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## ***In vitro* protein denaturation and membrane stabilizing anti-arthritis activity of aqueous extracts of bark of *Ficus benghalensis* L. against methotrexate**

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### **Abstract**

The *in vitro* anti-arthritis activity of the aqueous extract of bark of *Ficus benghalensis* L. were studied by protein denaturation inhibition assay and membrane stabilizing activity. Methotrexate, well-established and promising DMARDs which is commonly used in inflammatory conditions like rheumatoid arthritis was used as standard drug. In rheumatoid arthritis protein denaturation was the main cause of inflammation. HRBC membrane stabilization was similar to lysosomal membrane which influences the process of inflammation. The percentage of protein denaturation and membrane stabilization for aqueous extracts were done at different concentrations (100,200,400,800,1000 µg/ml). The maximum inhibition of protein denaturation and membrane stabilization of aqueous extracts of *Ficus benghalensis* L. was found to be 45.31 ±1.90 and 62.50 ±0.66 at dose of 1000 µg/ml respectively and standard inhibition of protein denaturation and membrane stabilization using methotrexate was found to be 87.50 and 81.25 at 100 µg/ml respectively. The aqueous extracts of *Ficus benghalensis* showed significant activity at the highest concentration.

**Keywords:** *Ficus benghalensis*, protein denaturation assay, membrane stabilizing activity, anti-arthritis activity, methotrexate

### **Introduction**

Inflammation is the defense mechanism in living tissues to get protection from injury, irritation and infection. The mechanism of inflammation is attributed in part to release of reactive oxygen species (ROS) from activated neutrophils and macrophages. Prolonged inflammation leads to rheumatoid arthritis, autoimmune disease and other infectious diseases [1].

Rheumatoid arthritis is a common chronic inflammatory autoimmune disease of joints accompanied by progressive destruction of bones, joints and affects other organs of body. It affects an estimated 1% population throughout the world. The cause of rheumatic arthritis is due to genetic and environmental factors which results in body's immune system attacks the joints [2, 3]. Progression of the disease results in joint destruction, deformity and significant disability [4]. It is characterized by autoantibody production, bone destruction, skeletal disorders and synovial inflammation [5].

Various classes of drugs which have been used to treat pain and inflammation in rheumatoid arthritis belong to the category of NSAIDs, Corticosteroids, DMARDs and Biological. All these drugs show severe side effects like ulceration, malignancies and infections [6]. Therefore, there is need to find new effective, economical, beneficial and safe alternative treatment for rheumatoid arthritis. The traditional plants used worldwide remains major source of active constituents for curing various diseases. World's most population relies on traditional medicine for primary healthcare needs and involves use of plant extracts or their components. The traditional medicine remains an alternative to modern medicine. The combination therapies of herbal products with DMARDs are gradually and widely accepted in management of rheumatoid arthritis. Various plants have been used for curing pain and inflammatory conditions like arthritis. Plants are excellent sources of antioxidants, anti-arthritis and anti-inflammatory agents [7].

Proteins lose its structure or become denatured when there is activation of various enzymes, migration of tissues and break down of tissues occurs [8, 9]. The hypo tonicity and heat induced hemolysis of erythrocytes is commonly used method for assessing anti-inflammatory activity.

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The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane. In addition, lysis of lysosomal membranes during chronic inflammation has been proven to release pro-inflammatory markers including activated neutrophils, proteases and histamines at the local site of tissue damage [10, 11, 12].

*Ficus benghalensis* L. commonly known as Banyan tree belongs to family Moraceae. Various parts of plants is claimed to possess anti-arthritic, antileprotic, anti-inflammatory, anti-spasmodic, laxative properties and useful in many other disease [13, 14]. In ayurveda it is used for diarrhoea, dysentery, piles, and diuretic, astringent and applied to gums to lessen inflammation. The various activities like antioxidant property [15], immunomodulatory activity of methanolic extract of roots [16] and anti-inflammatory activity of ethanolic bark extract [17] have been reported. The analgesic, anti-rheumatic and anti-oxidant activity of methanolic extract were studied at doses of 100,200,and 3000 mg/kg [18].

The present study is aimed to investigate anti-arthritic activity of aqueous extract of *Ficus benghalensis* L. by *in vitro* methods using methotrexate as a standard drug.

## Material and Method

### Collection of Plant

The barks of *Ficus benghalensis* L. were collected from local area of Sangli districts and authenticated by Dr. V. B. Awale, an approved Botanist, Dept. of Botany, Dr. Patangrao kadam Mahavidyalaya, Sangli. A specimen voucher no. (ACOP/SNG/101 to 120) has been stored for further reference in Department of Pharmacognosy, Adarsh College of Pharmacy, Vita, Sangli, MS, India.

### Extraction

The cleaned barks of *Ficus benghalensis* L. were dried at room temperature. Further it was pulverized and passed through sieve no.40. The obtained powdered material were then macerated for 7 days with water containing chloroform in the ratio 16:1. The filtered extract was concentrated and stored for further use.

### Method: *In vitro* anti -arthritic activity [19, 20]

The anti-denaturation study for investigating anti-arthritic activity was performed using bovine serum albumin (BSA). When BSA is heated, it undergoes denaturation and antigens are expressed which are associated with type-III hypersensitivity reaction, which in turn is related to various diseases like rheumatoid arthritis.

### Inhibition of protein denaturation [21]

The method was adopted from the previous article published by Pavithra, T.K *et al.* 2015 with slight modification. The reaction mixture consisted of the 100µl aqueous extracts of *Ficus benghalensis* (final concentration 100, 200, 400, 800, 1000 µg/ml) and 100µl of 5% aqueous solution of bovine serum albumin (BSA). The sample was incubated at 37 °C for 20 min and then temperature. Was increased to 70 °C in hot water bath for 10 min.

The mixture was allowed to cool under tap water for 10 min after which turbidity and absorbance was measured at 660 nm. The blank comprised of the distilled water. The results were compared with methotrexate.

**Formula: Formula:** The percentage inhibition of protein denaturation can be calculated as:

$$\text{Percentage inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

### Effect on Membrane Stabilization [22]

The method used was from procedure adopted by Vallabh Deshpande *et al.* with some modifications. Blood sample from healthy human volunteers who did not consumed NSAID for two weeks prior to this experiment was obtained. The reaction mixtures 4.5ml consists of 2ml hypotonic saline (0.25% NaCl) + 1ml 0.15M phosphate buffer (pH 7.4) + 1ml test solution (100-500µg/ml) in normal saline + 0.5ml of 10% HRBC in normal saline. The mixture was incubated at 56°C for 30 minutes. The test tubes were cooled under running tap water for 20 minutes. After that reaction mixture was centrifuged for 3000rpm for 10min and the absorbance of the supernatant was measured at 560nm. The methotrexate was used as standard drug.

**Formula:** The percentage membrane stabilization activity was calculated as per following formula

$$\% \text{ protection} = \frac{100 - \text{OD of sample}}{\text{OD of control}} \times 100$$

**Table 1:** The percentage inhibition of different concentration of aqueous extracts of *Ficus benghalensis* L. by Protein denaturation method (BSA)

Plant name	Conc (µg/ml)	OD	% inhibition	IC50
Blank		0.64		
<i>Ficus benghalensis</i>	100	0.57	10.94±0.80	1157.68
	200	0.53	17.19±1.03	
	400	0.47	26.56±1.14	
	800	0.41	35.94±1.06	
	1000	0.352	45.31±1.90	

Values represent in the results are mean±SD of three replicates; linear regression analysis was used to calculate IC50 value

**Table 2:** The percentage inhibition of different concentration of Methotrexate by Protein denaturation method (BSA)

Drug	Conc (µg/ml)	OD	% inhibition	IC50 (µg/ml)
Blank		0.64		
Standard Methotrexate	10	0.48	25.00	35.53
	20	0.31	51.56	
	40	0.29	54.68	
	80	0.17	73.43	
	100	0.08	87.50	

Values represent in the results are mean±SD of three replicates; linear regression analysis was used to calculate IC50 value

**Table 3:** The percentage stabilization of aqueous extracts of *Ficus benghalensis* by HRBC Membrane Stabilization method.

Plant name	Conc (µg/ml)	OD	% inhibition	IC50
Blank		0.32		
<i>Ficus benghalensis</i>	100	0.23	28.12 ±1.01	747.50
	200	0.21	34.37 ±1.19	
	400	0.19	40.62 ±1.10	
	800	0.17	46.87 ±0.93	
	1000	0.12	62.50 ±0.66	

Values represent in the results are mean±SD of three replicates; linear regression analysis was used to calculate IC50 value

**Table 4:** The percentage inhibition of different concentration of Methotrexate by HRBC Membrane Stabilization method.

Drug	Conc (µg/ml)	OD	% inhibition	IC50 (µg/ml)
Blank		0.32		
Standard (methotrexate)	10	0.15	53.12	>100
	20	0.12	62.50	
	40	0.10	68.75	
	80	0.08	75.00	
	100	0.06	81.25	

Values represent in the results are mean±SD of three replicates; linear regression analysis was used to calculate IC50 value

## Results and Discussion

The *in vitro* anti-arthritis activity of various concentrations of aqueous extract of bark of *Ficus benghalensis* L. was studied by protein denaturation and HRBC membrane stabilization method respectively. The inhibition of protein denaturation and inhibition of hypotonicity induced membrane stabilization was taken as a measure of the anti-arthritis activity. The percentage of inhibition of protein denaturation and percentage of membrane stabilization for aqueous extract of *Ficus benghalensis* and methotrexate were done at concentration (100, 200, 400, 800, 1000 µg/ml) and (10, 20, 40, 80, 100 µg/ml) respectively. Aqueous extracts of *Ficus benghalensis* are effective in inhibiting protein denaturation at different concentrations. It showed that maximum inhibition 65.62% ±1.10 at 1000 µg/ml of aqueous extract and 87.50% at 100 µg/ml of methotrexate. The inhibition of hypotonicity induced HRBC membrane lysis i.e., stabilization of HRBC membrane was taken as a measure of the anti-arthritis activity. Aqueous extracts of *Ficus benghalensis* are effective in inhibiting the heat induced hemolysis of HRBC at different concentrations. It showed that maximum inhibition 71.87 ±1.46 at 1000 µg/ml of aq. extract and 81.25% at 100 µg/ml of methotrexate. With increasing concentration, the inhibition of protein denaturation and membrane stabilization and protection is also increased. Hence anti-arthritis activity of extracts was concentration dependent.

Protein denaturation which is identified as a cause of inflammation occurs when living tissue is injured. Due to this disruption of electrostatic, hydrogen, disulphide bonds occurs which results in denaturation of proteins. The compounds which prevent these changes are having potential anti-arthritis activity. HRBC membrane stabilization was similar to lysosomal membrane which influences the process of inflammation. The inflammatory response of lysosomal membrane was important because it helps the inhibition process by inhibiting the release of lysosomal constituents of activated neutrophil. The aqueous extract of *Ficus benghalensis* may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation.

## Conclusion

Present research work was an attempt to find out the ant arthritic potential of aqueous extract of bark of *Ficus benghalensis* L. Protein denaturation and Stabilization of HRBC membrane by hypotonicity induced membrane lysis was carried out to check activities. Our present work on *in vitro* studies of *Ficus benghalensis* demonstrate significant and dose dependent ant arthritic activity. The presence of active principles in the aqueous extracts may be responsible for this activity. Further investigation should be carried out to find mechanism of action of aqueous extract of *Ficus benghalensis* L.

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