Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

COMPARATIVE EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF *PONGAMIA PINNATA* (LINN.) PIERRE AND *PUNICA GRANATUM* LINN. : AN *IN-VITRO* STUDY

RUPESH K. GAUTAM*[1,2], SANJAY SHARMA[1a], KOMAL SHARMA[3]

[1]Research Scholar, [1a]Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, [2]Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, [3]Department of Pharmacology, Bhupal Nobles' Institute of Pharmaceutical Sciences, Udaipur (Rajasthan), India. *Email: rgautam3906@yahoo.co.in

Received: 17 Oct 2013, Revised and Accepted: 08 Nov 2013

ABSTRACT

Objective: The present study was carried out to compare the anti-arthritic activity of ethanolic extract of seeds of *Pongamia pinnata* (linn.) pierre (EEPP) and methanolic extract of rind of *Punica granatum* linn. (MEPG) by *in-vitro* techniques.

Methods: Two in-vitro models i.e. inhibition of protein denaturation and Human red blood cell (HRBC) membrane stabilization were selected for the study. Diclofenac sodium was used as a standard drug.

Results: The results of both models exhibited that EEPP, MEPG and standard drug (diclofenac sodium) showed concentration dependent inhibition of protein (egg albumin) denaturation as well as stabilization towards HRBC membrane.

Conclusion: By comparing the present findings, it can be concluded that MEPG has more potent anti-arthritic activity than EEPP. The activity may be due to the presence of phytocompounds such as flavonoids, steroids etc.

Keywords: *Pongamia pinnata*, *Punica granatum*, Anti-arthritic activity, Karanja, Anar

INTRODUCTION

India has a rich assortment of medicinal plants distributed in different geographical and ecological conditions widespread in the country. Plants have been used since prehistoric times for treatment of various ailments. Today, according to World Health Organization (WHO) as many as 80% of the world's population depend on the traditional medicine for their primary healthcare needs [1, 2]. Rheumatoid arthritis is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of synovial cells and angiogenesis in affected joints, which ultimately leads to destruction of cartilages and bone. RA is characterized by inflammation of synovial joints infiltered by CD4 + T cells, macrophages, and plasma cells that play major role in pathogenesis of disease. T cells have direct impact on TNF - alpha, IL-6, IFN- gamma, induction in joints. TNF–alpha is known to play an important role in the pathogenic mechanism of a number of chronic inflammatory diseases, including RA. B cells may play role in pathogenesis of RA through cell-cell interaction with T cells, dendritic cells, synovial nurse like cells and fibroblasts. CD4+ CD25+ regulatory T cells are potent suppressors of T cell responses both in- vitro and in-vivo [3].

Pongamia pinnata (Linn.) Pierre (Family- Fabaceae) commonly known as karanja [4]. The seed and seed oil of the plant have been reported to be effective in various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, muscular and articular rheumatism, cutaneous affection including herpes and scabies etc. in folk medicine and Ayurveda, a traditional system of healing [5-7]. Anti-inflammatory activities of this plant for various parts have been reported in various models [7-10]. *Punica granatum* Linn. (Family: Punicaceae) commonly known as Anar. The plant is used in folklore medicine for the treatment of various diseases such as ulcer, hepatic damage, snakebite, etc [6, 11]. Anti-inflammatory and immunomodulatory activity of this plant are already reported in various models [12-22]. The anti-arthritic activity of these plants has not been reported yet in any of *in-vitro* models. With this background, the present study was carried out to investigate the anti-arthritic activity of *P. pinnata* and *P. granatum* in *in-vitro* models.

MATERIALS AND METHODS

Plant material

The seeds of *Pongamia pinnata* and fruits of *Punica granatum* were purchased from local vendor of Udaipur (Rajasthan), India in the month of April, 2011.The seeds of *P. pinnata* was identified and authenticated at Department of Botany, University of Rajasthan, Jaipur (Rajasthan), India. The fruit of *P. granatum* was identified and authenticated at Department of Horticulture, Rajasthan College of Agriculture, Udaipur, India

Preparation of extract

The seeds of *P. pinnata* and rind of *P. granatum* were powdered mechanically through mesh sieve. The powdered plant parts of *P. pinnata* were extracted with solvent ethanol by continuous hot percolation method using soxhlet apparatus. The filtrate of the extracts was concentrated to dryness. In case of *P. granatum*, methanol was used as solvent. For *P. pinnata*, powdered seeds were defatted with petroleum ether (40–60°C) before extraction with ethanol.

Evaluation of anti-arthritic activity

Following two *in-vitro* models were selected for the study-

Inhibition of protein denaturation model [23,24]

2ml of egg albumin (from fresh hen's egg), 28 ml of phosphate buffer (PBS, pH 6.4) and 20ml distilled water were used as control solution (50 ml). 2ml of egg albumin, 28 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) (10, 50, 100, 200, 400, 800, 1000 and 2000µg/ml) were served as standard drug solution (50 ml). 2ml of egg albumin, 28 ml of phosphate buffer and various concentrations of plant extract (10, 50, 100,200, 400,800, 1000 and 2000 µg/ml) were taken as test solution (50 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 and their viscosity was determined by using Ostwald viscometer. The percentage inhibition of protein denaturation was calculated using the following formula-

Percentage inhibition = (Vt/Vc-1) x 100

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

Human red blood cell (HRBC) membrane stabilization model

Preparation of reagents

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. This mixture was used as Alsevers solution. Hypotonic saline was prepared by dissolving 0.36 gm of sodium chloride in 100 ml of distilled water. Isotonic saline was prepared by dissolving 0.85 gm of sodium chloride in 100 ml of distilled water. 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. This was served as phosphate buffer (pH 7.4, 0.15 M) [25].

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 NSAID'S for 2 weeks prior to the experiment and was mixed with equal volume of sterilized Alsevers solution [26]. 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. This HRBC suspension was used for the study [27].

Assay of membrane stabilizing activity

The assay mixtures contains 1ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension & 0.5 ml different concentrations of extract, reference sample and control were separately mixed.

1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of plant extract of various concentration (100, 200, 400, 800 and 1600 µg/ml) and 0.5ml of 10% w/v human red blood cells were used as test solution. 1ml of phosphate buffer and 2ml of water and 0.5ml of 10%w/v human red blood cells in isotonic saline were served as test control.1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of standard drug (Diclofenac sodium) of various concentration (100, 200, 400, 800 and 1600 µg/ml) and 0.5ml of 10% w/v human red blood cells were taken as standard solution.

All the assay mixtures were incubated at 37^{o}c for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the hemolysis produced in control as 100% [28, 29]. 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 model

Diclofenac sodium was used as standard drug which at different concentrations (10 to 2000 µg/ml) showed inhibition of protein denaturation. EEPP and MEPG at different concentrations (10 to 2000µg/ml) also showed inhibition of protein (egg albumin) denaturation. The effect of MEPG was found to be more than EEPP as well as diclofenac sodium. The results are summarized in Table 1 and 2.

Table 1: Effect of diclofenac sodium against protein denaturation

Concentration (µg/ml)	% Inhibition	Viscosity (Cps)
Control	-	1.41
10	18.08	0.85
50	58.66	0.86
100	172.99	0.94
200	247.07	0.97
400	302.44	0.99
800	454.63	1.02
1000	624.92	1.06
2000	813.25	1.14

Human red blood cell (HRBC) membrane stabilization model

Diclofenac sodium was used as standard drug which at different concentrations (100 to 1600 µg/ml) exhibited stabilization towards HRBC membrane. EEPP and MEPG at different concentrations (100 to 1600µg/ml) also exhibited stabilization towards HRBC membrane. The effect of MEPG was found to be more than EEPP as well as diclofenac sodium. The results are summarized in Table 3 and 4.

Table 2: Comparative effect of EEPP and MEPG against protein denaturation

Concentration (µg/ml)	% Inhibition		Viscosity (Cps)	
	EEPP	MEPG	EEPP	MEPG
Control	-	-	1.41	1.41
10	22.12	24.84	0.84	0.82
50	112.16	130.42	0.85	0.84
100	223.65	242.72	0.92	0.89
200	412.72	457.96	0.94	0.91
400	516.56	686.32	0.97	0.93
800	623.23	927.27	0.98	0.94
1000	712.37	1189.69	1.03	0.96
2000	833.22	1357.73	1.05	1.02

EEPP- *Pongamia pinnata* ethanolic extract, MEPG- *Punica granatum* methanolic extract

Table 3: Effect of diclofenac sodium on HRBC membrane stabilization

Concentration (µg/ml)	% Protection
Control	-
100	21.76
200	43.60
400	59.59
800	71.48
1600	86.85

Table 4: Comparative effect of EEPP and MEPG on HRBC membrane stabilization

Concentration (µg/ml)	% Protection	
	EEPP	MEPG
Control	-	-
100	21.21	29.35
200	39.12	46.15
400	54.23	66.58
800	67.23	82.86
1600	74.28	92.71

DISCUSSION

The incredible development in the field of synthetic drugs during present era is accompanied by numerous undesirable side effects. Whereas plants still hold their own unique place, with lesser side effects [26]. For the preliminary study, two *in-vitro* models *i.e.* inhibition of protein denaturation and HRBC membrane stabilization were selected. Both are well established model for screening of anti-inflammatory and anti-arthritic activity. Inhibition of protein denaturation model has been used by Chandra et al., 2012 for the study of Ashwagandha, *Mikania scandens* and Coffee [23, 30, 31]. HRBC membrane stabilization has been used for the study of *Skimmia anquetilia*, *Gendarussa vulgaris*, *Thunnus alalunga* by Kumar et al., 2012; Saleem et al., 2011; Azeem et al., 2010, respectively [26-28].

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 viv*o. The increments in absorbances of test sample with respect to control indicate stabilization of protein i.e. inhibition of protein (albumin) denaturation by plant extract (EEPP and MEPG) and standard drug diclofenac sodium. [30] 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. However, the viscosities were found to decrease with concomitant decrease in concentration of test extract (EEPP and

MEPG) and standard drug as well. Although, the viscosities of the test samples of all concentrations were always less than that of control. Nevertheless, the viscosity data indicated inhibition of protein (albumin) denaturation [31, 32].

HRBC method was selected for the in vitro evaluation 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 [28]. Though the exact mechanism of the membrane stabilization by the extract is not known yet, 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 [26].

Literature revealed that *Pongamia pinnata* seed and seed oil contain isopongaflavone, pongol [3'-hydroxyl furano (2", 3", 7, 8)-flavone], lanceolatin-B, isopongachromene, a chromenochalone [4]. *Punica granatum* rind part contains phenolic punicalagins, gallic acid and other fatty acids, catechin, quercetin, rutin, and other flavonols, flavones, flavononesetc. [33]. Phytochemical investigation of EEPP and MEPG also reveals the presence of flavonoids, steroids etc. The compounds such as flavonoids and steroids are well known for their anti-inflammatory property and presence of these compounds in the extracts may behind the anti-arthritic activity shown by these plants [28, 34].

CONCLUSION

It can be concluded that both EEPP and MEPG showed anti-arthritic activity but by comparing the results of the both plant extracts in both *in-vitro* models, it can be stated that MEPG has more potent anti-arthritic activity than EEPP and standard drug. Further *in-vivo* study of both plants is in progress for confirmation of the results of the *in-vitro* study.

REFERENCES

1. Gautam RK. Ulcer protective action of *Punica granatum* Linn. in aspirin induced ulcer in diabetic rats. Journal of Pharmacy Research 2012; 5(8): 4389-91.
2. Kosalge SB, Fursule RA. Investigation of ethanomedicinal claims of some plants used by tribals of Satpura Hills in India. J Ethnopharmacol 2009; 121: 456-61.
3. Young CL, Seung HK, Seong SR. Suppressive effects of *Chelidonium majus* methanol extract in knee joints, regional lymph nodes, and spleen on collagen-induced arthritis in mice. J Ethnopharmacol 2007; 112: 40-8.
4. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in ayurveda, New Delhi: Central Council for Research in Ayurveda and Siddha: 2001.
5. Nadkarni KM. Indian materia medica. 3rd ed. Vol.- 1, Mumbai: Bombay popular prakashan; 1954 (Reprint 2007).
6. Satyavati GV, Gupta AK, Tandon N. Medicinal plants of India, New Delhi: Indian Council of Medical Research; 1987.
7. Srinivasan K, Muruganandan S, Lal J, Chandra S, Tandon SK, Raviprakash V. Evaluation of anti-inflammatory activity of *Pongamia pinnata* leaves in rats. J Ethnopharmacol 2001; 78:151-7.
8. Singh RK, Panday BL. Anti-inflammatory activity of seeds extracts of *Pongamia pinnata* in rats. Indian J Physio Pharmacol 1996; 40(4): 355-8.
9. Singh RK, Joshi VK, Goel RK, Gambhir SS, Acharya, SB. Pharmacological actions of *Pongamia pinnata* seeds- a preliminary study. Indian J Exp Biol 1996; 34(12): 1204-7.
10. Singh RK, Nath G, Acharya SB, Goel RK. Pharmacological actions of *Pongamia pinnata* roots in albino rats. Indian J Exp Biol 1997; 35(8): 831-6.
11. Gautam R, Sharma SC. Antiulcer activity of *Punica granatum* Linn. in diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4 (Supp 3): 459-61.
12. Venkatrao N, Korths MD, Satyanarayana S, Hemamalini R, Santakumar SM. Antidiarrhoeal and anti-inflammatory activity of fruit rind extract of *Punica granatum*. Indian Drugs 2007; 44(12): 909.
13. Sarker M, Das SC, Saha SK, Mahmud ZA, Bachar SC. Analgesic and anti-inflammatory activities of flower extracts of *Punica granatum* Linn. (Punicaceae). Journal of Applied Pharmaceutical Science 2012; 2 (4): 133-6.
14. Nain P, Saini M, Malik M. Evaluation of anti-inflammatory and analgesic activity of *Punica granatum* Linn. leaves. International Journal of Research in Ayurveda & Pharmacy 2011; 2(3): 987-90.
15. Das S, Singh RS, Ahmed S, Kanodia L. Analgesic and anti-inflammatory activities of ethanolic extract of leaves of *Punica granatum* on experimental animal models. Pharmacologyonline 2011, 3: 379-85.
16. Gupta KJ, Kumar SS, Misra V, Patel K. Evaluation of anti-nociceptive and anti-inflammatory activity of *Punica granatum* seed extract. Int Re J Pharm 2011; 2(12): 235-7.
17. Olapour S, Najafzadeh H. Evaluation of analgesic, anti-inflammatory and antiepileptic effects of hydro alcoholic peel extract of *Punica granatum* (pomegranate). Asian Journal of Medical Sciences 2010; 2(6), 266-70.
18. Gracious RR, Selvasubramanian S, Jayasundar S. Immunomodulatory of *Punica granatum* in rabbits - a preliminary study. J Ethanopharmacology. 2001; 78 (1), 85-7.
19. Ross RG, Selvasubramanian S, Samuel JJ. Effect of *Punica granatum* on dexamethasone induced immunosuppression in rabbits. Indian Journal of Animal Sciences 2004; 74 (2): 139-42.
20. Yamasaki M, Kitagawa T, Koyanagi N, Chujo H, Meada H, Kohano MJ, et al. Dietry effect of Pomegranate seed oil on immune function and lipid metabolism in mice. Nutrition 2006; 22 (1): 54-9.
21. Hedge VL, Venkatesh YP. Anaphylaxis to excipient mannitol: evidence for an immunoglobulin E-mediated mechanism. Clinical and Experimental Allergy 2004; 34(10): 1602-9.
22. Lee S, Kin BS, Kin KS, Lee S, Shin KS, Lim JS. Immunosuppressant activity of punicalagin via inhibition of NFAT activitation. Biochemical and Biophysical Research Communications 2008; 371(4): 799-803.
23. 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): 47-9.
24. 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): 822-7.
25. Narayanan BL, Rajkumar LAP, Arulanandham A, Babu NS, Gayathri T, 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): 474-8.
26. 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; 627-30.
27. 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; 147-9.
28. Azeem AK, Dilip C, Prasanth SS, Shahima VJS, Kumar S, Naseera C. Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*. Asian Pacific Journal of Tropical Medicine 2010; 794-6.
29. Chippada SC, Meena V. Antioxidant, an anti-inflammatory and anti-arthritic activity of *Centella asiatica* extracts. J Chem Bio Phy Sci 2011; 1(2): 260-9.
30. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Preliminary *in vitro* assessment of anti-inflammatory property of *Mikania scandens* flower extract. Journal of Advanced Pharmacy Education & Research 2012; 2(1): 25-31.
31. Chandra S, Dey P, Bhattacharya S. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine 2012; S178-S180.

32. Anson ML, Mirsky AE. The effect of denaturation on the viscosity of protein systems. Gen Physiol 1932; 15: 341-50.
33. Jurenka J. Therapeutic applications of Pomegranate (*Punica granatum* L.): A review. Alternative Medicine Review 2008; 13:128-44.
34. Paval J, Kaitheri SK, Potu BK, Govindan S, Kumar RS, Narayanan SN, et al. Comparing the anti-arthritic activities of the plants *Justicia gendarussa* Burm F. and *Withania somnifera* Linn.. International Journal of Green Pharmacy 2009; 4: 281-4.