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Screening of *Polyalthia longifolia* Leaves as Potential Immunomodulatory Target

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ABSTRACT
Polyalthia longifolia Thwaites (Annonaceae) (PL) is a well known plant with major focus as antifungal and antibacterial agent. The present study highlights the immunomodulatory prospects of PL leaves with its prior identification of rutin in previous studies. Ethanolic extract at three dose levels (100, 250, 500 mg kg^{-1}) was studied by Haemagglutination Antibody (HA) Titre and Delayed-Type Hypersensitivity (DTH) response using Sheep Red Blood Cells (SRBCs, 0.5×10^{9}) as antigens. Carbon Clearance (CC) test (Phagocytic Index) was estimated by Indian ink suspension. Complete Freud's Adjuvant (CFA) induced arthritic model was studied with estimation of rat paw edema, body weights, arthritic index and biochemical parameters after acute toxicity studies. Acute toxicity results revealed that the extracts were found to be therapeutically safe upto 5000 mg kg^{-1}. No significant immunoeffect was observed at 100 mg kg^{-1}. Dose levels of 250 and 500 mg kg^{-1} showed significant immunostimmulation with HA Titre and DTH reaction (after 48 h). Phagocytic index (0.0143±0.001 and 0.0180±0.001) and CFA model showed decreased paw volume and knee joint erosion. Enhanced body weights and satisfactory biochemical results with increasing dose of the extract with arthritic index (1.29±0.13 and 1.19±0.11), respectively suggested satisfactory immunomodulatory prospective. Results were interpreted by One-way ANOVA followed by Dunnett test was used to undergo analysis. *Polyalthia longifolia* leaves could serve as a promising immunostimulator for treatment and prevention of immunodeficiency disorders in future.

Key words: Haemagglutination antibody, delayed hypersensitivity, phagocytic index, freund's adjuvant, immunomostimulation

INTRODUCTION

Immune system plays a pivotal role against vast number of invading micro-organisms with skin acting as the first barrier of protection leading to a variety of reactions with antigens (living or non-living) followed by various outcomes such as increase in temperature and pH which may be beneficial or injurious to the host. Allergy and immunology, related to a variety of primary and secondary immunodeficiency disorders is one of the most rapidly increasing areas of medical research in humans (Ananthanarayan and Panikar, 2000a-c). Local plants under the umbrella of non-rasayana are explored as potential targets for immunotherapy since present allopathic drugs are supported with life threatening side effects of growth retardation, osteopenia, hyperglycemia, nephropathy, hypertension, wound healing and increased chances of risk of infection (Gupta, 2009; Thatte and Dahanukar, 1986). The concept reveals that these plants are thought to contain 'Ama' which serve as immunologically active complexes generated in intestine due to improper digestion of food having pathogenesis in ulcerative colitis, rheumatoid arthritis and multiple diseases involving liver which has crystal-clear background of immunology (Doshi *et al*., 2013). Some of the

plants with established immunomodulatory potential are *Glycyrrhiza glabra*, *Uncaria tomentosa*, *Tinospora cordifolia*, *Allium asativum*, *Aloe vera*, *Angrographis paniculata*, *Asparagus racemosus*, *Azadirachta indica*, *Curcuma longa*, *Nycanthes arbortristis*, *Ocimmum sanctum*, *Panax ginseng*, *Pylanthus embilica* etc (Gulati *et al*., 2002).

Thus, literature review revealed a need to explore local plants which are enriched with active biological molecules and can act as probable targets for immuno related therapies across the globe. The Annonaceous plants are well known in the literature as folk medicine as well as in treatment of septic infections, coughing, hepatospleenomegaly, hepatomegaly, diarrhea and cancer (Warrier *et al*., 1994). The plant has been reported as anticancer, antimicrobial, anti-inflammatory, antiulcer, analgesic and hepatoprotective (Malairajan *et al*., 2006; Ghosh *et al*., 2008; Sampath and Vasanthi, 2013; Jothy *et al*., 2012; Sharma *et al*., 2011; Katkar *et al*., 2010). In stepping up to previous research studies, a local plant *Polyalthia longifolia* Thwaites belonging to this family was screened for its probable immunomodulatory activity after a concise observation of reported active constituent such as rutin which plays an important role as in treatment of arthritis, cancer and infectious disorders (Maury *et al*., 2012).

The present study aimed to evaluate the immunomodulatory effects of *Polyalthia longifolia* leaves with its prior identification of rutin.

MATERIALS AND METHODS

Chromatographic studies: Selected part of the above mentioned local plant was subjected to extraction followed by analytical studies for isolation of active constituent (Doshi *et al*., 2014). These extracts were stored at 8-15°C for proceeding studies related to immunomodulatory models.

Standard drugs and chemicals: Ashwagandha Churna (Dabur, India Ltd.) used as standard herbal drug was procured from local market which is reported to possess well known immunomodulatory activity (Gupta *et al*., 2006). Indian ink was procured from Camlin India Ltd., Mumbai and Complete Freund's Adjuvant (CFA) from Difco Laboratories. All the chemicals used throughout the experimentation purpose were procured from local suppliers.

Animal studies: The protocol for animal studies was approved by Institutional Animal Ethics Committee of Vivekanand Education Society's College of Pharmacy, Mumbai, Maharashtra, India (VESCOP/07/2014). Albino Wistar rats of either sex obtained from the Haffkine institute, Mumbai, each weighing between 120-150 g were used. They were divided into five groups for each model.

- **Group I:** Served as control, which received 1% Sodium CMC solution at 1 mL kg^{-1} b.wt., p.o
- **Group II:** Received PL extract at 100 mg kg^{-1} b.wt., p.o
- **Group III:** Received PL extract at 250 mg kg^{-1} b.wt., p.o
- **Group IV:** Received PL extract at 500 mg kg^{-1} b.wt., p.o
- **Group V:** Received standard at 100 mg kg^{-1} b.wt., p.o

Experimental conditions: The animal experimental area was air-conditioned with adequate air changes per hour with a light cycle of 12 h light and 12 h dark, temperature 22±1°C and relative humidity 65±10%. They were housed in groups of three in polycarbonate cages with paddy husk bedding and fed with standard pellet food and Genpure RO water provided *ad libitum*. The animals were fasted for approximately 16 h before studies with free access to water.

Antigen: Fresh blood was collected from sheep sacrificed in the local slaughter house. Sheep red blood cells (SRBCs) were washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5×10^9 cells mL^{-1} for immunization and challenge.

Induction of arthritis: Arthritis was induced in rats by injecting 0.1 mL of CFA in to the left hind paw. The CFA used, consists of 5 mg *Mycobacterium butyricum* suspended in heavy paraffin oil by thoroughly grinding in mortar and pestle to give concentration of 5 mg mL^{-1}.

Acute toxicity: Acute toxicity studies were undertaken as per OECD guidelines 423 (OECD., 2001). The extract was dissolved in 0.5% carboxymethyl cellulose (vehicle). Female rats were administered a single dose of the extract at 5000 mg kg^{-1} b.wt., p.o. (administration volume was 1 mL/100 g):

- **Mortality/viability:** The condition was observed during the first 30 minutes and at approximately 1, 2, 3 and 4 h after administration of the extract on test day 0 (in common with the clinical signs) and twice daily during the acclimatization period (at least once on day of sacrifice)
- **Body weights:** The animals were weighed on test days 0 (prior to administration) and on day 7, 14 and at death
- **Clinical signs:** Clinical signs were monitored, during the first 30 min and at approximately 1, 2, 3 and 4 h after administration of the extract on test day 0 and once daily during the acclimatization period
- **Macroscopic findings:** All surviving animals were sacrificed at the end of the observation period by carbon dioxide in euthanasia chamber after the gross/macroscopic pathological changes were observed and recorded. No organs or tissues were retained

Animal models: Animal models were proposed by Shukla *et al*. (2009), Bader (1997) and Gaur *et al*. (2009) which are as follows:

Haemagglutination antibody (HA) titre: The rats were immunized by injecting 0.1 mL of SRBCs suspension containing 0.5×10^9 cells intraperitoneally on day 0. Vehicle, extracts and standard were given orally to the animals for 7 days. Blood samples were collected in micro-centrifuge tubes from individual animal by retro-orbital puncture on day 7. The blood samples were centrifuged and serum was obtained. Equal volumes of individual serum samples of each

group were pooled. Two-fold serial dilutions of pooled serum samples made in 25 µL volume of normal saline in microtitration plates were added to 25 µL of 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37°C for 1 h and examined for haemagglutination under microscope. Antibody levels were determined by taking the reciprocal of the highest dilution of the test serum agglutination as the antibody titre.

Delayed-type hypersensitivity (DTH) response: The rats were challenged by injection of 0.5×10^9 cells SRBCs in right hind foot pad. Foot thickness was measured after +24 and +48 h of this challenge. The extract was administered orally on day 0 and continued till day 7 of challenge. Right hind footpad thickness was measured with micrometer screw gauge on 7th (prior to injection), 8th and 9th day of the study. Difference between prior and post injection footpad thickness was reported as DTH response.

Phagocytic response (carbon clearance test): The rats were treated from day 0-7 with different concentrations of the extracts. On day 7, all the animals of the entire groups received the treatment of an intravenous injection of 0.2 mL/animal Indian ink dispersion (pre-warmed at 37°C) through tail vein. Blood samples were collected from retro-orbital plexuses immediately at 0, 5, 10 and 15 min after the injection of Indian ink. Twenty five microliter of blood samples were added to 2 mL of 0.1% sodium carbonate solution. Absorbance of the samples was measured at 660 nm using UV visible spectrophotometer. The rate of carbon clearance (phagocytic index, K) was calculated using the following equation:

$$\text{Rate of carbon clearance (K)} = \frac{\log OD_2 - \log OD_1}{T_2 - T_1}$$

where, OD_1 and OD_2 are the optical densities and T_1 and T_2 is time in minutes, respectively.

Adjuvant arthritis: Vogel (2002), Krensky *et al.* (2005), Petchi *et al.* (2013), Subash *et al.* (2012), Kuncha *et al.* (2014) and Chattopadhyay *et al.* (2004) stated the adjuvant arthritis methods. The rats, after induction of Complete Freund Adjuvant (CFA) were administered with extracts and standard from day 1 and continued dosing till 12 days.

Paw volume: Paw volumes of both the sides was measured with plethysmometer and body weights were recorded on the day of injection. Rats were not dosed with extracts or the standard from 13th to 21st day. Rat paw volume was measured on 1st, 3rd, 5th, 9th, 13th and 21st day in order to record the primary and secondary lesions. Mean changes in injected paw edema with respect to initial paw volume, were calculated using the equation as:

$$\text{Percentage inhibition of paw edema} = \left(1 - \frac{\text{Mean change in paw volume of treated rat}}{\text{Mean change in paw volume of untreated rat}}\right)\times 100$$

Primary and secondary lesions: Primary lesions refer to edema formation in the injected hind paw that peaks 3-5 days after injection of the phlogistic agent and is measured on day 5 by calculating percent inhibition of the edema volume of the injected left paw over the vehicle control. Secondary lesions are immunologically mediated changes characterized by inflammation of the noninjected sites (hind leg, forepaws, ears, nose and tail) paw over the vehicle control. Percent reduction in body weight is calculated using the equation:

$$\frac{B_0 - B_{21}}{B_0}\times 100$$

where, B_0 and B_{21} is body weight recorded on day 0 and 21, respectively.

Arthritic index: An arthritic index is calculated as the sum of the scores as shown in Table 1 for each animal. The average of treated animals is compared with the control group.

Biochemical parameters: Parasuraman *et al.* (2010) analysed the biochemical parameters. At the end of the 21st day, blood samples were withdrawn from all groups through retro-orbital plexus puncture by anaesthetizing the animals with diethyl ether and the biochemical parameters such as hemoglobin content, total WBC count and RBC count were analyzed.

Histological determination: Kilimozhi *et al.* (2009) determined the historical parameters on 21st day, the rats were subjected to appropriate euthanasia. The hind paws were amputated above the knee joint, fixed in 7.4% formalin, decalcified using 10% nitric acid, embedded in paraffin and sectioned in a mid-sagittal plane. The Transverse Section (TS) of knee joint of tarsals were stained with haematoxylin and eosin and examined microscopically for bone destruction, presence of mononuclear infiltration and pannus formation.

Table 1: Arthritic index for each animal

Lesion site and nature of lesion	Score
Ears	
Absence of nodules and redness	0
Presence of nodules and redness	1
Nose	
No swelling of connective tissue	0
Intensive swelling of connective tissue	1
Tail	
Absence of nodules	0
Presence of nodules	1
Forepaws	
Absence of inflammation	0
Inflammation of at least 1 joint	1
Hind paws	
Absence of inflammation	0
Slight inflammation	1
Moderate inflammation	2
Marked inflammation	3

Statistical analysis: The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnett test. The values were expressed as Mean±SEM and *p<0.05, **p<0. 01, ***p<0.001 when compared with control group was considered significant.

RESULTS

Acute toxicity

Mortality/viability: No mortality was reported up to 5000 mg kg^{-1} b.wt.

Body weights: All surviving animals had gained body weight by day 14 as compared to day 0.

Clinical signs: All the animals appeared normal throughout the experimental period.

Macroscopic findings: No abnormalities were detected for any of the animals at terminal sacrifice.

Animal models (Immunomostimulatory effects)

Haemagglutination antibody (HA) titre: The PL extract showed mild (100 mg kg^{-1}) and significant immunostimmulation (250 and 500 mg kg^{-1}) as depicted in Table 2.

Delayed-Type Hypersensitivity (DTH) response: The results interpreted showed dose dependent decrease in paw edema after 24 h of challenge which was observed at all dose levels of PL extract when compared to control group. The PL extracts (100, 250 and 500 mg kg^{-1}) after 48 h lowered the paw thichness depicting good immunostimulatory activity (Table 2).

Phagocytic response (carbon clearance test): The model showed prominent phagocytic index (0.0125, 0.0143 and 0.0180 at 100, 250 and 500 mg kg^{-1} b.wt., respectively) suggesting sufficient immunostimulatory activity of the extract as compared to control group (Table 3).

Adjuvant arthritis

Paw volume: The percentage of inhibition expressed that of PL extract (100 mg kg^{-1} mild and 250, 500 mg kg^{-1} moderate activity) and standard (100 mg kg^{-1} significant) b.wt., p.o reduced paw swelling induced by CFA when compared to the control group from days 5-21 (Table 4).

Primary and secondary lesions: *Polyalthia longifolia* extract at 250 and 500 mg kg^{-1} b.wt., p.o and standard (100 mg kg^{-1}) b.wt., gained body weights as compared to control group (Table 5).

Arthritic index: The PL extract showed good arthritic index as compared to control arthritic group at 250 and 500 mg kg^{-1} b.wt. (Table 5).

Biochemical parameters: PL extract at 500 mg kg^{-1} b.wt., significantly enhanced the levels of RBCs, WBCs and Hb as compared to the control arthritic group which showed decreased levels (Table 6).

Histological determination: Histological studies on transverse section of knee joint reports showed severe bone erosion with inflammatory infiltration in control group whereas PL extract (100 mg kg^{-1} showed the synovial tissue consists of focal loss of continuity in the synovial cells with inflammatory infiltration (Long-Arrow) with vascular spaces (Short-arrow), 250 mg kg^{-1} section studied shows bone trabeculae covered with intact articular hyaline cartilage

Table 2: *In vivo* assessment of immunomodulatory activity using HA titre and DTH

Groups	Treatment	Hemagglutination antibody titre (Mean±SEM)	Delayed hypersensitivity reaction (Mean±SEM)	
			24 h	48 h
Group I (Control)	Sodium CMC (0.5%)	117.3±10.67	0.54±0.03	0.52±0.02
Group II	PL (100 mg kg^{-1} b.wt., p.o)	106.6±13.50	0.46±0.01	0.44±0.02
Group III	PL (250 mg kg^{-1} b.wt., p.o)	256.0±57.25	0.56±0.01	0.42±0.01*
Group IV	PL (500 mg kg^{-1} b.wt., p.o)	341.3±53.98**	0.54±0.01	0.39±0.01 **
Group V (Standard)	100 mg kg^{-1} b.wt., p.o	384.0±57.12****	0.52±0.03	0.37±0.02****

Values are expressed as Mean±SEM (n = 6), *p<0.05, **p<0.01, ****p<0.001 when compared with control group. One-way ANOVA followed by Dunnett test

Table 3: Phagocytic index (PI)

Groups	Treatment	Phagocytic index (Mean±SEM)			
		0-5 min	0-10 min	0-15 min	Mean PI
Group I (Control)	Sodium CMC (0.5%)	0.0076±0.004	0.0109±0.002	0.0105±0.001	0.0097±0.002
Group II	PL (100 mg kg^{-1} b.wt., p.o)	0.0085±0.002	0.0149±0.001	0.0142±0.001	0.0125±0.001
Group III	PL (250 mg kg^{-1} b.wt., p.o)	0.0113±0.002*	0.0161±0.001*	0.0155±0.001*	0.0143±0.001*
Group IV	PL (500 mg kg^{-1} b.wt., p.o)	0.0166±0.001**	0.0183±0.002**	0.0191±0.002**	0.0180±0.001**
Group V (Standard)	100 mg kg^{-1} b.wt., p.o	0.0178±0.003****	0.0186±0.003****	0.0209±0.003****	0.0191±0.003****

Values are expressed as Mean±SEM (n = 6), *p<0.05, **p<0.01, ****p<0.001 when compared with control group using one-way ANOVA followed by Dunnett test

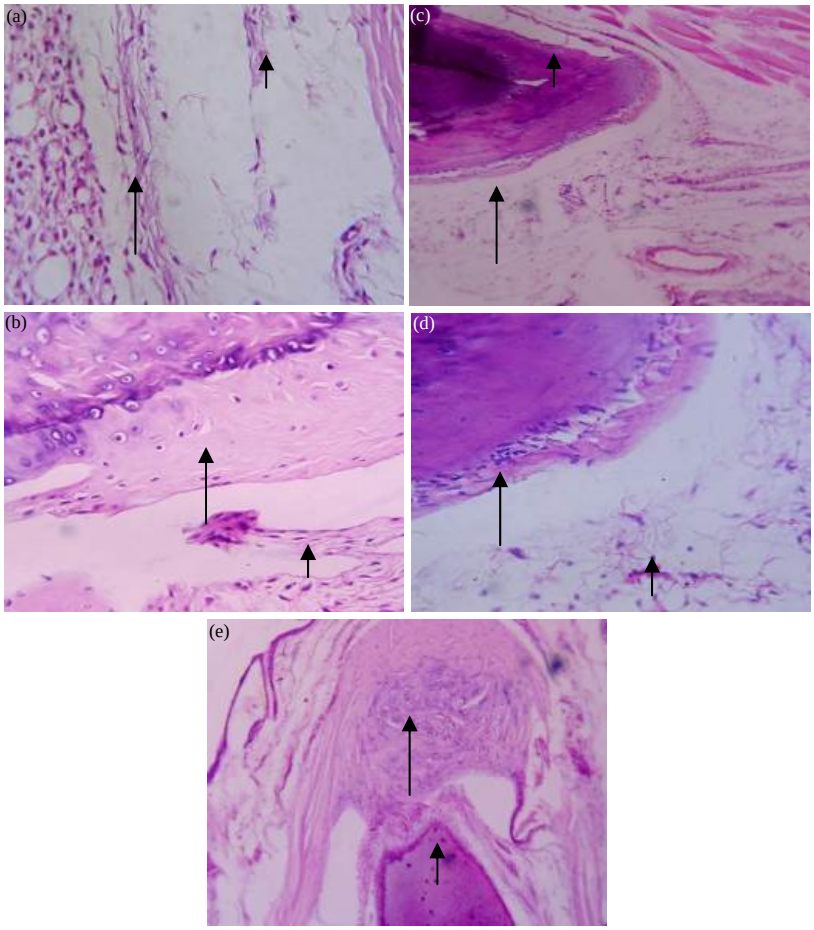

Fig. 1(a-e): TS of knee joint, (a) Control, (b) PL-100, (c) PL-250, (d) PL-500 and (e) STD

Table 4: Mean changes in paw volume using plethysmometer in adjuvant induced arthritis

		Mean changes in paw volume					
Groups	Treatment	Day 1	Day 3	Day 5	Day 9	Day 13	Day 21
Group I (Control)	Sodium CMC (0.5%)	1.483±0.08	2.700±0.03	3.000±0.06	3.183±0.07	3.033±0.06	3.100±0.05
Group II	PL (100 mg kg^{-1} b.wt., p.o)	1.367±0.05(7.9)	2.633±0.06(2.5)	2.750±0.07(8.3)	2.767±0.10***(13.0)	2.650±0.09***(12.62)	2.567±0.08***(17.1)
Group III	PL (200 mg kg^{-1} b.wt., p.o)	1.417±0.06(4.5)	2.617±0.03(3.0)	2.583±0.06***(13.9)	2.483±0.04***(21.9)	2.350±0.02***(22.51)	2.317±0.03***(25.25)
Group IV	PL (500 mg kg^{-1} b.wt., p.o)	1.400±0.07(5.5)	2.567±0.08(4.9)	2.467±0.04***(17.7)	2.383±0.04***(25.1)	2.267±0.04***(25.25)	2.183±0.05***(29.58)
Group V (Standard)	100 mg kg^{-1} b.wt., p.o	1.467±0.08(1.1)	2.400±0.03***(11.11)	2.233±0.07***(25.56)	2.067±0.06***(35.06)	1.167±0.04***(61.52)	1.1048±0.05***(66.19)

Values are expressed in Mean±SEM (n = 6), when compared with control, *p<0.05, **p<0.01, ***p<0.001, One-way ANOVA followed by Dunnett test.() means % inhibition

Table 5: Changes in body weight in adjuvant induced arthritis

		Mean changes in body weight (gms) (Mean±SEM)		
Groups	Treatment	Day 0	Day 21	Arthritic index (Mean±SEM)
Group I (Control)	Sodium CMC (0.5%)	160.3±2.23	148.3±1.80	2.10±0.39
Group II	PL (100 mg kg^{-1} b.wt., p.o)	152.0±2.26	145.0±2.25	1.73±0.64
Group III	PL (250 mg kg^{-1} b.wt., p.o)	153.2±2.21	159.7±2.70**	1.29±0.13**
Group IV	PL (500 mg kg^{-1} b.wt., p.o)	152.7±2.80	164.3±2.37***	1.19±0.11***
Group V (Standard)	100 mg kg^{-1} b.wt., p.o	157.8±2.61	168.2±2.52***	1.093±0.08***

Values are expressed in Mean±SEM (n = 6), when compared with control, *p<0.05, **p<0.01, ***p<0.001, One-way ANOVA followed by Dunnett test

(Long-arrow) and adjacent synovial tissue (Short-arrow), 500 mg kg^{-1} depicted that the synovial tissue consists of focal loss of continuity in the synovial cells with inflammatory infiltration (Long-arrow) and edema separating the fibrous tissue with vascular spaces (Short-arrow) in dose dependent manner and the standard showed section of the synovial tissue consists of intact synovial membrane with proliferation of synovial cells (long-arrow) and outer layer shows increased fibrous tissue (short-arrow) with vascular spaces and scattered inflammatory cells (Fig. 1a-e).

Table 6: Effect of hematological parameters

Groups	Treatment	Mean changes in Hematological parameters (Mean±SEM)		
		RBC ($\times 10^6$ cells/mm^3)	WBC ($\times 10^3$ cells/mm^3)	Hb (g %)
Group I (Control)	Sodium CMC (0.5%)	1.16±0.13	1.99±3.17	4.70±0.50
Group II	PL (100 mg kg^{-1} b.wt., p.o)	2.26±0.22	2.33±0.36	5.73±0.41
Group III	PL (250 mg kg^{-1} b.wt., p.o)	3.03±0.38*	3.38±0.30	7.01±0.65 *
Group IV	PL (500 mg kg^{-1} b.wt., p.o)	3.39±0.46***	4.91±0.72*	9.07±0.49 ****
Group V (Standard)	100 mg kg^{-1} b.wt., p.o	4.72±0.43****	8.68±1.05****	12.10±0.73****

Values are expressed in Mean±SEM (n = 6), when compared with control, *p<0.05, ***p<0.01, ****p<0.001, One-way ANOVA followed by Dunnett test

DISCUSSION

Leukocytes regarded as guard cells consists of two major divisional sections viz., granular and non-granular cells. The former encompasses of neutrophils, basophiles and eosinophiles responsible for macrophage phagocytic mechanism whereas, later includes lymphocytes (B-cells-Bone marrow and T-cells-Thymus) and monocyctes participate in specific and nonspecific responses. They are found in higher concentration in lymph nodes, spleen, bone marrow and thymus (Barar, 2009; Krensky *et al*., 2005; Pelczar, 1993). In present immunomodulatory studies, rats were selected which contains Ox49 antigen equivalent to functionally important protein as CD_2 in SRBC's receptor to which these antigens bind in an antigen-specific manner, catalyze cell activation and finally on incubation with SRBC's lead to rosettes formation (Bader, 1997).

Polyalthia longifolia Thwaites (Mast tree) cultivated as ornamental and noise pollution prevention tree in urban cities by residents and equally known for its vast pharmacological significance with many constituents isolated as cited in literature by many researchers guided us to select these easily available local tree for immuno studies which could serve as herbal remedy to various immune system related disorders either stimulation or suppression (Warrier *et al*., 1994; Wagner, 1984). The present results reveal that *Polyalthia longifolia* can act as potent immunostimulator acting on T and B cells by specific and nonspecific immune mechanisms in the host.

In humoral immunity, B-cells on their cell surface receptors stimulate cells with antigen specific antibodies. Short lived plasma cells recognize circulating soluble antigens in the host and under the chemical influence of T-cells secrete antibody molecules which are a product of B-lymphocytes and plasma cells. IgG and IgM are the major immunoglobulins which are involved in complement activation, opsonization, neutralization of toxins, etc. against the antigens (Miller and Peacock, 1991). In addition, long lived memory cells respond to the antigen if encountered subsequently. In case of ethanolic extract of PL leaves (Table 5) high values indicated by haemagglutination antibody titre immuno model suggested enhanced responsiveness of T and B lymphocyte subsets involved in the antibody synthesis (Benacerraf, 1978) and thereby stating the probable role of PL extract as an immunostimulator.

In case of Cell Mediated Immunity (CMI), T-lymphocytes with T specific-cell receptor (TcR) with antigen-specific molecules on their surface similar to antibodies recognize antigens when they are presented by another cell in combination with proteins of Major Histocompatibility Complex (MHC). By functioning as molecular markers they discriminate between cells that are self and nonself. CD_4 and CD_8 lymphocytes are the two major subpopulations of T-cells based on their functions and surface proteins. T-lymphocytes and their products (lymphokines) serve to carry out defense mechanisms against infectious organisms, infectious foreign grafts, tumor immunity and delayed-type hypersensitivity reactions involving effectors mechanisms due to release of mediator such as histamine (Miller and Peacock, 1991). Diagnosis of vast number of cell mediated hypersensitivities and auto-immune diseases rely on T-cell population scrutinization. Therefore, in PL extract the reaction was notified by elevation in response to T-cell dependent antigen supported the same underlying role of the PL extract as in humoral immunity related to B-cells.

Phagocytosis is a process in which phagocytes ingests and removes micro-organisms, malignant cells, inorganic particles and tissue debris. The concept of swift clearance of Indian ink has been correlated with the enhanced phagocytic activity (Miller and Peacock, 1991) further suggested by linear growth in the statistical data which interpreted that PL extract is supporting B and T-cells concepts of the immunity with more focus on macrophage activity.

Patients on steroid therapy suffer from associated side effects and hormonal imbalance due to synthetic nature of the compounds. Hence, researchers urge for plant derived drugs in order to reduce these complications. In CFA induced arthritic model, since paw swelling is one of the major factor, it has been used to assess the degree of inflammation and therapeutic efficacy (Krensky *et al*., 2005; Vogel, 2002; Habila *et al*., 2010) of the PL extract and standard groups at different time intervals and dose levels with respect to control group. Animals under the experimental study conditions developed severe inflammation and typically, hind limbs became intensely red and oedematous within 16 to 24 h time course with peak severity of arthritic condition within 3-4 day after the onset of disease. PL extract demonstrated moderate (100 mg kg^{-1} Day 9-21) and significant (250 and 500 mg kg^{-1} Day 5-21) inhibition in paw volume on respective days in comparison to the control. The mechanism suggests probable suppression of T cell-mediated immune functions involving the release of inflammatory mediators due to induction of CFA during the phase of primary lesions. Increase in body weight on 21st day and inhibition of secondary lesions suggested

significant immunomodulatory activity of the extract and standard groups. The condition of arthritis was severe in the control whereas the extract at various dose levels and standard groups gained b.w. Mean arthritic index results suggested good role of the selected part of the plant in arthritic condition. In arthritic control patients, there is a mild to moderate increase in the WBC count due to a release of IL-IB which increases the production of both granulocyte and macrophage colony stimulating factors (Parasuraman *et al*., 2010; Kumar *et al*., 2008). Researchers over the years have drawn common conclusion that migration of leukocytes produces a significant reduction in the WBC count. The model depicts the involvement of pathway in which prostaglandins, cyclooxygenase products and various cells are involved in significant inflammatory changes (Parasuraman *et al*., 2010). Hence, our research studies reveal that the arthritic rats exhibited decreased RBC, WBC counts and Hb levels correlated to indicate an anaemic condition, which is a common diagnostic feature in patients with chronic arthritis (Tiwari *et al*., 2004; Sharma and Kumar, 2009). The treatment with the extract and standard improved the RBC, WBC counts and Hb levels closer to normal suggesting significant recovery from anaemic condition and arthritic progress. Improved knee joint results indicated that the extract has dose dependent response in arthritic condition as compared to 100 mg kg^{-1} of standard.

CONCLUSION

The present investigation suggests that ethanolic extract of *Polyalthia longifolia* Thwaites leaves exert immunostimulatory effect on B and T lymphocyctes. Further studies on the constituents identification as well as different immunomodulatory models on the selected plant extract could be explored.

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