACEMOSA LINN. ON HUMAN POLYMORPHONUCLEAR CELL

Sudha Patil [1], Mahesh Ramu Patil [2], Prabhakar Patil *[3], Rohit Dixit [4], Rajesh C Sharma [5].

[1,*3,4,5] Navodaya Medical College Raichur, Karnataka, India.
[2] R.C. Patel College of Pharmacy, Shirpur, Dhule, Maharashtra, India.

ABSTRACT

The objective of present study was to investigate Petroleum ether, ethyl acetate and hydroalcoholic extracts of B. racemosa fruits in vitro on human polymorphonuclear (PMN) cells to screen their effects on phagocytosis and chemotaxis. Ethyl acetate extract of B. racemosa fruits was found to be a stimulant of PMN cell phagocytosis of Nitroblue tetrazolium (NBT) dye and candida albicans. It also stimulated intracellur killing capacity of PMN cells. It was further found to increase the chemotaxis of human PMN cells. While, petroleum ether extract and hydroalcoholic extract were lesser active as far as these activities are concerned.

KEY WORDS: Barringtonia racemosa, Phagocytosis, Chemotaxis, Polymorphonuclear cells.

Address for Correspondence: Dr. Prabhakar Patil, Navodaya Medical College, Raichur, Karnataka, India. Mobile No. +919900346851. **E-Mail:** drprpatil2006@gmail.com

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INTRODUCTION

Different activities e.g. antifungal, anti-inflammatory and antibacterial activity of B. racemosa were already studied [1,2,3]. One recent reports claim that B. racemosa possesses significant anti-tumor activity [4]. In previous study we have reported its immunomodulatory effects [5]. But its action on polymorphonuclear cells was not reported. In present investigation the hydroalcoholic, petroleum ether and ethyl acetate extract of fruits of B. racemosa has been evaluated for its action on human polymorphonuclear (PMN) cell function.

MATERIALS AND METHODS

Preparation of extracts: Fruits of B. racemosa were purchased from a local vendor and were authenticated at Botanical Survey of India, Pune, India. The fruits were coarsely powdered and macerated with methanol: water (1:1) for 8 hours with frequent stirring. At the end of maceration, the extract was filtered and the filtrate was dried under vacuum in a rotary evaporator under reduced pressure at 40^0C- 45^0 C [4]. Fresh powder was similarly macerated with petroleum ether and ethyl acetate and the extract was concentrated at room temperature.

Chemicals: Minimum Essential Medium (MEM), casein, Haematoxylin stain, Giemsa's stain, tryptan blue dye and Nitroblue tetrazolium dye (NBT) (Himedia Labs, Mumbai, India), Bacterial endotoxin (210SE Sigma)

Dosage and concentrations: For in vitro studies, the concentrations of the extract that were non toxic to the cells were used. These concentrations were determined by incubating the PMN

cell suspensions for three hours with different concentrations of the extracts and studying the viability of cells by tryptan blue dye exclusion method.

Nitroblue tetrazolium (NBT) test: Fresh blood sample was withdrawn from a healthy human volunteer in anticoagulant EDTA (Ethylene Diamine Tetraacetic acid) containing tubes. PMN cells were separated from the blood sample by dextran sedimentation method. The blood was added to sterile centrifuge tubes containing equal volume of 6% w/v dextran solution in saline. The tube was set aside for 15 minutes at room temperature. The supernatant and turbid ring above the RBCs (Red Blood Cells) layer was collected with an autopipette, centrifuged and the resultant pellet was washed thrice with PBS and was finally re-suspended in Minimum Essential Medium (MEM) at a concentration of 5 X 10^6 cells per ml.

For NBT assay, 100 µl of PMN cell suspension was taken in each eppendroff tube; to this, 40 µl l of 0.3 % Solution of NBT in saline was added. Different fruit extracts were added to these tubes in such a way that final concentrations of the extract in the assay mixtures were 125 µg/ ml, 250 µg/ ml, 625 ìg/ ml, 1250 µg/ ml, 2500 ìg/ ml. The final study mixture contained MEM, PMN cells, NBT and extract. In positive control assay tube instead of extract, bacterial endotoxin solution (50 µl) was taken.

All these test tubes were incubated for 20 min at 37^{0}C and at room temperature for further 20 minutes. At the end of the incubation, the assay tubes were manually shaken and a 20 µl of assay mixture was taken on microscopy slides and smears were prepared. The slides were then air dried, stained with Giemsa stain and observed under oil immersion microscope (using Labomed CXL Plus Sl. Microscope, Model No-038086) for the NBT positive cell containing blue deposits of the phagocytosed NBT dye. Percentage of NBT positive cells was determined [6,7]

Phagocytosis and candidacidal assay: The PMN cell suspension was prepared as stated above. The count of PMN cells was adjusted approximately to 5 X 10^6 cells/ ml. For preparation of candida albicans suspension, twelve hour old, unicellular culture of C. albicans was used. The candida cell suspension was prepared in PBS at a concentration of about 5 X 10^6 cells/ ml.

In the final assay tubes, 100 µl of PMN cell suspension and equal volume of candida cell suspension were taken. Various fruit extracts were added to separate tubes so that the final concentrations in the assay mixtures, in case of each extract were 125 µg/ml 250ìg/ml, 625 µg/ ml, 1250 µg/ml, 2500 µg/ml. In the positive control assay unit, instead of fruit extract, 100 µl of serum derived from the same human volunteer was taken.

The assay tubes were incubated for 30 min at 37^{0}C. At the end of the incubation period, the tubes were centrifuged and small amounts of the residues were taken on separate microscopy slides. Smears were prepared, air dried and stained with Giemsa's stain.

Neutrophils were examined for the number of ingested and associated Candida albicans with each cell. Average number of candida albicans associated PMN cell was determined for each assay unit [8].

The pellets in the assay tubes were again suspended in 100µl MEM and further incubated at 37^{0}C for 30 minutes. At the end of the incubation period, 0.25 ml Sodium deoxycholate (2.5% in PBS) was added to each tube to lyse the leucocytes. After this, 0.25 ml 0.01 % methylene blue was added to each tube and mixed well. The tubes were centrifuged, supernatant was decanted and smears were prepared on microscopic slides using resultant pellets. The percentage of dead candida cells (stained) in each case was determined [9] . The time schedule was adjusted in such a way that after addition of methylene blue to an assay tube, the readings were taken within next 5 minutes.

Chemotaxis: In vitro effects of different extracts of *B. racemosa* on human PMN cell chemotaxis was studied using fabricated Boyden's chambers consisting of tuberculin syringes as upper compartments separated through a 0.5 µm pore size filter (Millipore, Cat.No.SMWP-04700) from the lower compartment, a 5 ml beaker. The upper compartments of the assay units contained PMN cell suspension in MEM at concentration of about 1 X 10^6 cells/ ml. The lower compartments

contained 2.5 ml PBS consisting different concentrations of the fruit extracts as 60ìg/ml, 100ìg/ml, 300ìg/ml, 500ìg/ml, 1000 µg/ml. In a positive control assay unit, lower compartment contained 2.5 ml PBS and 0.5 ml casein solution (5 mg/ml). The negative control assay unit contained only 3 ml of PBS in the lower compartment.

All the assay units were incubated at 37^{0}C for 75 minutes. At the end of incubation period, the filters attached to the tuberculin syringe in each assay unit were separated, fixed with 70% methanol and stained with Harris Haematoxylin stain. The leukocyte migration was determined microscopically (using Labomed CXL Plus Sl. Microscope, Model No-038086) [10].

Statistical analysis: The data obtained was analyzed for statistical significance using one-way ANOVA followed by Bonferroni's Multiple Comparison test using Graphpad Prism 4.0 software.

RESULT

Effect on Nitroblue tetrazolium reduction: Ethyl acetate extract of *B. racemosa* fruit was found to cause stimulation of PMN cells which was evident from concentration dependent rise in the percentage of PMN cells containing reduced NBT. Petroleum ether extract and hydroalcoholic extract also exerted similar but less potent effect as compared to ethyl acetate extract. The effect of hydroalcoholic extract was the least and resembled to that of negative control group. (Table 1)

Table 1: Effect on NBT Reduction by human PMN cells (% of Neutrophils Stimulated).

Sr. No	Concentration (ug/ml)	Ethyl acetate Extract Mean ± SEM (%)	Petroleum Ether Extract Mean ± SEM (%)	Hydroalcoholic Extract Mean ± SEM (%)
1	125	42 ± 1.2**	45 ± 0.58**	37 ± 0.0**
2	250	67 ±1.2**	48 ± 0.58**	38 ± 1.2**
3	625	68 ± 1.2**	52 ± 1.2**	40 ± 0.0**
4	1250	84 ± 0.0*	68 ± 0.0**	43 ± 1.2**
5	2500	86 ± 0.58	70 ± 0.58**	46 ± 0.58**

For Positive control: Mean ± SEM - 88 ± 0.0% Negative control: Mean ± SEM- 32 ± 0.58% *** P < 0.001** - P < 0.01, * - P < 0.05

Effect on Phagocytosis: Phagocytosis activity was estimated by determining average number of candida cells associated with or engulfed by PMN cells. The results were expressed as 'Minimum Particle Number' (MPN), the average number of candida cells associated with PMN cells.

In case of all the extracts, the MPN increased with increase in concentration of extract in assay medium up to 1250 ug/ml, above this concentration the activity was either constant or decreased. Hydroalcoholic extract showed lesser stimulation of phagocytosis as compared to other two extracts. (Table 2)

Table 2: Effect on phagocytosis of Candida albicans by human PMN cells (Minimum Particle Number- MPN).

Sr. No.	Concentration (ug/ml)	Ethyl acetate Extract (MPN)	Petroleum Ether Extract (MPN)	Hydroalcoholic Extract (MPN)
1	125	3	2	2
2	250	3	2	2
3	625	3	3	2
4	1250	3	4	3
5	2500	3	3	2

Positive Control: Minimum Particle Number (MPN) = 5

Candidacidal Assay: In case of all the three extracts, the intracellular killing capacity of PMN cells was found to be increased in a concentration dependent manner. Petroleum ether extract showed more stimulation of candidacidal activity as compared to other two extracts. The hydroalcoholic extract exerted minimum stimulation of this activity. (Table3)

Table-3: Effect on candidicidal capacity of human PMN cells.

Sr. No.	Concentration (ug/ml)	Ethyl acetate Extract Mean ± SEM (%)	Petroleum Ether Extract Mean ± SEM (%)	Hydroalcoholic Extract Mean ± SEM (%)
1	125	10 ± 0.0*	15 ± 0.0*	20 ± 0.0*
2	250	28 ± 0.58*	30 ± 0.58*	20 ± 0.58*
3	625	30 ± 0.0*	30 ± 0.0*	25 ± 0.0*
4	1250	45 ± 0.58*	40 ± 1.2*	30 ± 0.58*
5	2500	45 ± 0.0*	60 ± 0.0*	35 ±1.2

Positive Control: % of Candida albicans cell killed: Mean ± SEM 35 ± 0.0%

Chemotaxis: Ethyl acetate and Petroleum ether extract were found to increase the PMN cell chemotaxis in a concentration dependent manner upto 500 ug/ ml. Maximum chemotactic activity was observed in case of ethyl acetate extract. Hydroalcoholic extract showed no

chemotactic activity, the migration of cells in this case was similar to that of negative control readings. (Table 4)

Table 4: Effect on human PMN cell chemotaxis.

Sr. No	Concentration (ug/ml)	Ethyl acetate Extract Mean ± SEM (µm)	Petroleum Ether Extract Mean ± SEM (µm)	Hydroalcoholic Extract Mean ± SEM (µm)
1	60	40*	45*	20
2	100	78**	60**	23
3	300	114***	65**	25
4	500	118***	70**	28
5	1000	60*	30	22

Positive Control (casine): cell migration in µm: 150µm
Negative Control (MEM): cell migration in µm: 28µm ,*** P < 0.001** - P < 0.01, * - P < 0.05

DISCUSSION

In in-vitro assays, the other extracts of fruit were found to exert stimulation of PMN cell phagocytosis of NBT and candida albicans cells, PMN cell intracellular candidacidal capacity as well as Chemotaxis in a concentration dependent manner. The ethyl acetate and petroleum ether extracts showed more potent activities in both these assays as compared to hydroalcoholic extract. The hydroalcoholic extract of fruit B. racemosa was found to exert in-vitro models it did not exert any significant alteration of human PMN cell functions.

It is proposed that, the extracts of B. racemosa studied in present investigation may be further fractionated and investigated using more sensitive in vivo and in vitro models related to immune system disorders.

CONCLUSION

Ethyl acetate extract of fruit of B. racemosa can alter PMN cell functions. This should be investigated further in different inflammatory and immunological diseases.

Conflicts of Interests: None

REFERENCES

[1]. N. M. Hussin, R. Muse, S. Ahmad, J. Ramli, M. Mahmood, M. R. Sulaiman, M. Y. A. Shukor, M. F. A. Rahman and K. N. K. Aziz Antifungal activity of extracts and phenolic compounds from Barringtonia racemosa L. (Lecythidaceae) African Journal of Biotechnology 2009;8(12):2835-2842.

[2]. Mandana Behbahani, Abdul Manaf Ali, Radzali Muse and Noorjahan Banu Mohd. Anti-oxidant and anti-inflammatory activities of leaves of Barringtonia racemosa. Journal of Medicinal Plants Research 2007;1(5):095-102.

[3]. Khan S, Jabbar A, Hasan CM, Rashid MA. Antibacterial activity of *Barringtonia racemosa*. J Ethnopharmacol 2001;72:162-164.

[4]. Thomas T.J, Panikkar B, Subramoniam A, Nair M, Panikkar K.R. Short communication, antitumor property and toxicity of Barringtonia racemosa roxb seed extract in mice .J Ethnopharmacol. 2002;82:223-227.

[5]. Prabhakar R. Patil , Mahesh R. Patil, , Abhay Mane, Sudha Patil. Immunomodulatory effects of fruits of Barringtonia racemosa Linn. International Journal of Basic & Clinical Pharmacology 2013;2 (2):216-219.

[6]. Wilkinson P.C. Neutrophil leukocyte function test. Chapter 13 In: Thomson RA, editor. Techniques in clinical Immunology .Oxford London: Blackwell Scientific Publications 1981a, II nd edition; p. 276-278.

[7]. Manosroj A, Saraphanchotiwitthaya A, Manosroj J. Immunomodulatory activities of Clausena excavata Burm.f.wood extracts. J Ethnopharmacol. 2003;89:155-160.

[8]. Wilkinson P.C. Neutrophil leukocyte function test. Chapter 13 In: Thomson RA,editor. Techniques in clinical Immunology. Oxford London: Blackwell Scientific Publications 1981b, II nd edition; p.287-289.

[9]. Wilkinson P.C. Neutrophil leukocyte function test Chapter 13 In: Thomson RA,editor. Techniques in clinical Immunology .Oxford London: Blackwell Scientific Publications 1981c, II nd edition; p. 290-.291.

[10]. Wilkinson P.C. Neutrophil leukocyte function test Chapter 13 In: Thomson RA, editor. Techniques in clinical Immunology. Oxford London: Blackwell Scientific Publications 1981d, II nd edition; p. 279-.283.

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