Indian Journal of Traditional Knowledge
Vol. 16(4), October 2017, pp. 689-693

Immunomodulatory effect of leaf extracts of *Barringtonia acutangula* (L.) Gaertn.

Rashmi K[a], Karunakar Hegde[b] & K Bhasker Shenoy[c]*

[a]Department of Applied Zoology, Alva's College, Vidyagiri Moodbidri, DK - 574 227, Karnataka, India;

[b]Department of Pharmacology, Srinivas College of Pharmacy, Valachil Post- Parangepete, Mangalore- 574 143, Karnataka, India;

[c]Department of Applied Zoology, Mangalore University, Mangalagangothri- 574 199, Karnataka, India

E-mails: bshenoyk@gmail.com, rashmirajk82@gmail.com, khegde_sh2003@yahoo.co.in

Received 25 July 2016, revised 30 November 2016

The presence of immunostimulant compounds in higher plants has been extensively reviewed but only a limited number of immunomodulatory products of plant origin have been reported. The present investigation was designed to get an in depth understanding of the efficacy of crude extracts of leaf of *Barringtonia acutangula* as immunomodulatory agent on experimental rats. The oral administration of aqueous and methanolic leaf extracts (LWBA and LMBA) of *B. acutangula* for 14 days was found to stimulate the non specific arm of immunity. Haemagglutinating antibody (HA) titre test was performed to know humoral antibody response of LWBA and LMBA extracts at 200 mg/kg and 400 mg/kg doses. Results obtained showed significant ($p < 0.001$) increase in antibody production in response to sheep red blood cells (SRBCs) at both doses when compared with Cyclophosphamide treated control group. Cyclophosphamide induced suppression of humoral immune response was significantly attenuated by daily oral treatment of LWBA and LMBA extracts at a dose of 400 mg/kg. LWBA extract showed slightly more HA titre than LMBA extract. The results justify that the LWBA and LMBA extracts of *B. acutangula* have a strong potential to be explored further as an immune-based herbal therapy.

Keywords: *Barringtonia acutangula*, Cyclophosphamide, HA titre, Immunomodulation, Vit. E

IPC Int. Cl.[8]: A61K, A61K 31/00, C07D 311/92, A61K 39/00, G01N 33/53

Modulation of immune response to maintain a disease free state has been an interesting topic in recent times and the concept of *Rasayana* in *Ayurveda* is based on this principle. The function and efficacy of immune system may be influenced by many exogenous factors like food and pharmaceuticals resulting in either immune-stimulation or immune-suppression. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and immunomodulatory activity[1]. Studies conducted by Bamunnarachi & De Silva[2] have shown that such drugs are well tolerated by the patients. *Barringtonia acutangula* (L.) Gaertn, commonly known as *Samudraphala*, belongs to the family Barringtonaceae (Lecythidaceae). It is found throughout India, Sri Lanka and North Australia. The root, leaves and fruits of *B. acutangula* have been known in *Ayurveda* to be beneficial for jaundice, liver disorders, stomach disorders, etc.[3]. Previously the authors have reported the hepatoprotective effect of its leaf and root extracts against CCl_4 and paracetamol induced liver damage[4,5]. A hydroalcoholic extract of *Barringtonia acutangula* root extract (EBA) has been studied by Babre *et al*. for the hypolipidemic activity on streptozotocin-induced rats[6]. Nothing much has been studied about the immunomodulatory potential leaf extracts of *B. acutangula*. Despite the popular uses of this plant in traditional medicine, there is dearth of information on its immunomodulatory potentials. Hence, the present study aims to investigate the immunomodulatory activity of different crude leaf extracts of *B. acutangula*.

Methodology

Plant materials

The leaves of *B. acutangula* were collected from Kanwathirtha, Kerala and authentication of the material was done by Department of Dravyaguna, Alva's Ayurveda Medical College, Moodbidri. A

*Corresponding author

voucher specimen No. D.G. 90 was deposited in Department of Dravyaguna, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India. The leaves were washed thoroughly in tap water, shade dried and powdered mechanically.

Preparation of extracts

The leaf powder of *B. acutangula* was subjected to soxhlet extraction using methanol for 24 h, since the per cent yield of the extract was found to be almost similar to that of 48 h and 72 h extraction procedures. The extract was concentrated under reduced pressure at 40 °C to yield a semisolid mass and further evaporated at room temperature and stored in refrigerator till use. It was referred as LMBA (percentage yield- 13.81). Water extract (LMBA) was prepared by boiling dried leaf powder in distilled water for 45 min. The extract was filtered and concentrated in water bath (percentage yield- 20.68).

Chemicals

All the chemicals and solvents used for the present investigation were of analytical grade and procured from E. Merck (India) Ltd., Mumbai.

Animals

Wistar strain, male albino rats (*Rattus norvegicus*, 150-200 g body weight) were bred and maintained under standard conditions (12 h light / dark cycle; 25 ± 2 °C, 45–60 % humidity) and were fed standard rat feed and water *ad libitum*. Care of the animals was taken as per the committee for the purpose of control and supervision on experiments on animals (CPCSEA)[7] regulations. The animals were acclimatized to laboratory conditions for a week before commencement of experiments. All experimental protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiments (Mangalore University Reg. No. 232/ CPCSEA).

Acute toxicity study

The acute toxicity study was carried out in male Wistar albino rats (200 g body weight) as per Organization for Economic Cooperation and Development (OECD) guidelines No. 425[8]. The animals were fasted overnight and next day the water extract of the leaves of *B. acutangula* suspended in gum acacia was administered orally at different dose levels (2000 mg/kg and 4000 mg/kg body weight). The animals were observed continuously for 3 h for any behavioral changes and then every 30 min for next 3 h and then upto 24 h. The animals were further monitored for next 14 days for mortality and general behaviour of animals, signs of discomfort and nervous manifestations.

Immunomodulatory study

Humoral immune response

The humoral immune response was measured as Haemagglutination titre (HA) to sheep red blood cells (SRBC) using a modified method of Mediratta *et al*.[9] as indicated in Table 1. Fresh SRBCs in Alsever's solution was obtained from authentic sources. SRBCs were washed three times in 30 mL of 0.9 % normal saline and adjusted to a concentration of 0.5 X 10^9 cells/mL for immunization. Haemagglutinating Antibody (HA) titre was carried out following the method of Puri *et al*.[10]. Subramonium *et al*. method[11] was used for total leucocyte count. Cynomethemoglobin method given by Bin- Hafeez *et al*.[12] was used for Hb count.

Statistical analysis

The data were expressed as mean ± SE, (n=6). Data for each test were analyzed using One way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test (SPSS 10.0 for Windows). Values of $p < 0.05$ were considered statistically significant.

Results

Acute toxicity study

According to the acute toxicity study, there was no mortality or behavioural changes at a dose level of 2000 mg/kg and 4000 mg/kg thus indicating a wide margin of the safety of the drug used. According to the study conducted by Mishra *et. al.*, 2011[13] *B. acutangula* extracts did not produce any mortality up to a dose of 5000 mg/kg. This finding suggests that

Table 1 — Experimental protocol for testing immunomodulatory effect of leaf extracts (LWBA & LMBA) of *B. acutangula*

Treatment groups	Dose and route of treatment
I. Normal control	A single daily dose of 1 mL saline p.o. for 14 days.
II.Toxic (cyclophosphamide) control	A single daily dose of 1 mL saline p.o.for 14 days. cyclophosphamide 100 mg/kg, p.o. on 9th day.
III Standard (Vit. E) Control	A single daily dose of 150 mg/ kg Vit. E p.o. for 14 days.
IV Test Groups (LWBA & LMBA)	A single daily dose of 200 mg/kg or 400 mg/kg of extracts, p.o. for 14 days.

the leaf extract was safe or non-toxic to rats and hence the doses of 200 mg/kg and 400 mg/kg, po were selected for the study of hepatoprotective activity. The middle dose (200 mg/kg) was approximately one tenth of the maximum dose used during acute toxicity studies and a high dose (400 mg/kg) was twice that of one tenth dose.

Immunomodulatory study

One of the earliest immune response can be seen and measured by studying the hematological parameters of an animal. Blood cells are the first cells to respond to invading non self materials. Haemagglutinating antibody (HA) titre test was performed to know humoral antibody response of LWBA and LMBA extracts at 200 mg/kg and 400 mg/kg doses. Results obtained showed significant ($p < 0.001$) increase in antibody production in response to SRBCs at doses 200 mg/kg and 400 mg/kg when compared with Cyclophosphamide treated control group. Cyclophosphamide induced suppression of humoral immune response (HA titre 5.8) was significantly attenuated by daily oral treatment of LWBA and LMBA extracts (7.1 & 7.5 respectively) at a dose of 400 mg/kg. The values obtained were more or less equal to normal control and Vit. E control groups as presented in Table 2. LWBA extract showed slightly more HA titre than LMBA extract.

Fig. 1 provides data on effect of LWBA and LMBA extracts on total leukocyte count. When compared to cyclophosphamide treated control group total leukocyte number was found increased in the 200 mg/kg and 400 mg/kg of LWBA and LMBA extracts in dose dependent manner. More number of leukocytes was found in LMBA extract than LWBA. In the present study both LWBA and LMBA extracts caused significant increase in hemoglobin level in treated animals (13.4 g/mL & 12.9 g/mL, respectively). The group which received 400 mg/ kg of drug showed highest hemoglobin level than the one which got 200 mg/kg of drug (Fig. 2). The values were found to be almost similar to Vit. E control group.

Table 2 — Effect of the leaf extracts of *B. acutangula* (viz LWBA & LMBA) on HA titre

Group	Dose (mg/kg po)	HA titre
I. Normal control	5 mL	8.2 ± 0.23
II. Cyclophosphamide	100	5.8 ± 0.23[a]
III. Vitamin E	150	8.5± 0.51[b]
IV. LWBA	200	6.8± 0.16[b]
	400	7.1± 0.29[b]
V. LMBA	200	6.6± 0.38[b]
	400	7.5± 0.57[b]

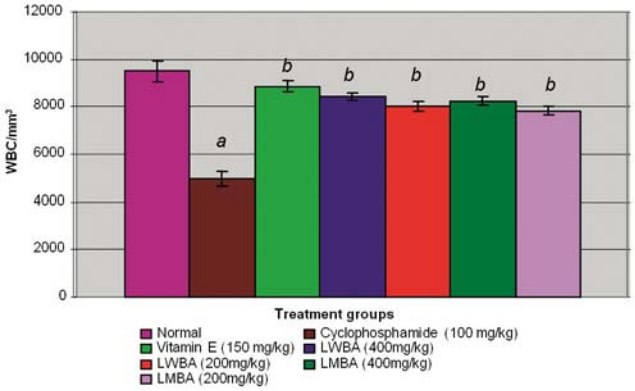

Fig. 1— Effect of LWBA and LMBA extracts on total leukocyte count [Values are mean ± SE, n=6] [a]$p < 0.001$ when compared with normal control group, [b]$p < 0.001$ when compared with Cyclophosphamide treated control group.

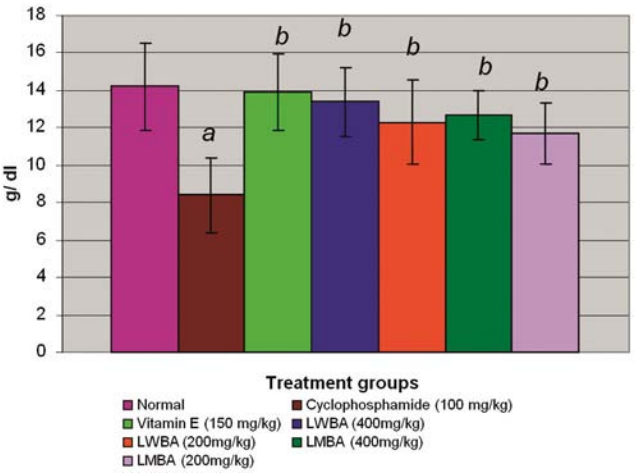

Fig. 2 — Effect of LWBA and LMBA extracts on Hb count [Values are mean ± SE, n=6] [a]$p < 0.001$ when compared with normal control group, [b]$p < 0.001$ when compared with Cyclophosphamide treated control group.

Discussion

Immune system is a remarkably sophisticated defense system seen in vertebrates to protect them against invading agents. Varieties of cells and molecules were generated by the immune system to recognize and eliminate the foreign substances. Modulation of the immune system involves induction, expression, amplification or inhibition of any part of the immune system. Immunopharmacology is a comparatively new and developing branch of pharmacology which aims at searching for immunomodulators. According to the studies conducted by Alamgir & Uddin[14], medicinal plants

and their active components have shown to be an important source of such immunomodulators. Immunomodulatory agents isolated from plants generally enhance the immune responsiveness of an organism by activating the immune system. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional treatment modalities[15]. Several medicinal plants have been investigated for immunomodulatory potentials and they are proved to be beneficial for the alteration in the immune system[16].

In the present study, *B. acutangula* showed an overall stimulatory effect on humoral immunity in rats. The HA titre test, total leukocyte count and Hb count was performed to assess the immunomoduatory effect of *B. acutangula*. The enhancement of antibody responsiveness to SRBC in rats in this study indicated the increased activation of macrophages and B lymphocyte subsets involved in the antibody synthesis as shown by Benacerraf[17]. Both LWBA and LMBA extracts, when administered orally, significantly ($p < 0.001$) increased the immune response as evidenced by an increase in the HA titre, total leukocyte and Hb count. The increase in all the three parameters showed dose response relationship since the values were found to be more at a dose of 400 mg/kg (Table 2 & Figs. 1-2). Similar observations were made by Halder *et al*.[18] while working with immunomodulatory activity of *Terminalia arjuna* bark powder on experimental rats.

Our earlier studies have shown the presence of various phytochemicals such as steroids, alkaloids, tannins, triterpenoids, saponins and phytosterols in *B. acutangula*[4]. Some of these compounds might have immunostimulatory effects as demonstrated by Mediratta *et al*.[9]. These constituents also possess antioxidant properties and they may induce the lmmunostimulant effect, as several antioxidants have been reported to possess immunomodulatory properties[19,20].

In this study, the oral administration LWBA and LMBA extracts of *B. acutangula* for 14 days was found to stimulate the non specific arm of immunity. Moreover, both the extracts were found to enhance the antibody-mediated immune response. This was evident by high titer value in HA assay, increased leukocyte count and Hb count. Hence, the LWBA and LMBA extracts of *B. acutangula* have a strong potential to be explored further as an immune-based herbal therapy. However, based on the results obtained in the present investigation, it seems that more detailed studies need to be conducted regarding the mechanism of immunomodulation of this plant.

Immunomodulation using medicinal plants can provide an alternative to conventional therapies for a variety of diseases. There is a great potential for the discovery of more specific immunomodulators which mimic or antoginize the biological effects of cytokines and interleukins. The results obtained in the present investigation show that LWBA and LMBA extracts of *B. acutangula* produces stimulatory effect on the humoral immune response in the experimental animals thus suggesting its therapeutic usefulness in treating disorders of immunological origin**.**

Acknowledgement

The authors are thankful to the authorities of Alva's Education Foundation, Moodbidri, Karnataka, India, Department of Applied Zoology, Mangalore University, Mangalagangotri, Karnataka, India and A Shama Rao Foundation Mangalore, Karnataka, India for the facilities.

References

1 Patwardhan B, Kalbag D, Patki PS & Nagsampagi BA, Studies on Immunomodulatory agents, A review, *Indian Drugs*, 28 (1991) 249-254.

2 Bamunnarachi A & De Silva KT, An ethnopharmacognostic approach to the search for immunomodulators of plant origin, *Planta Med*, 55 (1989) 339- 348.

3 Kirtikar KR & Basu BD, Indian medicinal plants, 3rd edn, Vol V, edited by Blatter E, Caius JF, Mhaskar K, (Lalit Mohan Basu, Allahabad) 1935.

4 Rashmi K, Shenoy KB, Hegde K & Shabaraya AR, Heaptoprotecive effect of various extracts of *Barringtonia acutangula* (L.) Gaertn. against CCl_4 induced hepatic damage, *J Pharm Res*, 4 (2) (2011) 540-542.

5 Rashmi K, Shenoy KB & Hegde K, Hepatoprotective and *in vitro* antioxidant studies of *Barringtonia acutangula* (L.) Gaertn., *J Pharm Rese*, 5 (7) (2012) 3622-3625.

6 Babre NP, Debnath S, Manjunath YS, Deshmaukh G, Hariprasath K, *et al.*, Hypolipidemic effect of hydro-alcoholic effect of *Barringtonia acutangula* L. root extract on streptozotocin-induced diabetic rats, *J Pharmacuet Sci Tech*, 2 (11) (2011) 369-371.

7 CPCSEA-http.//envfor.nic.in/divisions/awd/cpcsea_laboratory.pdf.

8 OECD, Guidelines for testing of chemicals, Acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Adjustment No. 425 (2001) 1-30.

9 Mediratta PK, Sharma KK & Singh S, Evaluation of immunomodulatory potential of *Ocimum sanctum* seed oil and its possible mechanism of action, *J Ethnopharmacol*, 80 (2002) 15 - 20.

10 Puri A, Saxena RP, Saxena KC & Srivastava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbortristis* L., *J Ethnopharmacol,* 42 (1994) 31 - 37.

11 Subramonium A, Rajasekaran S & Latha PG, Evans DA, Pushpangadan P. Immunomodulatory and antitumor activities of *Janakia arayalpathra* root, *Fitoterapia*, 67 (1996) 140- 145.

12 Bin- Hafeez B, Haque R, Parvez S, Pandey S, Syeed I, *et al.*, Immunomodulatory effect of *Trigonella foenumgraecum* L. extract in mice, *Int Immunophamacol*, 3 (2003) 257- 265.

13 Mishra S, Sahoo S, Rout KK, Nayak SK, Mishra SK, *et al*., Hepatoprotective effect of *Barringtonia acutangula* Linn. leaves on carbon tetrachloride-induced acute liver damage in rats, *Indian J Nat Prod Res*, 2 (4) (2011) 515- 519.

14 Alamgir M & Uddin SJ, Recent advances on the ethnomedicinal plants as immunomodulatory agents, *Ethnomedicine*, (2010) 227-244.

15 Wagner H & Proksch A, Immunomodulatory drugs of fungi and higher plants in economic and medicinal plant research, (Academic Press, London), 1983.

16 Mukherjee PK, Neema NK, Bhadra S, Mukherjee D, Braga FC, *et al.*, Immunomodulatory leads from medicinal plants, *Ind J Tradit Knowle,* 13(2) (2014) 235-256.

17 Benacerraf B, A hypothesis to relate the specificity of T lymphocytes and the activity of I region specific Ir genes in macrophages and B lymphocytes, *J Immunol,* 120 (1978) 1809 - 1812.

18 Halder S, Bharal N, Mediratta PK, Kaur I & Sharma KK, Anti- inflammatory, Immunomodulatory and antinociceptive activity of *Terminalia arjuna* Roxb bark powder in mice and rats, *Ind J Exp Biol*, 47 (2009) 577 - 583.

19 De La Fuente M & Victior MM, Antioxidants as modulators of immune system. *Immunol Cell Biol*, 78 (2000) 49-54.

20 Devasagayam TPA & Sainis KB, Immune system and antioxidants, especially those derived from Indian medicinal plants, *Ind J Exp Biol*, 40 (2002) 639-655.