



## Evaluation of Anti-Arthritic Effect of Various Fraction of Methanolic Extract of *Punica granatum* Rind: A Preliminary *In Vitro* Study

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### ABSTRACT

Rheumatoid arthritis (RA) is a chronic, relapsing autoimmune disorder. *Punica granatum* (Punicaceae family) is a plant, which is reported to have anti-inflammatory activity. In view of its potent anti-inflammatory activity, present investigation was designed to evaluate anti-arthritic potential of various fraction of methanolic extract. The present investigation was carried out to determine the possible chemical components from methanolic fraction of *Punica granatum* rind extract. The evaluation of anti-arthritic activity was done by Protien denaturation method. n-hexane, aqueous and butanolic fraction of methanolic extract showed potent anti-arthritic activity, but chloroform fraction did not showed anti-arthritic activity. Inhibition of protein denaturation was studied to establish the mechanism of Anti-arthritic effect of *Punica granatum*. From the present findings it can be concluded that *Punica granatum* possessed marked anti-arthritic effect against denaturation of protein *in vitro*. The effect was plausibly due to flavonoid, alkaloid and terpenoids contents of *Punica granatum*.

**Keywords:** *Punica granatum*, Rheumatoid arthritis, Protien denaturation method.

### INTRODUCTION

Rheumatoid arthritis is highly inflammatory poly arthritis, often leading to joint destruction, deformity and loss of function which has a worldwide distribution with an estimated prevalence of 1 to 2%. Prevalence increases with age, approach hing 5% in women over age 55<sup>1</sup>. Rheumatoid arthritis, or RA, was first described clinically in a 1800 doctoral thesis by Landre-Beauvais, a French medical student, who called the condition "Primary Aesthenic Gout." Sir Alfred Garrod established the distinction between RA and Gout in 1859 and gave the condition its present name.<sup>2</sup>Inflammatory diseases including different types of rheumatic diseases are a major cause of morbidity of the working force throughout world. This has been called the 'King of Human Miseries'<sup>3</sup>.

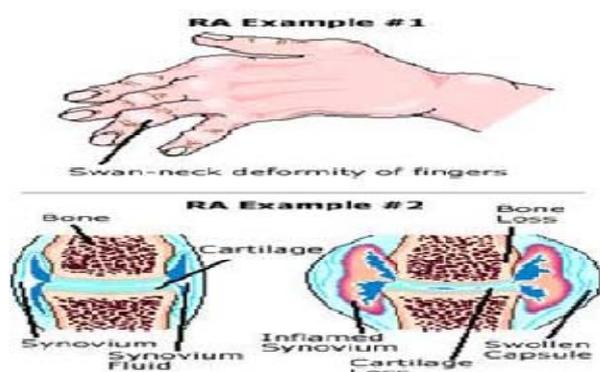
Rheumatoid arthritis has 19th century roots and a 20th century pedigree. Although its name was introduced in the 1850s. Rheumatoid arthritis is characterized by persistent synovitis, systemic inflammation and auto antibodies (particularly to rheumatoid factor and citrullinated peptide)<sup>4</sup>. It. caused by no of pro-inflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotrines and cytokines. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like cox and lox are the potential target for chronic inflammatory conditions<sup>5</sup>. The production of auto antigens in certain arthritic diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic,hydrogen, hydrophobic and disulphide bonding.<sup>6</sup>

### Pathophysiology

Like many other forms of arthritis, RA is initially characterized by an inflammatory response of the synovial membrane, including hyperplasia, increased vascularity and infiltration of inflammatory cells, primarily antigen-driven CD4+ T cells. These cells, through cell-cell contact and production of different cytokines, such as interferon- $\gamma$  (IFN- $\gamma$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), then activate monocytes, macrophages and synovial fibroblasts to overproduce the proinflammatory cytokines IL-1, IL-6, and TNF- $\alpha$ , which appear to play a pivotal role in the progression of RA. Consequently, these cytokines are now very popular therapeutic targets for the treatment of RA.<sup>7</sup> (doc.)

Interleukin-1 (IL-1) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) stimulate the expression of adhesion molecules on endothelial cells and increase the recruitment of neutrophil into the joints. TNF- $\alpha$  causes bone destruction in RA (Figure 1). Neutrophil release elastase and proteases, which degrade proteoglycan in the superficial layer of cartilage. The depletion of proteoglycan enables immune complexes to precipitate in the superficial layer of collagens and exposes chondrocytes. Chondrocytes and synovial fibroblasts release matrix metalloproteinase (MMPs) when stimulated by IL-1, TNF- $\alpha$ , or activated CD4+ T cells. MMPs, in particular stromelysin and collagenases, are enzymes that degrade connective-tissue matrix and are thought to be the main mediators of joint damage in RA. Thus result cartilage and bone destruction and to the fibrosis.<sup>8</sup>





**Figure 1:** Joint deformity in fingers and bone<sup>9</sup>

RA progresses in three stages.

- The first stage is the swelling of the synovial lining, causing pain, warmth, stiffness, redness and swelling around the joint.
- Second is the rapid division and growth of cells, or pannus, which causes the synovium to thicken.
- In the third stage, the inflamed cells release enzymes that may digest bone and cartilage, often causing the involved joint to lose its shape and alignment, more pain and loss of movement.

This disease also affects the tissues surrounding the joints such as skin, blood vessels, and muscles.<sup>10</sup>

#### Currently available treatment treatments for RA

Improved understanding of the pathogenesis of rheumatoid arthritis has led to the development of various RA treatments. The current therapies for RA are divided into four categories: non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, non-biologic disease-modifying anti-rheumatic drugs (DMARDs) and biologic DMARDs.

Current study aimed to find out the possible role of this plant extracts against protein denaturation method induced arthritis and give a scientific rationale for their use.

#### MATERIALS AND METHODS

##### Preparation of extracts

The fruit of *Punica granatum* fruits were collected from local market of Udaipur. The fruit were identified by Dr. D. K. Sarocia, Assistant Professor, Department of Horticulture, Rajasthan College of Agriculture, Udaipur, India. Rind portion of fruit was separated out from the fruit. The rind were dried under the shed and powdered into the coarse form. It was successively extracted with 70% alcohol using soxhlet apparatus. The extract was concentrated under reduce pressure and preserved under reduced pressure and the residues were then successively partitioned between water and n-hexane followed by chloroform and n-butanol. The solutions were completely evaporated to give the respective fractions.

#### Chemicals and Instruments

All chemicals used in the estimation were of analytical grade. Shimadzu 1800 UV Visible spectrophotometer was used for the in vitro study.

#### Anti arthritic activity by inhibition of protein denaturation method

1. **Control solution** (50 ml) consists of 2 ml of egg albumin and 28 ml of phosphate buffer and 20 ml distilled water.
2. **Standard Drug** (50 ml) consists of 2 ml of egg albumin and 28 ml of phosphate buffer and various concentrations of standard drug (Diclofenac Sodium at conc. of 10, 50, 100, 200, 400, 800, 1000 and 2000 µg/ml).
3. **Test solution** (50 ml) consists of 2ml of Egg albumin and 28 ml of phosphate buffer and various concentrations of plant extract (PG fraction at conc. of 10, 50, 100, 200, 400, 800, 1000 and 2000 µg/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 416nm. The percentage inhibition of protein denaturation was calculated using the formula.<sup>11</sup>

$$\text{Percentage inhibition} = \left[ \frac{V_t}{V_c} - 1 \right] \times 10$$

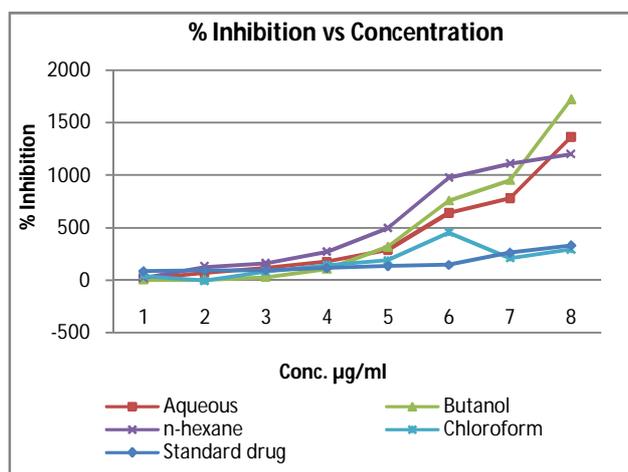
Where,  $V_t$  = absorbance of test sample,  $V_c$  = absorbance of control.

#### RESULTS

Plants continue to serve as possible source for new drug and chemicals. They are extremely useful as a lead structure for synthetic modification and optimization of bioactivity.

**Table 1:** Anti-inflammatory activity *Punica granatum* rind by protein denaturation method

Conc. (µg/ml)	% Inhibition				
	Aqueous	Butanol	n-Hexane	Chloroform	Standard drug
10	13.09	2.38	20.68	37.93	82.14
50	67.85	3.57	125.28	-8.04	85.71
100	116.66	28.57	159.77	78.16	90.07
200	176.19	103.57	270.45	142.52	110.71
400	285.71	315.47	496.53	187.35	129.76
800	640.47	754.76	977.01	452.87	140.87
1000	779.76	950	1106.89	209.19	258.33
2000	1365.54	1721.42	1201.14	37.93	327.77



**Graph 1:** Anti-inflammatory activity *Punica granatum* rind by protein denaturation method.

## DISCUSSION

In the present study the protein denaturation bioassay was selected for in vitro assessment of anti-arthritis property of methanolic extract of *Punica granatum* rind. 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. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-arthritis drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by extract and reference drug diclofenac sodium<sup>12</sup>.

*In vitro* studies on *P.Granatum* demonstrate suppression in arthritis. The fraction of methanolic extract of the rind of *P.Granatum* must contain some principles like steroids, alkaloids, tannins and flavonoid type of compounds, which possess anti-arthritis activities. Hence attempt was made to compare the methanolic extract and its fraction for anti-arthritis activity. The present study concludes that three fraction (Aqueous, n-Hexane and butanol) shows more anti-arthritis activity compared to standard Diclofenac sodium.

## CONCLUSION

Inhibition of protein denaturation and membrane stabilisation was studied to establish the mechanism of anti-arthritis effect of fraction of methanolic extract of *P.granatum*. Therefore, our present *in-vitro* studies on *P.granatum* extracts demonstrated the significant anti-arthritis activity. Due to the presence of active principles

such as flavonoids, steroid and triterpenoids may responsible for this activity. Hence *P.granatum* can be used as a potent anti-arthritis agent.

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