

Effect of *Pterospermum acerifolium* Willd on Inflammation and Pro-Inflammatory Cytokines

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ABSTRACT

In our present study, the effect of alcoholic fraction of *Pterospermum acerifolium* seeds on inflammation and inflammatory cytokines was evaluated in-vitro by using human peripheral blood mononuclear cells (PBMCs) and in vivo by croton oil induced mouse ear edema (acute study) and Carrageen induced rat paw edema. The extract exhibited significant inhibition of the production of tumor necrotic factor- α and interleukin-6 by PBMCs stimulated lipopolysaccharides (LPS) in a dose dependent manner. The extract, at the selected dose of 150 and 300mg/kg body weight per oral exhibited significant dose dependent anti-inflammatory response with 27.77% and 47.17% inhibition of inflammation in croton oil induced mouse ear edema and Carrageen induced rat paw edema respectively.

Keywords: *Pterospermum acerifolium*, ear edema, pro-inflammatory cytokines.

INTRODUCTION

Inflammation is regarded as multiple physiological events coupled with series of biochemical alteration. It is also regarded as protective response to a variety of stimuli which includes trauma, infection, tissue damage or hypoxia.¹ Inflammation is a group of several discrete physiological events taking place over a longer period of time. Chemicals released from the inflammatory tissue are generally believed to be responsible for the spread of inflammatory reaction. These chemicals are called endogenous chemical mediators which are capable of producing vasodilatation, increased vascular permeability, migration of neutrophils and chemotaxis of other inflammatory agents. Various endogenous autacoids namely Histamine, 5-HT, Bradykinin, Prostaglandin etc participate in inflammatory process². Prostaglandins (PGE₂) are ubiquitous substances that indicate and modulate cell and tissue response involved in inflammation. Most of the available anti-inflammatory drugs are inhibitors of prostaglandin synthesis.

Although inflammatory diseases are the oldest known to mankind, there is no substantial progress in the therapeutic regimen of inflammation in terms of efficacy and safety. Many commercially available products have produced a dramatic symptomatic improvement in inflammatory condition but cannot arrest the progress of the disease process and all of them shared some undesirable side effects³. *Pterospermum acerifolium* Willd (Sterculiaceae) commonly known as 'Kanak champa' is a shrub distributed in tropical Asia. It has been traditionally used for blood troubles, inflammation, ulcer, tumors, leprosy and for small pox eruptions⁴. Thus, the present study was attempted to explore scientifically the anti-inflammatory activity of the seeds of said plants.

The anti-inflammatory activity was evaluated in vitro by inhibition of pro-inflammatory cytokines produced by human peripheral blood mononuclear cells (PBMCs). The activity was also measured in vivo in rats by inflammation assay induced by Carrageen induced rat paw edema and mouse ear edema (acute) induced by croton oil.

MATERIALS AND METHODS

Plants materials

Seeds of *Pterospermum acerifolium* Willd were collected in the month of January, 2009, from the hill area near the bank of Subarnarekha riverside in the district of Mayurbhanj, Odisha. The collected seeds with complete herbarium was authenticated at Botanical survey of India, Central National Herbarium, Botanical Garden, Howrah, Kolkata vide Letter No. CNH/1-1(15)/2009/Tech 11/413. A sample specimen was deposited there.

Preparation of extract:

One kilogram of the air dried seeds was blended to a fine powder and extracted with Petroleum ether, chloroform, ethanol and water for 6 days (144hours). The extract was concentrated using a rota evaporator. The powder was defatted with pet ether followed by extraction with chloroform. The marc left after chloroform extraction was dried in an air oven below 30°C. The dried material was then packed in soxhlet apparatus and extracted successively with ethanol followed by water. The extraction was continued till the completion of 30 siphons each. The extract was filtered while hot, concentrated and dried under vacuum. Ethanolic extract was dissolved in normal saline for oral administration to the rats in the experiment.

Table 1: Effect of alcoholic extract of *P. acerifolium* extract on the LPS stimulated **IL-6** production, in peripheral blood mononuclear cells (PBMC).

Agent	Dose ($\mu\text{g/ml}$)	Appearance of IL-6 (Pg/ml)	% Inhibition of IL-6 production
Control	0	58467.15 \pm 3932.01	-
	1	48621.42 \pm 2134.43**	16.84
P. acerifolium extract	5	39376.14 \pm 446.60*	32.65
	10	31461.28 \pm 998.56*	46.18
	0.004	49376.47 \pm 2272.29**	15.54
Dexamethasone	0.04	19842.36 \pm 3119.87*	66.06
	0.4	11369.57 \pm 4666.78*	80.55

Note: P value VS control (by student 't' test ** $p < 0.05$, * $p < 0.01$)

Table 2: Effect of *P. acerifolium* extract on LPS stimulated **TNF- α** production in peripheral blood mononuclear cells (PBMC).

Agent	Dose ($\mu\text{g/ml}$)	Appearance of TNF- α (Pg/ml)	% Inhibition
Control	0	7981.26 \pm 412.68	-
	1	7267.22 \pm 282.31	8.94
P. acerifolium extract	5	6521.42 \pm 328.72	18.29
	10	5764.28 \pm 7.93	27.77
	0.004	6533.02 \pm 148.27	18.14
Dexamethasone	0.04	3317.04 \pm 438.88**	58.43
	0.4	1662.012 \pm 741.02**	79.17

Note: Values are expressed as mean \pm SEM (n=6)

Note: P value VS control (by student 't' test ** $p < 0.01$)

Table 3: Effect of *P. acerifolium* extract on Croton oil induced mouse ear edema.

Groups	Croton oil induced mouse ear oedema expressed as percentage	% Inhibition
Control	136 \pm 14.6	-
Acetone	2.88 \pm 8.2	-
T1	42.14 \pm 16.2**	72.13
T2	32.11 \pm 9.8*	79.22
S	22.18 \pm 12.6*	85.51

Note: P value VS control (by student 't' test ** < 0.01 , * < 0.001)

[Results are mean \pm S.E., n=6) (Dose of *P. acerifolium* extract, T1 - 150mg/kg and T2- 300mg/kg oral). Indomethacin (s- 20mg/kg, oral) and acetone 1 μl /ear.]

Animals

Wistar rats (140 – 150g) of both sexes were used for the studies. The rats were obtained from the Department of Pharmacology, Jadavpur University, Kolkata. Animals were housed in cages under standard laboratory conditions (12:12 hours light/ dark cycle at $25 \pm 2^{\circ}\text{C}$). They had free access to standard commercial diet and water. The animals were divided into groups of six and fasted for 12 hours before the experiments. The ethical guidelines for the investigation of animals used in experiments were followed in all tests.

Isolation and stimulation of human PBMCs

Blood samples were collected from healthy, overnight fasted human volunteers (age range 20-40) years. Human peripheral blood mononuclear cells (PBMCs) were isolated by ficoll- hypaque centrifugation⁵. After

centrifugation, PBMCs were collected from the interphase layer and washed four times with 10% heat activated foetal calf serum, 25ml HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2ml L-glutamine and Penicillin/ streptomycin (penicillin 5U MI^{-1} and streptomycin 50 μg MI^{-1}) procured from Sigma-Aldrich. PBMCs (9% monocytes, 76% lymphocytes with respect to total number of PBMCs) were maintained at 37°C in RPMI 1640 medium.

In vitro bioassay

Effect on lipopolysaccharides (LPS) stimulated cytokines (interleukin-6 and tumor necrosis factor - α) produced by PBMCs.

Peripheral blood mononuclear cells count was adjusted to 1×10^6 cells per ml using RPMI 1640 medium. A total of 200 μl of this suspension was added to 96 wells made in the plate. Extracts of varied concentration were added to this cell suspension. After incubation for 30min, the cells were stimulated with LPS (1 $\mu\text{g}/\text{mL}$) for 4 hrs at 37°C in an atmosphere of 5% carbon dioxide (CO_2)⁶. Each concentration was assayed in triplicates. Similarly, unstimulated cells (control) were also maintained and assayed in triplicates. After 4 hrs the plates were centrifuged at 2000 rpm for 5min, supernatant was collected and stored at 70°C and assayed using Interleukin-6 (IL-6) and tumour necrotic factor α (TNF- α) assay kit. Dexamethasone was used as reference standard.

In vivo Bioassay

Croton oil induced mouse ear oedema

In this method, the mice were divided into four groups with six mice in each group. Croton oil (0.02 mg in 25 ml acetone; topical) was applied to the left ear of each

Table 4: Effect of *P. acerifolium* extract and indomethacin on time course of carrageenin induced rat paw oedema.

Treatment	Mean Oedema Volume (ml) at different intervals following Carrageenin injection					
	1 st Hr.	2 nd Hr.	3 rd Hr.	4 th Hr.	5 th Hr.	6 th Hr.
Control	0.34 ±0.04	0.53 +0.02	0.67 +0.03	0.68 +0.01	0.57+0.02	0.55 +0.01
T ₁	0.31 +0.01	0.43 +0.01	0.46 +0.03	0.42 +0.02	0.36 +0.03	0.35 +0.03
T ₂	0.14 +0.03	0.24 +0.02	0.15 +0.02	0.06 +0.03	0.06 +0.03	0.06 +0.01
N	0.016+0.02	0.25 +0.01	0.14 +0.01	0.04 +0.02	0.03 +0.03	0.02 +0.03

Note: *P value VS control (by student 't' test ** <0.01, *<0.001)

*Mean oedema volume (ml) at different intervals following carrageenin injection

[Results are mean + SE, n=6] [Dose of *P. acerifolium* extract (T₁ = 150mg/kg, T₂ = 300mg/kg oral) and Indomethacin N = 20mg/kg oral].

animal. After 30 minutes two groups were treated with *P. acerifolium* extract (150 and 300mg/kg respectively; oral), one group with standard drug indomethacin (20mg/kg; oral) and one group with control vehicle. The right ear of the mice served as control and was treated with 25ml acetone. Animals were sacrificed after five hours under ether anesthesia and then the difference in weight of the left and right ear pouches were measured⁷.

Carrageenin induced rat paw edema

The animals were divided into four groups of six animals each. Carrageenin (0.1 ml of 1% w/v solution) was injected subcutaneously into the planter of the right hind paw of the rats. After 30 minutes, the control group received only distilled water orally, test groups received *P. acerifolium* extract. (150 or 300 mg/kg oral) and standard group were fed indomethacin (20 mg /kg) orally. The paw volume was then measured after one hour interval by using the volume displacement method with the help of plethysmometer.

RESULT AND DISCUSSION

The extract showed concentration dependent inhibition of IL-6(Table-1) and TNF - α (Table-2) production by PBMCs. Pro-inflammatory cytokine like TNF - α and IL-6 can initiate the inflammatory cascade. TNF - α has been found to stimulate the synthesis and secretion of various cytokines. Blocking of TNF - α interrupts the inflammatory process by inactivation of T-cells, macrophages, and fibro blasts⁸. IL-6 is a major mediator of inflammation and activator of signal transducer and serves to block apoptosis in cells during the inflammatory processes. Thus, the cytokine blockers made its presence felt in the treatment of various inflammatory disease mainly arthritis.

Croton oil and also its active principle 12-o-tetradecanoyl phorbol acetate (TPA), has been widely used for edema formation following topical application. In experimental models, TPA has been found to induced significant anti-inflammatory responses characterized by prostaglandin production, increased vascular permeability leading to neutrophil infiltration. Topical application of TPA may result in transient increase in arachidonic acid release and subsequent prostanoids production. It has observed that various phospholipase, lipoxygenase inhibitors as well as gluco-corticoids were effective in reducing edema, produced in response to topical application of TPA⁹. In our study oral administration of *P. acerifolium* extract significantly decrease TPA (Croton oil) induced ear

edema in mice. Thus, croton oil induced model is a suitable test procedure to screen anti-inflammatory agents. The *P. acerifolium* extract, at selected dose of 150 and 300mg/kg inhibited significant anti inflammatory activity four hrs. onwards (Table-3).

Carrageenin induced rat paw oedema is usually biphasic in nature¹⁰ and it has been observed that 2nd phase of the oedema is effectively inhibited by commonly used steroids and NSAIDs¹¹, that the early phase in carrageenin in paw might be a result of trauma caused by injection. Although, the phase is found to be transient in nature and it could be detected following the measurement of paw volume in the first hour following carrageenin administration. Further it has been suggested that the first phase is mainly mediated by release of various inflammatory mediators, namely histamine, serotonin, and bradykinin, surrounding the damaged tissue while the 2nd phase is due to generation of eicosanoids derived from the metabolism of arachidonic acid¹². Studies with *P. acerifolium* extract showed that, following oral administration there is significant inhibition of carrageenin induced paw at doses of 150 and 300mg/kg (oral) (Table-4). These observations indicate the effectiveness of the *P. acerifolium* extract against edema formation.

It was further observed that the paw volume in the control group of animals significantly increased with time and attends a maximum value at the end of four hours. Thus, our observation is in conformity with earlier reports¹². However, *P. acerifolium* extract and Indomethacin (standard anti-inflammatory agent) significantly reduced the paw volume in both phases of inflammation. Thus, our finding indicated the effectiveness of orally administered *P. acerifolium* in reducing the edemogenic activity of the various mediators involved in the early phase of inflammation.

Plant polyphenolic are well known to play major role to counteract inflammatory response¹³. Some polyphenolics like polymethoxy flavonoids suppressed IL-induced production of prostaglandin in human synovial fibroblasts cells and decrease the expression of pro-inflammatory cytokines. Quercetin and luteolin produce anti-inflammatory effect through inhibition of LPS stimulated cytokines production¹⁴. Some polyphenolics were found to be active inhibitors against both human lipoxygenase and pro-inflammatory cytokines¹⁵. Thus, literature suggested that plant polyphenols act as anti-inflammatory agent through multiple approaches chiefly through

inhibition of pro-inflammatory cytokines. In the present study the extract was found to inhibit LPS stimulated cytokines production by PBMCs. The extract also exhibited significant control on both acute and chronic animal inflammations. So, the anti-inflammatory activity may be due to the presence of polyphenol compounds and /or flavonoids in the said extract through inhibition of Pro-inflammatory cytokines.

Therefore, considering the present step of affairs with conventional NSAIDS, *P. acerifolium* extract with its probable inhibition may offer itself as a cheaper and convenient alternative against inflammatory states at least as a nutritional supplements with definite preventive value.

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