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Research Article

IN-VIVO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *PONGAMIA PINNATA* (L.) PIERRE SEED

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ABSTRACT

Objective: The purpose of this study was to evaluate the in vivo anti-inflammatory and anti-arthritic activity of hydroalcoholic extract of *Pongamia pinnata* (L.) Pierre seeds.

Methods: The *P. pinnata* hydroalcoholic extract (PPHE) was tested against Carrageenan-induced paw edema inflammation and Freund's s Complete Adjuvant (FCA) induced arthritic rats. The standard drugs and PPHE (250 and 500 mg/kg) doses were administered orally in all experiments. Arthritis assessment and body weight were measured daily till day 28th whereas Determination of haematological parameters was measured on last day 28th.

Results: Subplantar administration of Carrageenan and FCA results increased in paw volume, joint diameter whereas it reduced motor incoordination and nociceptive threshold. Treatment with PPHE showed significant ($P < 0.001$) inhibition of edema in Carrageenan induced paw edema. PPHE significantly ($P < 0.001$) decreased the arthritis which was evident with arthritis index, paw volume and joint diameter. It also significantly ($P < 0.001$) increased the mechanical hyperalgesias and nociceptive threshold. The hematological parameters also revealed the control in arthritis with PPHE.

Conclusion: The present study was suggestive to that PPHE has prominent anti-inflammatory and anti-arthritic activity which may be mediated through the phytochemical constituents of the plant.

Keywords: Anti-arthritic, Anti-inflammatory, Carrageenan-induced rat paw edema, Freund's complete adjuvant-induced arthritis, *Pongamia pinnata* (L.) Pierre.

INTRODUCTION

Many mediators coordinate inflammatory and allergic reaction. The non-steroid ant-inflammatory drugs (NSAIDSs reduce mainly those components of the inflammatory and immune response in which prostaglandins, mainly derived from COX-2, play a significant part [1].Rheumatoid Arthritis is chronic inflammation which spreads to the surrounding tissues, and can eventually damage cartilage and bone. Other than genetic (inherited) factors and environmental factors, hormonal factors are also involved.

Women are more likely to develop rheumatoid arthritis than men, pregnancy may improve the disease, and the disease may flare after a pregnancy. The common signs & symptoms often affects the wrist joints and the finger joints closest to the hand, also other parts of the body besides the joints and causes pain, swelling, stiffness, and loss of function in the joints [2, 3].

The fresh bark is used internally in bleeding piles. A decoction of the bark is given in beriberi. The flowers are used as a remedy for diabetes; whereas the leaves in the form of a poultice are applied in case of worm-infested ulcers. The seeds are mainly valued for the oil obtained from them which has many industrial and medicinal uses. The seeds crushed to paste are used for leprous sores, skin disease, and painful rheumatic joints.

The seeds contain 27-39 percent of fatty oil which is used for leather dressing, soap making, lubrication, illumination, and for medicinal purposes [4]. The plant is considered to be useful by tribals in leprosy [5]. The oily is highly esteemed for medicinal purpose. It is applied in scabies, herpes, leucoderma and other cutaneous disease. Internally, it has sometimes been used as stomachic and cholagogue in case of dyspepsia with sluggish liver.

It has been reported to be useful in the treatment of rheumatism [6]. Ethanolic extract of leaves showed anti-inflammatory activity [7]. In this study, we investigated the anti-arthritic and anti-inflammatory activity of hydroalcoholic extract of *Pongamia pinnata* (L.) Pierre seeds by in vivo model.

MATERIALS AND METHODS

Collection and authentication of plant

The seeds of *Pongamia pinnata* were collected from local vendors of Udaipur in April 2012. A voucher specimen (Voucher no. RUBL21095) was kept at the Department of Botany, University of Rajasthan, Jaipur after identified and authenticated of the plant.

Preparation of hydro-alcoholic extract of seeds of *P. pinnata*

Dried Seeds were powdered mechanically through mesh sieve. The seeds powder were first defatted with petroleum ether (40–60°C) and extracted with hydro-alcoholic mixture by continuous hot percolation method using Soxhlet apparatus. The filtrates of the extracts were concentrated to dryness.

Preliminary phytochemical screening

The Preliminary phytochemical screening of the *Pongamia pinnata* hydroalcoholic extract (PPHE) was carried out according to previously described methods [8, 9, 10].

Chemicals

Freund's Complete Adjuvant (FCA) (Sigma Aldrich, USA), Diclofenac sodium (Sun pharma, India) and Dexamethasone (Zydus cadila, India) were used. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

Animals

Wistar albino rats of either sex of weighing 150-200g were procured from animal house of Jaipur College of Pharmacy, Jaipur, and Rajasthan. The animals were maintained under standard environmental conditions, fed with standard pellet diet and water (*ad libitum*).

They were housed in polypropylene cages maintained under standard conditions. All animal experiments were carried out under the institute ethics committee approval.

Experimental procedure

Anti-inflammatory and anti-arthritic activity of *Pongamia pinnata* hydro-alcoholic extract of seeds carried out by the following in vivo screening animal models.

Carrageenan-induced paw edema in rats [11, 12]

Group I: Normal animals, 1% aqueous solution of Tween 80, p.o.

Group II: Vehicle control animals, 1% aqueous solution of Tween 80, p.o. (1 h before carrageenan injection)

Group III: Drug treated animals, Diclofenac (4 mg/kg, p.o.) (1 h before carrageenan injection)

Group IV: Drug treated animals with *Pongamia pinnata* hydroalcoholic extract (PPHE) (250 mg/kg, p.o.) (1 h before carrageenan injection)

Group V: Drug treated animals with PPHE (500 mg/kg, p.o.) (1 h before carrageenan injection)

Paw edema in the rats were induced by an injection 0.05 ml of 1% (w/v) carrageenan in saline in to subplantar region of left hind paw on day 1st under light ether anesthesia.

The paw volume of each rat was measured before a carrageenan injection and then at hourly intervals up to five times with a Plethysmometer. The drug treatment was given at 1 h before carrageenan injection.

Freund's Complete Adjuvant induced Arthritis in rats

Non-arthritic animals:

Group I: Vehicle normal animals, 1% aqueous solution of Tween 80, p.o. (non-arthritic)

Arthritic animals:

Group II: Arthritic control, 1% aqueous solution of Tween 80, p.o.

Group III: Arthritic standard treated, 5mg/kg Dexamethasome, p.o.

Group VI: Arthritic animals treated with PPHE 250 mg/kg, p.o.

Group VII: Arthritic animals treated with PPHE 500 mg/kg, p.o.

Experimental arthritis was induced in rats; each rat was injected with 0.1ml of Freund's complete adjuvant (FCA) into subplantar region of left hind paw on day 1st. The dosing of all the groups was started from day 12th once daily orally.

Anti-arthritic activity of extract was evaluated on joint diameter, paw volume, pain withdrawal latency, fall off time and arthritic score on day 0, 4th, 7th, 10th, 12th, 14th, 17th, 19th, 21th and day 28th. On last day (28th day), blood was withdrawn by retro-orbital puncture for assessment of biochemical parameters [11, 13, 14].

Behavioral assessment

Arthritic score

The morphological feature of the arthritis like redness, swelling and erythema was monitored by set visual criteria as follows [15]

Normal paw = 0

Mild swelling and erythema of digits = 1

Swelling and erythema of the digits = 2

Severe swelling and erythema = 3

Gross deformity and inability to use the limb = 4

Paw volume

The left hind paw volumes of all animals were measured just before FCA injection on day 0 and thereafter the same has been carried out at different time intervals till day 28th using a Plethysmometer.

The change in paw volume was measured as the difference between the final and initial paw volumes [16].

Motor incoordination test (Rota-Rod test)

Rats were placed for 1 min on the rotating rod of rota rod apparatus. The time taken for the falling of the rat from the roller, during the period of 1 min was recorded [17].

Paw thickness

Paw thickness was measured by compressing the joint by rotating the screw of micrometer screw gauge till the pain elicited as indicated by squeaking or leg withdrawal. The distance moved by the screw gauge was recorded [18].

Anti-nociceptive activity

The apparatus consists of a hot plate on which the rats were placed for testing (Eddy's Hot Plate Method). Pain threshold was determined by the latency for nociceptive response (withdrawal of any paw) with a maximum cut-off time 15 sec for all groups [19].

Determination of haematological parameters

On last day 28th, the blood was withdrawn by retro-orbital puncture. The parameters analyzed were of follows [11]

1. White blood cell (WBC) count
2. Red blood cell (RBC) count
3. Hemoglobin (Hb) concentration
4. Determination of erythrocyte sedimentation rate (ESR)

Statistical analysis

All the results were expressed as mean ± S.E.M. Statistical comparisons were made between drug-treated groups and arthritic control groups. The data of disease activity index and haematological parameters was statistically analyzed by one-way ANOVA followed by Dunnett's test Graph Pad Prism 5.0 software. The values of $P < 0.05$ were considered statistically significant [11].

RESULTS

Preliminary phytochemical screening

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit pharmacology activities. The *Pongamia pinnata* hydroalcoholic extract of seed (PPHE) was screened for various chemical tests as per the reported methods and was found to contain triterpenes, flavonoids, alkaloids, steroids and tannins. The tests results are summarized in Table 1.

Table 1: Phytochemical screening of *Pongamia pinnata* seeds

S. No.	Tests	Hydroalcoholic extract
1.	**Alkaloids**	
	A Mayer's test	+ve
	B Dragendarff's	+ve
2.	**Carbohydrate**	
	A Molisch test	+ve
3.	**Flavonoids**	
	A Shinoda test	+ve
4.	**Tannins/ Phenolic compounds**	
	A Ferric chloride test	-ve
5.	**Steroids**	
	A Salkowski test	+ve
6.	**Irioid glycosides**	-ve
7.	**Terpenoid**	+ve
8.	**Saponin** +ve	

("+" means present, "-" means absent)

Carrageenan-induced paw edema in rats

Anti-inflammatory effect of hydroalcoholic seed extract of *Pongamia pinnata* was evaluated after subplantar injection of carrageenan in rats. The standard drug Diclofenac sodium (4 mg/kg) and PPHE (250 and 500 mg/kg) groups were compared to control group. Diclofenac sodium (4 mg/kg) showed significantly and dose-dependent decrease in paw edema on 3rd ($P < 0.05$), 4th ($P < 0.01$), 5th ($P < 0.01$), 6th ($P < 0.01$) and 24th ($P < 0.001$) hours as compared to control rats. PPHE (500 mg/kg) showed significantly decrease in

paw edema as compared to control rats on 4th (P < 0.05), 5th (P < 0.05), 6th (P < 0.01) and 24th (P < 0.001) hours. PPHE (250 mg/kg) showed significantly decrease in paw edema as compared to control rats on 5th (P < 0.05), 6th (P < 0.01) and 24th (P < 0.01) hours (Table 2).

Table 2: **Anti inflammatory activity of PPHE on Carrageenan induced rat paw edema.**

Groups	1 h	2 h	3 h	4 h	5 h	6 h	24 h
Normal	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Control	0.20±0.0	0.3±0.06	0.40±0.0	0.46±0.06	0.46±0.06	0.53±0.06	0.46±0.06
Standard drug	0.20±0.0	0.20±0.0	0.20±0.0*	0.13±0.06**	0.13±0.06**	0.06±0.06**	0.0±0.0***
PPHE (250mg/g)	0.06±0.06	0.13±0.06	0.33±0.06	0.33±0.0	0.20±0.0*	0.13±0.06**	0.13±0.06**
PPHE (500mg/kg)	0.06±0.06	0.13±0.06	0.33±0.06	0.20±0.06*	0.20±0.0*	0.13±0.06**	0.0±0.0***

Values are expressed as Mean ± SEM

*P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group animals.

FCA induced arthritis

Subplantar administration of Freund's complete adjuvant in rat paw resulted in significant production of inflammation primarily which was steadily maintained for 28 days. After the 12 days of FCA injection immune respond was occurred this induced secondary arthritis.

The first manifestation of disease was erythema of one or more ankle joints followed by involvement of the metatarsal and interphalangeal joints.

Effect of PPHE on body weight:

Standard drug was significant increased in body weight from day 14th (P < 0.05), 17th (P < 0.05), 19th (P < 0.01), 21th and 28th (P < 0.001) as compared to the control.

Treatment with PPHE (500mg/kg) was significant increased in body weight from day 14th (P < 0.05), 17th (P < 0.05), 19th (P < 0.01), 21th and 28th (P < 0.001) as compared to the control. PPHE (250mg/kg) showed significant increase in body weight on 19th (P < 0.05), 21th and 28th (P < 0.01) (Table 3).

Table 3:-Effect of PPHE on average body weight (gm)

Group	0 Day	4th day	7th day	10th day	12th day	14th day	17th day	19th day	21st day	28th day
Normal	80 ± 5	85 ± 7.63	85 ± 7.63	101.6 ± 13.02	101.6 ± 13.02	108.3 ± 8.33	108.3 ± 8.33	108.3 ± 8.33	108.3 ± 8.33	116.6 ± 8.33
Control	130.8 ± 4.72	125 ± 5	118.3 ± 4.01	98.3 ± 3.07	103.3 ± 6.66	119.1 ± 13.07	119.1 ± 10.36	133.1 ± 12.02	129.1 ± 10.03	125 ± 9.12
Standard drug (5mg/kg)	150 ± 0	126.7 ± 1.66	123.3 ± 1.66	111.7 ± 7.26	120 ± 5	166.7 ± 8.33*	166.7 ± 8.33*	191.7 ± 8.33**	206.7 ± 15.90***	218.3 ± 9.28***
PPHE (250mg/kg)	150 ± 0	118.3 ± 4.41	116.7 ± 8.33	115 ± 7.63	120 ± 5.77	135 ± 7.63	141.7 ± 8.33	175 ± 0.0*	185 ± 2.88**	188.3 ± 6.00**
PPHE (500mg/kg)	150 ± 0	125 ± 0	115 ± 7.63	106.7 ± 6.66	113.3 ± 6.66	165 ± 7.63*	165 ± 7.63*	188.3 ± 6.00**	206.7 ± 6.66***	208.3 ± 8.33***

Values are expressed as Mean ± SEM

*P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

Table 4:-Effect of PPHE on average arthritic score

Groups	0 day	4th day	7th day	10th day	12th day	14th day	17th day	19th day	21st day	28th day
Normal	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Control	0 ± 0.0	2 ± 0.0	1.83 ± 0.16	2 ± 0.0	2 ± 0.36	1.83 ± 0.16	1.83 ± 0.16	2.16 ± 0.30	2.33 ± 0.21	1.83 ± 0.16
Standard drug (5mg/kg)	0 ± 0.0	1.66 ± 0.33	1.66 ± 0.33	2.33 ± 0.33	2 ± 0.00	1.0 ± 0.0*	1.00 ± 0.0*	0.333 ± 0.33**	0.333 ± 0.33***	0 ± 0.0***
PPHE (250mg/kg)	0 ± 0.0	2.66 ± 0.33	2.66 ± 0.33	2.66 ± 0.33	2.66 ± 0.33	1.66 ± 0.33	1.66 ± 0.33	0.66 ± 0.33*	0.66 ± 0.33**	0.66 ± 0.33**
PPHE (500mg/kg)	0 ± 0.0	2 ± 0.57	2.33 ± 0.33	2.33 ± 0.33	1.66 ± 0.33	1.000 ± 0.0*	1.000 ± 0.0*	0.333 ± 0.33**	0.33 ± 0.33***	0 ± 0.0***

Values are expressed as Mean ± SEM, *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

Effect of PPHE on arthritic score

All the groups of animals administered with FCA started showing signs of clinical inflammation in one or more hind paws, which was a biphasic response. The arthritic score was significantly increased from day 7 to 12 in control rats which remained significantly increased till the end of the study i.e. up to 28th day. On comparison from control rats, Standard rats showed significant and dose dependant decreased in arthritic score from day 14th ($P < 0.05$), 17th ($P < 0.05$), 19th ($P < 0.01$), 21th and 28th ($P < 0.001$). Treatment with PPHE (500mg/kg) showed significant decreased in arthritic score from day 14th ($P < 0.05$), 17th ($P < 0.05$), 19th ($P < 0.01$), 21th and 28th ($P < 0.001$). Treatment with PPHE (250mg/kg) showed significant decreased in arthritic score on 19th ($P < 0.05$), 21th and 28th ($P < 0.01$) as compared to control rats (Table 4).

Effect of PPHE on paw volume

There was significant decreased in paw volume from day 17th and 19th ($P < 0.01$), 21th and 28th ($P < 0.001$) in Standard rats (5mg/kg) as compared to control rats. Standard rats did not significant decreased in paw volume from day 12th to 14th. Treatment with PPHE (500mg/kg) showed significant decreased in paw volume 17th ($P < 0.05$), 19th ($P < 0.01$), 21th and 28th ($P < 0.001$) as compare to control rats. Treatment with PPHE (250mg/kg) was showed significant decreased in paw volume as compare to control rats on 19th day ($P < 0.05$) and 21th and 28th ($P < 0.01$) (Table 5).

Table 5:-Effect of PPHE on change of paw volume (ml)

Group	0 day	4th day	7th day	10th day	12th day	14th day	17th day	19th day	21st Day	28th day
Normal	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Control	0.0 ± 0.0	0.36 ± 0.33	0.36 ± 0.33	0.40 ± 0.05	0.46 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.46 ± 0.04	0.46 ± 0.06
Standard drug (5mg/kg)	0.0 ± 0.0	0.20 ± 0.0	0.40 ± 0.0	0.46 ± 0.06	0.40 ± 0.11	0.33 ± 0.06	0.20 ± 0.0**	0.13 ± 0.06**	0.06 ± 0.06***	0.0 ± 0.0***
PPHE (250mg/kg)	0.0 ± 0.0	0.53 ± 0.06	0.53 ± 0.06	0.33 ± 0.06	0.33 ± 0.06	0.33 ± 0.06	0.33 ± 0.06	0.26 ± 0.06*	0.20 ± 0.0**	0.06 ± 0.06**
PPHE (500mg/k)	0.0 ± 0.0	0.46 ± 0.06	0.46 ± 0.06	0.46 ± 0.06	0.40 ± 0.0	0.33 ± 0.06	0.26 ± 0.06*	0.20 ± 0.0**	0.066 ± 0.06***	0.0 ± 0.0***

Value expressed as Mean ± SEM, *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

Table 6:-Effect of PPHE on joint diameter (mm)

Groups	0 day	4th Day	7th Day	10th day	12th day	14th day	17th day	19th day	21st day	28th day
Normal	0 ± 0	0.09 ± 0.07	0.056 ± 0.073	0.03 ± 0.05	0 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.02	0.01 ± 0.01	0.02 ± 0.02
Control	0 ± 0	2.41 ± 0.39	2.33 ± 0.36	4.37 ± 0.65	3.12 ± 0.5	2.83 ± 0.32	2.35 ± 0.1	2.25 ± 0.38	2.18 ± 0.2	2.17 ± 0.21
Standard drug (5mg/kg)	0 ± 0	1.19 ± 0.15	2.4 ± 0.23	3.28 ± 0.12	2.57 ± 0.25	1.32 ± 0.06*	1.15 ± 0.02*	0.47 ± 0.24**	0.32 ± 0.19***	0.28 ± 0.19***
PPHE (250mg/kg)	0 ± 0	2.55 ± 0.21	3.08 ± 0.16	3.11 ± 0.17	3.18 ± 0.27	2.04 ± 0.25	1.94 ± 0.25	0.86 ± 0.10*	0.86 ± 0.10**	0.74 ± 0.03**
PPHE (500mg/kg)	0 ± 0	1.61 ± 0.6	2.14 ± 0.6	2.56 ± 0.31	2.37 ± 0.1	1.24 ± 0.56*	1.23 ± 0.56*	0.48 ± 0.16**	0.48 ± 0.16***	0.45 ± 0.17***

Values are expressed as Mean ± SEM, *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

Effect of PPHE on joint diameter

Treatment with Standard rats (5mg/kg) showed significant and dose dependant decreased in joint diameter from day 14th ($P < 0.05$), 17th ($P < 0.05$), 19th ($P < 0.01$), 21th and 28th ($P < 0.001$) as compared to control rats. There was significant decreased in joint diameter from day 14th ($P < 0.05$), 17th ($P < 0.05$), 19th ($P < 0.01$), 21th and 28th ($P < 0.001$) of PPHE (500mg/kg) treated rats as compared to the control rats.

Treatment with PPHE (250mg/kg) was showed significant decreased in joint diameter on 19th ($P < 0.05$), 21th and 28th ($P < 0.01$) (Table 6).

Effect of PPHE on fall off time

The motor incoordination was assessed by determining the mean fall off time in rota rod test. There was significant decrease in pain threshold on administration of FCA which continued till day 12th. When compared to control rats, the significant increased in fall off time of standard rats from day 14th and 17th ($P < 0.05$), 19th ($P < 0.01$), 21th and 28th ($P < 0.001$). Treatment with PPHE (500mg/kg) was showed significant increase in fall off time as compared to control rats from day 14th and 17th ($P < 0.05$), 19th, 21th ($P < 0.01$) and 28th ($P < 0.001$). Treatment with PPHE (250mg/kg) was showed significant increase in fall off time as compared to control rats on 17th day and 19th day ($P < 0.05$), 21th and 28th ($P < 0.01$) (Table 7)

Table 7:-Effect of PPHE on fall off time (sec.)

Groups	0 day	4th day	7th day	10th day	12th day	14th day	17th day	19th day	21st day	28th day
Normal	24.28 ± 2.767	23.4 ± 1.28	23.92 ± 1.22	24.16 ± 1.19	24.17 ± 1.19	24.09 ± 1.36	24.01 ± 1.32	24.84 ± 1.49	25.69 ± 1.49	26.24 ± 1.43
Control	23.66 ± 7.83	16.5 ± 4.9	12.86 ± 2.26	19.41 ± 7.03	14.46 ± 5.79	16.17 ± 6.21	18.93 ± 4.64	17.78 ± 3.79	15.87 ± 4.46	19.86 ± 2.4
Standard drug (5mg/kg)	36.73 ± 3.21	34.34 ± 3.62	33.23 ± 3.61	32.78 ± 3.49	33.74 ± 2.43	40.64 ± 1.74*	41.9 ± 2.19*	43.91 ± 1.12**	46.77 ± 1.09***	47.08 ± 0.91***
PPHE (250mg/kg)	22.9 ± 0.98	16.27 ± 4.39	9.55 ± 2.83	10.08 ± 2.7	10.02 ± 2.91	32.76 ± 1.68	37.93 ± 2.67*	37.93 ± 1.52*	39.06 ± 2.65**	33.54 ± 1.05**
PPHE (500mg/kg)	34.19 ± 7.3	19.91 ± 3.02	20.16 ± 12.87	12.88 ± 4.26	16.54 ± 3.93	42.4 ± 0.74*	40.06 ± 1.60*	40.5 ± 0.49**	44.29 ± 1.31**	43.18 ± 1.73***

Values are expressed as Mean ± SEM, *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

Table 8:-Effect of PPHE on paw withdrawal latency (Sec.)

Groups	0 day	4th day	7th day	10th day	12th day	14th day	17th day	19th day	21st day	28th day
Normal	23.1 ± 2.33	25.79 ± 2.74	27.31 ± 2.05	30.3 ± 0.65	30.29 ± 0.23	30.93 ± 1.12	29.63 ± 1.12	29.5 ± 2.01	30.42 ± 2.53	31.26 ± 2.67
Control	22.8 ± 2.31	14.88 ± 1.51	14.88 ± 1.3	13.81 ± 1.12	8.58 ± 1.4	11.77 ± 1.42	15.1 ± 6.25	14.79 ± 2.34	12.76 ± 1.76	16.9 ± 0.78
Standard drug (5mg/kg)	13.6 ± 0.58	12.22 ± 0.62	13.14 ± 0.93	15.91 ± 0.3	8.477 ± 0.24	18.28 ± 0.86*	38.96 ± 1.01*	35.07 ± 1.67***	37.82 ± 2.83***	40.52 ± 1.07***
PPHE (250mg/kg)	18.6 ± 0.8	16.24 ± 0.55	16.3 ± 1.15	15.38 ± 1.47	10.76 ± 0.38	15.92 ± 1.78	19.8 ± 0.77	26.12 ± 1.32*	26.19 ± 1.34**	27.26 ± 0.37**
PPHE (500mg/kg)	25.6 ± 5.69	24.19 ± 5.57	23.16 ± 5.15	21.5 ± 4.88	11.57 ± 0.62	18.17 ± 1.59*	36.67 ± 3.28*	29.43 ± 3.69**	30.76 ± 2.38***	33.04 ± 3.74***

Values are expressed as Mean ± SEM, *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

Table 11:- Effect of chronic treatment of PPHE on haematology and ESR

Groups	RBC(mil/cumm)	WBC (th/mm^3)	HGB (gms/dl)	ESR (mm/hr)
Normal	2.6 ± 0.185	6.2 ± 0.577	6.93 ± 0.218	6.00 ±1.155
Control	1.88 ± 0.060	7.2 ±0.057	4.90 ± 0.1979	6.67 ± 0.333
Standard drug (5mg/kg)	2.8 ± 0.057***	4.6 ± 0.088**	7.5± 0.152***	3.33 ± 0.333**
PPHE (250mg/kg)	2.233 ± 0.066*	5.067 ±0.809**	6.10 ± 0.208**	4.0 ± 1.155 *
PPHE (500mg/kg)	2.467 ±0.166**	4.80 ± 0.115**	7.20 ± 0.208***	3.333±0.881**

Values are expressed as Mean ± SEM, *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

Effect of PPHE on nociceptive threshold

There was a significant decrease in mechanical withdrawal threshold observed in all the animals treated with FCA. When compared to control rats, the significant increased in paw withdrawal latency of standard rats from day 14th (P < 0.05), 17th (P < 0.05), 19th (P < 0.001), 21th and 28th (P < 0.001). Treatment with PPHE (500mg/kg) was showed significant increase in paw withdrawal latency as compared to control rats from day 14th (P < 0.05), 17th (P < 0.05), 19th (P < 0.01), 21th and 28th (P < 0.001). Treatment with PPHE (250mg/kg) was showed significant increase in paw withdrawal latency as compared to control rats on 19th (P < 0.05), 21th and 28th (P < 0.01) day (Table 8)

Hematological Parameters

There was decrease in RBCs along with Hb and increase in WBCs in control rats as compared to normal rats. Rats treated with Standard drug Dexamethasone (5mg/kg) showed significant decrease (P < 0.01) in WBCs and ESR when compared to control rats where as significantly increase (P < 0.001) in the RBCs and Hb level as compared to control rats. There was significant (P < 0.05 and P < 0.01) decrease in ESR and WBC in rat treated with PPHE (250mg/kg). Whereas significantly increase (P < 0.05 and P < 0.01) in the RBCs and Hb level. Treatment with PPHE (500mg/kg) significantly increases (P < 0.01 and P < 0.001) in the RBCs and Hb level and significant decrease (P < 0.01) in ESR and WBC as compared to control rats (Table 9).

DISCUSSION

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic event. The primary acute phase (up to 2 h) involves generation and release of the inflammatory mediators including histamine, bradykinin, 5- hydroxytryptamine where as secondary chronic phase (2 to 5 h) is regulated by neutrophil infiltration and sustained production of arachidonic metabolites (prostanoids) or nitric oxide.

The carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism. PGE 2, a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to the redness and increased blood flow in areas of acute inflammation [11, 12]. This experimental

model is considered closest to stimulating human rheumatoid arthritis [20]. Freund's Complete Adjuvant (FCA) is inactivated and dried mycobacteria which are mainly responsible for stimulation of cell-mediated immunity which ultimately increased the production of certain immunoglobulins. FCA induced arthritis is a primary and secondary chronic arthritis. Primary is inflammatory phase where generation of prostaglandin occurs and secondary immunological state in which autoantibodies is generated. In this model, the affected articulations are infiltrated by blood-derived cells, mainly neutrophils, macrophages and dendritic cells. In response to activation, these cells generate free radicals, which are released in large amounts in the surrounding tissue [21]. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and pause in body weight gain; during the acute period, hind paw and fore paw joint diameters increase. In the later, acute stages of disease (day 12+), rats with adjuvant arthritis are often relatively immobile due to the severity of paw swelling [22]. Body weight, food intake and metabolism are affected by immunity and inflammation and they are regulated by a cytokine-like hormone known as Leptin. In FCA induced arthritis, within 24 hrs of the administration of FCA the plasma leptin levels were rapidly increased which led to anorexia and body weight loss. The arthritis score is index of the joint inflammation after immunization. This model in rats has been extensively used in the study of inflammatory processes and validated as a model of chronic pain. The hot plate test measures the response to a brief, noxious stimulus thus bears a closer resemblance to clinical pain. The increase in reaction time in the hot plate test suggests the anti-nociceptive effect. In this model the decreased levels of Hemoglobin (Hb) and Red blood cell count (RBCs) associated with the reduced erythropoietin levels caused due to decreased response of the bone marrow erythropoietin and destruction of premature RBCs in FCA induced arthritis. Erythrocyte sedimentation rate (ESR) is an index of suspension stability of RBC's in plasma. The number and size of RBC is associated with ESR. It also involved in the accelerated formation of endogenous proteins including plasma proteins such as fibrinogen, alpha and beta globulins. ESR is elevated during the inflammation, stress and cell necrosis. In elevated level of the IL-1α inflammatory response in FCA induced arthritis results in increase in granulocyte and macrophages colony stimulating factors which is associated with elevated level of White blood cell count (WBC) which plays a major role in body defense mechanism [11]. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit pharmacology activities. The presence of many biologically active phytochemicals such as triterpenes, flavonoids, alkaloids, steroids, tannins, and glycosides, in various plant extracts may be responsible for their pharmacological properties [23, 24, 11].

CONCLUSION

For further confirmation of the results, in vivo model i.e. Carrageenan-induced paw edema and Freund's complete adjuvant model have also been carried out. Both models are established and well known screening models for evaluation of anti-inflammatory and anti-arthritic activity. The studies of various parameters such as body weight, arthritic score, joint diameter, paw volume and hematological parameters were also occur. In these models, It was found that PPHE decrease the paw volume, arthritic score, joint diameter, increase in body weight and it could significantly normalize hematological in adjuvant induced arthritic rats. On the basis of these parameters, it can be concluded that PPHE has potent anti- inflammatory and anti-arthritic. The phytochemical study of PPHE revealed that presence of phytoconstituents such as flavonoids, saponins, steroids, terpenoids and alkaloids. The presence of these phytoconstituents might be responsible for the anti-inflammatory and anti-arthritic effect. In future isolation of lead molecules responsible for the activity will be carried out which may be beneficial for the development of new anti-inflammatory and anti-arthritic agent.

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