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Original Research Article

Anti-Arthritic Activity of the Leaves of *Urena lobata* Linn

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ABSTRACT

A study was carried out to ascertain the *in vitro* anti-arthritic activity of the aqueous extract of the leaves of *Urena lobata*. The activity was evaluated by means of protein denaturation method. The aqueous extract of the leaves of the plant produced remarkable anti-arthritic activity and the activity produced was comparable to the activity produced by acetyl salicylic acid which was used as the reference standard during the evaluation.

Key words: *Urena lobata*, Arthritis, Protein denaturation

INTRODUCTION

Rheumatoid arthritis is a chronic systemic inflammatory disorder that may affect many tissues and organs like skin, blood vessels, heart, lungs and muscles, but principally attacks the joints, producing a non-suppurative, proliferative and inflammatory synovitis that often progress to destruction of the articular cartilage and ankylosis of the joints. Although the cause of rheumatoid arthritis remains unknown, autoimmunity places a pivotal role in its chronicity and progression. [1] In about half of the patients, rheumatoid arthritis may begin slowly and insidiously with malaise, fatigue and generalized musculoskeletal pain, likely mediated by interleukin-1(IL-1) and Tumour necrosis factor(TNF). After several weeks to months the joints become involved. The involved joints are swollen, warm, painful and particularly stiff when rising in the morning or following inactivity. [2]

Protein denaturation involves changes within the molecule that cause the protein to become insoluble in solvents in which it was originally soluble. Proteins may be denatured by numerous agents and processes. Denaturation may be effected by solution in acid or alkali and standing for sometime at ordinary temperature or by briefly heating. Treatment with alcohol, acetone and other organic solvents leads to denaturation. The denaturation of protein is caused by various physical agencies besides heat .It is caused by exposure to X-rays or ultraviolet light and by visible light in the presence of a photosensitizer. Violent shaking of a protein solution leads to denaturation as a result of surface action. It has been established that protein films adsorbed into surfaces and interfaces may be acted upon by the unbalanced surface forces to cause denaturation of the protein in the film. Proteins may also be denatured by subjection to very high pressures. [3]

Urena lobata is a shrub from malvaceae family [4,7] Traditionally the plant is being used as diuretic, febrifuge and rheumatism. It is useful for wounds, toothache, gonorrhoea and for food for animals as well humans [5,7] The leaf of the plant contains secondary metabolites like alkaloids, flavonoids, saponins and tannins. [6]

Source of the plant

The leaves were collected from Pariyaram Medical College campus in the month of March and the same was authenticated by Dr. A. K. Pradeep, Asst. Professor, Department of Botany, University of Calicut, Kerala. It was then shade dried and a specimen of bearing voucher no. UL(L) 01/18 has been deposited in the Department of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur District, Kerala State, South India.

Fig.1. ***Urena lobata***

Preparation of aqueous extract

About 500gms of the dried and powdered leaves were macerated with chloroform water for seven days. The extract was filtered and concentrated in vacuo to syrupy consistency and dried in vacuum desiccators. [8]

Anti-arthritic Studies

Protein denaturation by bovine albumin

The aqueous extract of the leaves of *Urena lobata* at different concentrations and 1% of aqueous solution of bovine albumin were incubated at 37^0C for 20 minutes and then heated at 57^0C for 20 minutes. After cooling the samples, the absorbance of turbidity was measured at 660nm. The percentage of inhibition of protein denaturation was calculated by using the following formula; [9]

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Protein denaturation by egg albumin

The 5ml of reaction mixture consists of 0.2ml of egg albumin obtained from the fresh hen's egg, 2.8ml of phosphate buffered saline of P^H 604 and 2ml of varying concentrations of aqueous extract of the leaves of *Urena lobata* so that the final concentration become 100, 200, 400,600, 800 and 1000µg/ml. Distilled water of similar volume can be used as control. The reaction mixtures were incubated at 37±2 ^{0}C in a BOD incubator for 15 minutes and then heated at 70^0C for 15 minutes. After cooling, their absorbance was measured at 660nm. Acetyl salicylic acid was used as reference standard. The percentage of inhibition of protein denaturation was calculated by using the following formula; [10]

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

RESULTS

In the present study, the aqueous extract of the leaves of *Urena lobata* were investigated for anti-arthritic activity by protein denaturation method. Acetyl salicylic acid was used as the standard during the evaluation. The maximum anti-arthritic activity was observed in the concentration 1000µg/ml, while the minimum activity was observed in the concentration 100 µg/ml. The *in vitro* anti-arthritic activity by bovine albumin method is shown in table 1, where, the percentage of arthritic protection for the extract was found to be 101.5 in 1000ml concentration and 79.6 for acetyl salicylic acid. Similar results were obtained in the protein denaturation method using egg albumin method and are tabulated in table 2. The inhibition of protein denaturation of egg albumin for the

extract was found to be 109.9 and that of standard drug was found to be 78.9. From the findings the aqueous extract of the leaves of *Urena lobata* exhibited a dose dependent response. The effects of the anti-arthritic activity of the aqueous extract of the leaves were comparable with the reference standard used in the evaluation.

Table.1 Inhibition of protein denaturation (%) of the leaves of *Urena lobata*

Anti-arthritic evaluation	Concentration (µg/ml)	Percentage of Inhibition (Bovine albumin)
Aqueous extract of the leaves of *Urena lobata*	100	12.3
	200	29.7
	400	44.5
	600	66.9
	800	86.6
	1000	101.5
Acetyl salicylic acid (Reference standard)	50	79.6

Table.2Inhibition of protein denaturation (%) of the leaves of *Urena lobata*

Anti-arthritic evaluation	Concentration (µg/ml)	Percentage of Inhibition (Egg albumin)
Aqueous extract of the leaves of *Urena lobata*	100	17.6
	200	30.5
	400	53.8
	600	71.5
	800	89.2
	1000	109.9
Acetyl salicylic acid (Reference standard)	50	78.9

DISCUSSION

From the findings we can say that the aqueous extract of the leaves of *Urena lobata* can inhibit the denaturation of proteins. The drugs which can prevent the protein denaturation can be utilized in the development of anti-arthritic drugs. As in other autoimmune diseases, genetic predisposition and environmental factors contributes to the development, progression and chronicity of the disease. The pathological changes are mediated by antibodies against self antigens and cytokinine-mediated inflammation predominantly secreted by CD4+ T cells. CD4+ T helper (T_H) cells may initiate the autoimmune response in rheumatoid arthritis by reacting with an arthritogenic agent, perhaps microbial or a self-antigen. The T cells produce cytokines that stimulates other inflammatory cells to effect tissue injury. Although a large cytokines can be isolated from inflamed joints, of which TNF has been most infirmly implicated in the pathogenesis of rheumatoid arthritis and TNF antagonists have proved to be remarkable effective therapies for the disease. [2]

In addition the flavonoids are capable of inhibiting the denaturation of protein. [11] Further PuspalDe et al reported the antiprotein denaturation effects of alkaloids, flavonoids etc, in *Piper betle.* The leaf of *Urena lobata* is also rich in bioactive compounds like flavonoids, alkaloids and tannins. Hence the probable mechanism of anti-arthritic activity by inhibiting the denaturation of protein exhibited by the aqueous extract of the leaves of *Urena lobata* could be due to these bio active compounds.

CONCLUSION

Plants are considered to be one of the major sources for getting medicinally important bio active compounds. The plant *Urena lobata* was traditionally utilized for treating arthritis [5,7] but it has not been scientifically proved, That was the rationale in selecting this particular plant species for the above said activity. Our study revealed that the aqueous extract of the plant possess potent anti-arthritic effect. However, further investigations are required to isolate the active constituents responsible for the observed effect, and to elucidate the possible mechanism of action responsible for the anti-arthritic activity of the aqueous extract of the plant.

Conflict of Interest: Nil

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