Research Article

In-Vitro Anti-Inflammatory and Anti-Arthritic Activity of Hydroalcoholic Extract of *Pongamia Pinnata* (L.) Pierre Seed

*Divya Singh, Rahul Nainwani, Tripta Sharma, Rupesh K. Gautam

Department of Pharmacology, Jaipur College of Pharmacy, ISI-15, RIICO Institutional Area, Tonk Road, Sitapura, Jaipur, India.

ABSTRACT

Objective: To evaluate Anti-inflammatory and Anti-arthritic Activity of seed extract of *Pongamia pinnata* (L.) Pierre by in vitro model. **Materials and methods:** *P. pinnata* (L.) Pierre (Family: Leguminoseae) is a medicinal plant which is indicated for the treatment of arthritis in folklore medicine. The present study was aimed at the investigation of anti-arthritic and anti-inflammatory activity in hydroalcoholic extract of *P. pinnata.* The anti-arthritic and anti-inflammatory activity of *P. pinnata* hydroalcoholic extract was done by Inhibition of protein denaturation and Human red blood cell membrane stabilization (HRBC) in vitro methods. The hydroalcoholic extract of *P. pinnata* was subjected to in vitro Inhibition of protein denaturation in various concentrations i.e. 10, 50, 100, 200, 400, 800, 1000 and 2000µg/ml. HRBC method was also used for the estimation of anti-inflammatory activity from in various concentrations 100, 200, 400, 800 and 1600 µg/ml. **Results:** *P. pinnata* hydroalcoholic extract exhibited a concentration dependent inhibition of protein (albumin) denaturation. The stabilization of HRBC membrane showed a concentration dependent anti-inflammatory activity, and the protection percent increased with increase in the concentration of the *P. pinnata* hydroalcoholic extract. **Conclusion:** The present study is support to the isolation and use of phytoconstituents from seed of *P. pinnata* in treatment of inflammation and arthritis.

Keywords: Anti-arthritic, human red blood cell membrane stabilization, inhibition of protein denaturation, phytoconstituents, *pongamia pinnata* (L.) pierre

Received 30 Sept 2013 Received in revised form 19 Oct 2013 Accepted 21 Oct 2013

***Address for correspondence:**
Divya Singh
Near clark amer hotel, Durgapura, Jaipur, Rajasthan, India.
E-mail: pharmadivya027@gmail.com

INTRODUCTION

Pongamia pinnata (L.) Pierre is a tree belonging to the family Fabaceae [1, 2]. *P. pinnata* have several uses which ascribed in traditional medicine. The plant is considered to be useful by tribals in leprosy. The juice of roots is used to clean teeth, strengthening gums, gonorrhea and cleansing foul ulcers. The seeds are used for external application in skin disease. The oil from the seeds is used in leucoderma, cutaneous affecting including herpes and scabies, also used in rheumatism. The fresh bark is used internally in bleeding piles. A decoction of the bark is given in beriberi. The flowers are used as remedy for diabetes; whereas the leaves in the form of a poultice are applied in case of worm–infested ulcers [3].

The ethanolic extract of *P. pinnata* seeds have reported to be anti-inflammatory activity [4]. The seeds paste of *P. pinnata* is used for leporus sores, skin disease and painful rheumatic joints [2]. In the present work was undertaken check to the potential of *P. pinnata* seed in the treatment of inflammation and arthritis.

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 powdered were first defatted with petroleum ether (40–60°C) and extracted with hydro-alcoholic mixture by continuous hot percolation method using Soxhlet apparatus. The filtrate of the extract was concentrated to dryness.

Chemicals

Diclofenac sodium (Sun pharma, India) was used. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

Experimental procedure

Inhibition of protein denaturation method

The following procedure was followed for evaluating the percentage of inhibition of protein denaturation:-

- Control solution (50 ml): 2 ml of Egg albumin (from fresh hen's egg), 28 ml of phosphate buffer (pH 6.4) and 20 ml distilled water.
- Standard drug (50 ml): 2 ml of Egg albumin, 28 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) conc. of 10, 50, 100, 200, 400, 800, 1000 and 2000µg/ml.
- Test solution (50 ml): 2 ml of Egg albumin, 28 ml of phosphate buffer and various concentrations of plant extract (*Pongamia pinnata* hydro-alcholic seed extract) conc. of 10, 50, 100, 200, 400, 800, 1000 and 2000µg/ml.

All of the above solutions were adjusted to pH 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling the absorbance of the above solutions was measured using UV-Visible spectrophotometer at 660nm their viscosity was determined by using Ostwald viscometer. The percentage inhibition of protein denaturation was calculated using the following formula **[**5, 6].

$$\text{Percentage inhibition} = \left[\frac{Vt}{Vc} - 1\right] \times 100$$

Where, Vt = absorbance of test sample, Vc = absorbance of control

Human red blood cell membrane stabilization method

Preparation of reagents

- Alsevers solution: 2 gm Dextrose, 0.8 gm Sodium citrate, 0.05 gm Citric acid and 0.42 gm Sodium chloride were dissolved in distilled water. The final volume was made up to 100 ml with distilled water.
- Hypotonic Saline: 0.36 gm of Sodium chloride in 100 ml of distilled water.
- Isotonic Saline: 0.85 gm of sodium chloride in 100 ml of distilled water.
- Phosphate buffer (pH 7.4, 0.15 M): 2.38 gm disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8 gm of sodium chloride were dissolved in 100 ml of distilled water [7].

Preparation of Suspension (10% v/v) of Human Red Blood cell (HRBC)

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and was mixed with equal volume of sterilized Alsevers solution [8]. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline [9].

Assay of Membrane stabilizing activity

HRBC membrane stabilization method, the following solutions were used:-

- Test solution: 1 ml of phosphate buffer, 2 ml of hypotonic saline, 0.5 ml of plant extract of various concentration (100, 200, 400, 800 and 1600 µg/ml) and 0.5 ml of 10% w/v human red blood cells.
- Test control: 1 ml of phosphate buffer and 2ml of water and 0.5ml of 10%w/v human red blood cells in isotonic saline.
- Standard solution: 1 ml of phosphate buffer, 2 ml of hypotonic saline, 0.5 ml of plant extract of various concentration (100, 200, 400, 800 and 1600 µg/ml) and 0.5 ml of 10% w/v human red blood cells.

All the assay mixtures were incubated at 37ºc for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was separated and the hemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the hemolysis produced in content as 100% [10, 11]. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:-

Percentage protection = [100- (optical density sample/optical density control)] × 100

RESULTS

Inhibition of protein denaturation method

The anti-arthritic effect of *Pongamia pinnata* hydroalcoholic extract (PPHE) was evaluated against the denaturation of egg albumin *in vitro*. The result is summarized in (**Table 1**) (**Fig. 1**). PPHE and Diclofenac sodium were exhibited concentration dependent inhibition of protein (albumin) throughout the concentration range of 10 to 2000 µg/ml. All concentration of PPHE and Diclofenac sodium viscosities were always less than of control. The observed increase in viscosities may be due to increase in concentration of the PPHE and Diclofenac sodium.

The *Pongamia pinnata* hydroalcoholic extract of seed showed a concentration dependent anti-inflammatory activity, and the protection percent increased with increase in the concentration (100, 200, 400, 800 and 1600). The result is compared with Diclofenac sodium (standard) in (**Table 2, Fig. 2**).

Table 1: In vitro activity of PPHE and Diclofenac sodium inhibition of protein denaturation method

Group	Concentration (µg/ml)	% Inhibition (±SEM)	Viscosity (cps)
Pongamia pinnata	**Control**	-	1.41
hydroalcoholic extract	**10**	18.86±0.016	0.996
of seed	**50**	24.52±0.011	0.993
	100	52.83±0.006	1.014
	200	116.98±0.004	1.029
	400	238.99±0.003	1.034
	800	382.38±0.003	1.112
	1000	580.50±0.005	1.115
	2000	689.93±0.004	1.153
Diclofenac sodium	**10**	98.08±0.012	1.330
	50	107.66±0.005	1.525
	100	112.99±0.010	1.580
	200	147.07±0.002	1.635
	400	202.44±0.014	1.642
	800	254.63±0.026	1.656
	1000	324.92±0.034	1.670
	2000	363.25±0.030	1.677

Fig. 1: In vitro anti arthritic activity by inhibition of protein denaturation method

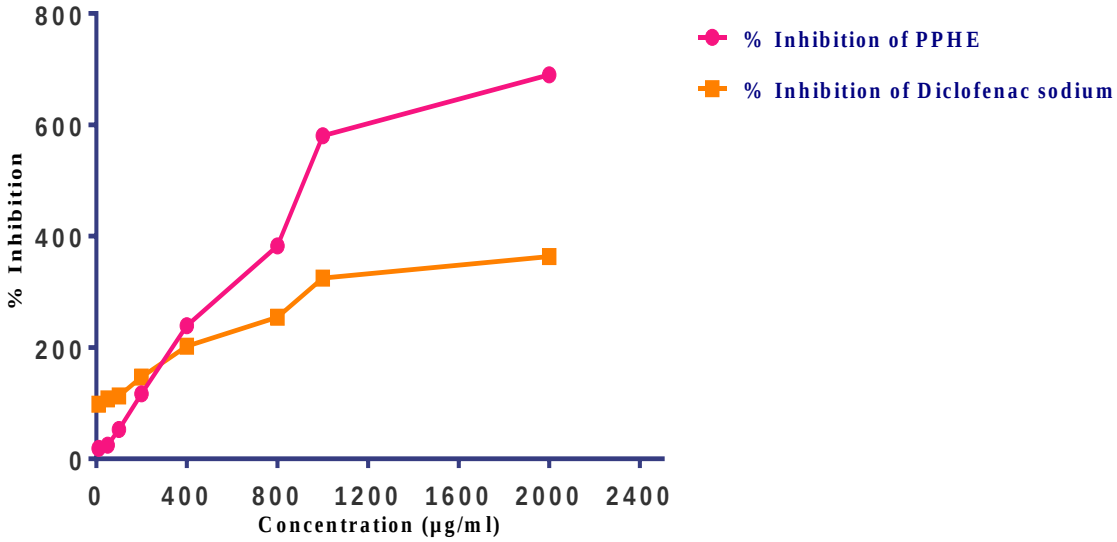
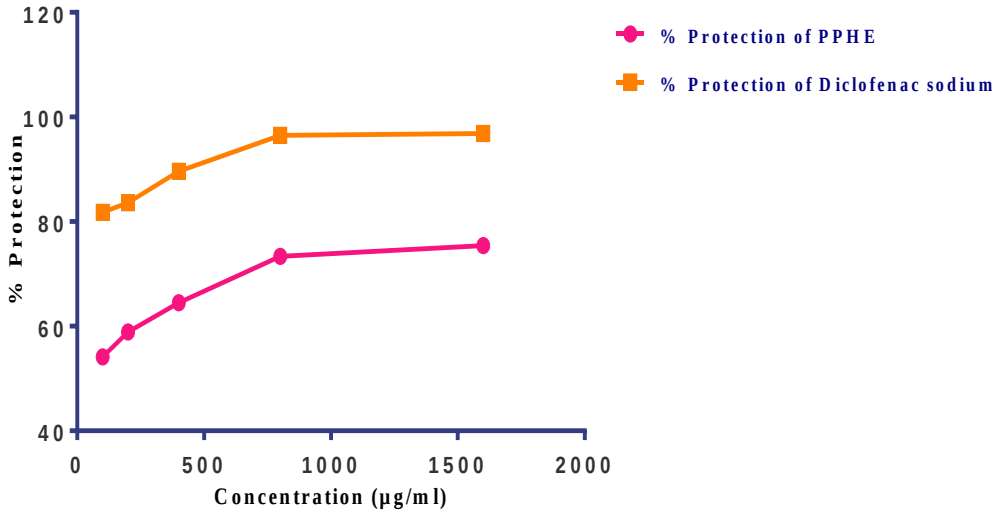


Table 2: In vitro activity of PPHE and Diclofenac sodium by HRBC Method

Group	Concentration (µg/ml)	% Protection (±SEM)
Pongamia pinnata **hydroalcoholic extract of seed**	**100**	54.34 ± 0.0
	200	58.95 ± 0.0057
	400	64.51 ± 0.0003
	800	73.40 ± 0.0014
	1600	75.42 ± 0.0014
Diclofenac sodium	**100**	81.76 ± 0.0003
	200	83.60 ± 0.0015
	400	89.59 ± 0.0008
	800	96.48 ± 0.0003
	1600	96.85 ± 0.0015

Figure 2: In vitro activity by HRBC Method



DISCUSSION

Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo*. The mechanism of denaturation probably involves alteration I electrostatic hydrogen, hydrophobic and disulphide bonding. The increments in absorbance of plant extract and reference drug with respect to control indicated the stabilization of albumin protein. This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation [5, 6].

HRBC method was selected for the in vitro evaluation of Anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [10, 11]. The hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [8].

CONCLUSION

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have various side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against arthritis and inflammation so as to exploit them as herbal anti-arthritic agents.

In Inhibition of protein denaturation method, PPHE extract exhibited concentration depended inhibition of protein denaturation throughout the concentration range from low to high. This anti-denaturation effect was further supported by increase in the viscosities, while in Human red blood cell membrane stabilization method, the PPHE exhibited protection of the membrane lysis throughout the conc. range from low to high. The finding of in vitro model suggested that PPHE has potent anti-inflammatory and anti-arthritic potential. 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.

REFERENCES

1. Sangwan S, Rao DV and Sharma RA. A Review on *Pongamia Pinnata* (L.) Pierre: A Great Versatile Leguminous Plant. Nature and Science. 2010;8(11):130- 131.
2. Bhattacharjee SK. Handbook of Medicinal Plants. 4th ed., Pointer Publishers, Jaipur, India;2004. p.281.
3. Satayavati GV, Gupta AK, Tandon N. Medicinal Plants of India. 2nd vol., Indian council of medical research, New Delhi;1987. p.490-498.
4. Singh RK, Pandey BL. Anti-inflammatory activity of seed extracts of *Pongamia pinnata* in rat. Indian J Physiol Pharmacol. 1996; 40(4):355-8.
5. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of Anti-inflammatory Effect of Ashwagandha: A Preliminary Study *in vitro.* Pharmacognosy Journal, 2012; 4 (29):47-49.
6. Sangeetha M, Kousalya K, Lavanya R, Sowmya C, Chamundeeswari D, Maheswara UC.In-vitro Anti-inflammatory and Anti-arthritic Activity of Leaves of *Cleodendron Inerme*. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2011;2(1):822-827.
7. Narayanan BL, Pradeep LAR, Arulanandham A, Babu NST, Gayathri and Raju A. Anti-oxidant and anti-inflammatory activity of synthesized 3(substituted) chromen-2-one.International Journal of Pharmaceutical Sciences and Research. 2012;3(2):474-478.
8. Kumar V, Bhat ZA, Kumar D, Khan NA, Chashoo IA. Evaluation of anti-inflammatory potential of leaf extracts of *Skimmia anquetilia*. Asian Pacific Journal of Tropical Biomedicine. 2012;627-630.
9. Saleem TK, Azeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extacts of *Gendarussa vulgaris Nees*. Asian Pacific Journal of Tropical Biomedicine. 2011;147-149.

10. Azeem AK, Dilip C, Prasanth SS, Junise Hanan Shahima V, Kumar S, Naseera C. Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*. Asian Pacific Journal of Tropical Medicine. 2010; 3(10):412-420.
11. Chippada SC, Meena V. Antioxidant, an anti-inflammatory and anti-arthritic activity of *Centella asiatica* extracts. J. Chem. Bio. Phy. Sci. 2011;1(2):260-269.