EVALUATION OF IN VITRO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF *PHASEOLUS VULGARIS* L AQUEOUS EXTRACT

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Abstract

The aim of present study was to evaluate the significance of *Phaseolus vulgaris L* seed extract for anti-arthritic activity using in vitro inhibition of protein denaturation model and anti-inflammatory activity by HRBC (Human Red Blood Cell) membrane stabilization method assay whereby Diclofenac sodium was used as a standard drug for comparisation. Ant-arthritic activity was carried out using 250-1000 µg/ml concentration, whereas anti-inflammatory activity at the concentration of 250- 1000 µg/ml. The results of the present study represents that the aqueous seed extract of *Phaseolus vulgaris* L demonstrates dose dependent inhibition. The maximum ant-arthritic activity of *Phaseolus vulgaris L* was found at the concentration of 1000 µg/ml with 80.66% inhibition. Correspondingly maximum anti-inflammatory activity of *Phaseolus vulgaris* L was observed at the concentration of 1000 µg/ml with 93.06% inhibition. It is apparent that the cause of inflammation and arthritis is mainly due to denaturation of tissue proteins and the present study explains that denaturation of proteins is inhibited, inhibiting the progression of arthritis and reducing the cause of inflammation.

Keywords: *Phaseolus vulgaris* L; anti-inflammatory; anti-arthritic; HRBC

1. INTRODUCTION

Twenty percent of the world's elderly suffer with arthritis yet the issues they face get a bit of consolidation and remedy other than some indicative relief from the pain. Rheumatism means joint inflammation; it is a chronic, progressive and disabling autoimmune disease. The disease mostly affects the ageing population although it can affect anyone with malfunctioning immune system or genetic degenerative bone disorder. Rheumatism can progress very rapidly causing swelling and damaging cartilage and bone around the joints. Any joint may be affected but it is commonly the hands, feet and wrists. It is a systemic disease which means that it can affect the whole body and internal organs (although this is not the case for everyone with rheumatoid arthritis) such as the lungs, heart and eyes (Hegan et al., 2008; Murugananthan et al., 2011).

Rheumatism can cause severe disability and ultimately affects a person's ability to carry out everyday tasks. Any part of the body can become inflamed or painful from arthritis. The two most-common types of arthritis are osteoarthritis and rheumatoid arthritis. Osteoarthritis is a degenerative joint disease, resulting from the wear and tear from day to day life. It leads to pain, tenderness, swelling, and decreased function of joints. The joints most often affected by osteoarthritis are knees, hips, hands, and spine. Rheumatoid arthritis is an

autoimmune disease that occurs when the body's own immune system mistakenly attacks the synovium (cell lining inside the joint). It causes joint pain, stiffness, swelling, and loss of joint function. Fortunately, nature has a remedy for this condition and there are a number of herbs that work synergistically to reduce chronic joint inflammation, such as osteoarthritis and rheumatoid arthritis (Bang *et al*., 2009).

Traditional folklore medicine can be of great value when used in a program of health care and highly effective preventive medicine when compared to expensive synthetic drugs. The WHO notes that from 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. About 25 percent of today's prescription drugs are at least partially derived from plants (Hegan *et al.*, 2008).

Phaseolus vulgaris L is the most popular bean in the United States and northwestern Mexico(Maize 2003 CGC Meeting, 2013), and is most often eaten whole (sometimes in broth), or mashed and then *refried*. Studies have indicated pinto beans can lower the levels of both HDL and LDL cholesterol. (Pinto beans have also been shown to contain the phytoestrogen coumestrol, which has a variety of possible health effects. (Bhagwat *et al*., 2008). Hence, the present study was undertaken to evaluate the potential of in vitro anti-arthritic activity and anti-inflammatory activity of *Phaseolus vulgaris L* seed extract.

2. MATERIALS AND METHODS

2.1. Collection and preparation of plant material

The healthy seeds of the *Phaseolus vulgaris L* seed from Erode market, Tamilnadu, India. The plant specimen was identified by botanist in department of Agricultural and forestry, Botanical survey, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2018/Tech,3321). The specimens were stored in Department of Biochemistry, Bharathidasan College of Arts and Science, Erode, Tamil Nadu, India.

2.3. Preparation of seed Extract

The seeds were cleaned by removing unhealthy seeds, shaded dried and then powdered. 50g of the powder was filled in the thimble and extracted successively using water 500ml as solvent for 16 hours and was concentrated under reduced pressure in a rotary evaporator at 60±10℃ to yield the required quantity of crude extract. The extract was stored and used for further studies.

2.4. In vitro anti-arthritic activity by inhibition of protein denaturation method

1. The Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% W/V aqueous solution) and 0.05ml of test solution of each seed extract of various concentrations (250, 500, 1000 µg/ml).

2. Test control solution (0.5ml) consists of 0.45ml of bovine serum albumin (5% W/V aqueous solution) and 0.05ml of distilled water.

3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.

4. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% W/V aqueous solution) and 0.05ml of diclofenac sodium (250µg/ml).

All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UVVisible spectrophotometer at 416 nm (Shravan Kumar N *et al*., 2011, Deshpande V *et al.*, 2009, Kokila N *et al.*, 2013) .

The percentage inhibition of protein denaturation can be calculated as;

Percentage Inhibition = [100 - (optical density of test solution – optical density of product control) / (optical density of test control) × 100.

The control represents 100% protein denaturation. The results were compared with diclofenac sodium (250µg/ml)

2.5. In vitro anti-inflammatory activity by HRBC membrane stabilization method

The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline (0.36%), 0.5 ml HRBC suspension (10% v/v) with 0.5 ml of seed extract of various concentrations (250, 500, 1000 µg/ml), standard drug diclofenac sodium (250, 500, 1000 µg/ml) and control distilled water instead of hypo saline to produce 100 % hemolysis were incubated at 37oC for 30 min and centrifuged respectively 1.(Shravan Kumar N *et al.*, 2011, Yogesh VU *et al* .,2013). The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. The percentage hemolysis produced in the presence of distilled water was taken as 100 %.

Percentage of HRBC membrane stabilization or protection was calculated using the formula;

Percentage stabilization = 100 - [(optical density of test solution) ÷ (optical density of control) × 100].

3. RESULT AND DISCUSSION

Anti-arthritic and Anti-inflammatory effect of aqueous extract of *Phaseolus vulgaris* L was studied significantly by using in-vitro inhibition of protein denaturation model and In vitro anti-inflammatory activity by HRBC membrane stabilization method.

Table:1 In vitro anti-arthritic activity of *Phaseolus vulgaris L seed* extract on inhibition of protein denaturation

	% inhibition of protein denaturation	
Concentration (µg/ml)	Plant parts seed	Standard Diclofenac-sodium
250	47.22	51.20
500	75.52	80.36
1000	80.66	86.50

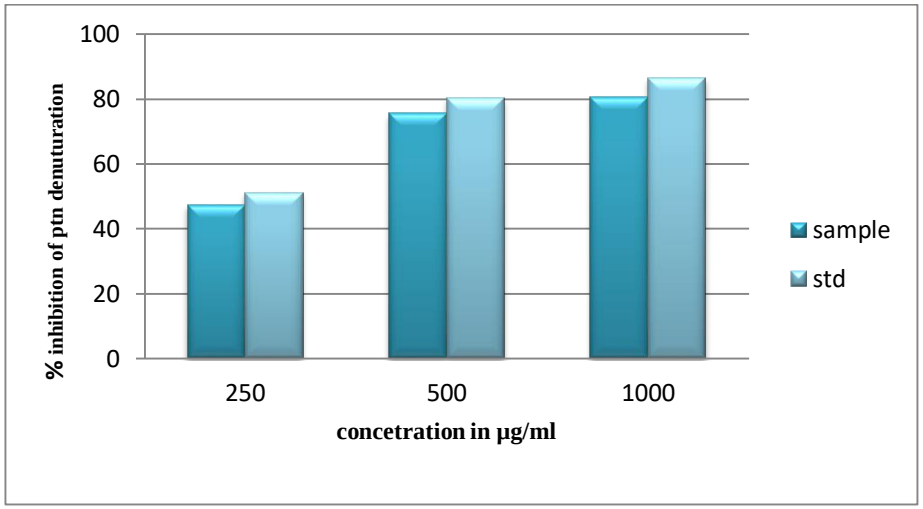

Fig 1: In vitro anti-arthritic activity of *Phaseolus vulgaris L seed* extract on inhibition of protein denaturation

In vitro anti-arthritic activity by inhibition of protein denaturation method: Table 1 displayed the effect of aqueous extract of *Phaseolus vulgaris L seed* on inhibition of protein denaturation. Extract of *Phaseolus vulgaris L* at different concentrations (dose levels) provided significant protection against denaturation of proteins. The maximum percentage inhibition was observed at (80.66% at 1000µg/ml) concentration as compared to other concentrations.

It possesses significant activity comparable with that of the standard diclofenac sodium. The most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins (Arya D *et al.*, 2013, Singh M *et al.*, 2011)

Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. From the results of present study it can observed that aqueous extracts of *Phaseolus vulgaris L seed* is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

Table:2 In vitro anti-inflammatory activity of *Phaseolus vulgaris L seed* extract on stabilization of HRBC membrane

	% stabilization on HRBC membrane	
Concentration (µg/ml)	Plant parts seed	Standard Diclofenac-sodium
250	76.42	80.60
500	80.5	86.39
1000	93.06	95.25

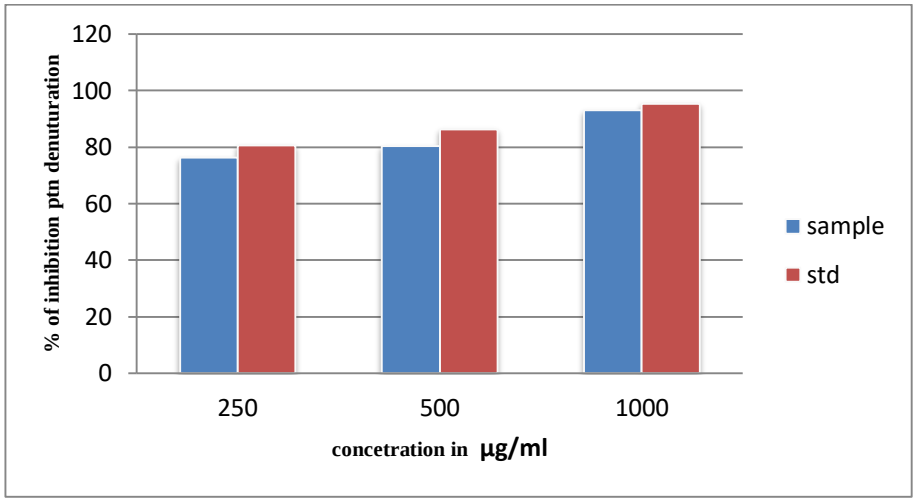

Fig: 2 In vitro anti-inflammatory activity of *Phaseolus vulgaris L seed* extract on on stabilization of HRBC membrane

In vitro anti-inflammatory activity by HRBC membrane stabilization method: The investigation is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. The effect of aqueous extracts of *Phaseolus vulgaris L seed* on stabilization of HRBC membrane is shown in table 2

The maximum percentage stabilization was observed in aqueous extracts of *Phaseolus vulgaris L seed* (93.06% at 1000µg/ml) as compared to other concentrations. It possesses significant activity comparable with that of the standard diclofenac sodium (Rajalakshmi GR *Et al*., 2013, Arya D *et al*., 2013). *Phaseolus vulgaris L seed* extract has significant anti-inflammatory activity which may be due to presence of chemical profile such as flavonoids, triterpenoids and phenols.

4. CONCLUSION

Owing to the global distress against the side effects caused by allopathic medicine there is essential need of returning back to nature, focusing on traditional folklore medicine to cure various diseases among the scientific community. The current research explores the usage of *Phaseolus vulgaris L* against arthritis and inflammation experimentally representing significant activity. Hence it could be beneficial for further research work to be carried out on *Phaseolus vulgaris L* seed extract to explore its potential in anti-arthritic and anti-inflammatory activity.

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