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Research Article

IN VITRO ANTI-ARTHRITIC ACTIVITY OF ETHANOLIC EXTRACT OF *CALLICARPA MACROPHYLLA* FLOWER

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ABSTRACT

The present study is aimed to evaluate the *in-vitro* anti-arthritic activity of ethanolic extract of *Callicarpa macrophylla* flower using inhibition of protein denaturation model and human red blood cell Membrane stabilization model. Diclofenac sodium was used as a standard drug. Results revealed that the ethanolic extract of *Callicarpa macrophylla* at different concentrations possessed significant anti-arthritic activity as compared to standard drug used as Diclofenac sodium. The results obtained in the present investigation Indicate that ethanolic extract of *Callicarpa macrophylla* flower showed anti-arthritic activity.

Key words: *Callicarpa macrophylla,* anti-inflammatory, Anti-arthritic, protein denaturation, Membrane stabilization.

INTRODUCTION

Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage.[1] Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells[2] It is a common disease having peak incidence in 3rd to 4th decades of life with 3-5 times higher preponderance in female.[3] Its prevalence depends upon age.[4] The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers.[5,6] Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity.[7] The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas.[8] Number of synthetic medicines has been derived from medicinal herbs.[9] The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

Callicarpa macrophylla of family Verbenaceae, is an indigenous plant of India, have with a wide spectrum of therapeutic properties. Its leaves are reported to have anti-inflammatory, analgesic and antipyretic effects.[10,11] while roots have are anti-inflammatory and analgesic effects.[12] Its stems of C. macrophylla has been evaluated for its anti-fungal activity and results are very significant.[13]

Previously we had reported the presence of glycosides, saponins, flavanoids, tannins and carbohydrates in the aqueous extract of stems of C. macrophylla Vahl. While their alcoholic extracts have significant glycoside, flavanoid, tannins, carbohydrates and steroid content. [14]

MATERIALS AND METHODS

Plant material

The flower of plant *Callicarpa macrophylla* were collected from local area of agra, U.P., India, and authenticated by Dr P N Sharma, department of Botany, Dr. B R Ambedkar university, Agra, U.P. where a voucher specimen No. has been submitted. (Voucher specimen No. BRAU3171)

Preparation of plant extract

Collected flower of *Callicarpa macrophylla* were converted into moderately coarse powder and extracted with solvent ethyl alcohol for 27 hours by soxhlet. The solvent was removed under reduced pressure.

Drugs and chemicals

Diclofenac sodium was obtained from Medley Pharmaceutical Pvt. Ltd., Jammu, J&K, India. Double distilled water from all-glass still was used throughout the study.

Assessment of in-vitro Anti-arthritic activity

Inhibition of albumin denaturation [15,16]

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of extract so that final concentrations become 50, 100, 200, 400, 800 µg/ml. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37 ± 2ºC in a BOD incubator for 15 mins and then heated at 70ºC for 5 mins. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Diclofenac sodium at the final concentration of (50, 100, 200, 400, 800µg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ of Inhibition} = 100 \text{ X } [Vt / Vc - 1]$$

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

Membrane stabilization test

Preparation of Red Blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the heparin zed centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.[17,18]

Heat induced hemolysis

The reaction mixture (2 ml) consisted of 1 ml of test drug solution and 1 ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56^{o}C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates. Percent membrane stabilization activity was calculated by the formula mentioned above.[18,19]

RESULT

Anti-arthritic effect of *Callicarpa macrophylla* was studied significantly by testing various in-vitro parameters. The effect of *Callicarpa macrophylla* on inhibition of protein denaturation and membrane stabilization is shown in table. *Callicarpa macrophylla* at different dose levels (50, 100, 200, 400 and 800µg/ml) provided significant protection against denaturation of proteins and hypotonic saline induced RBC membrane damage.

Table 1: *In vitro* Anti-arthritic activity by inhibition of Protein denaturation method

Test Sample	Conc. ((µg/ml)	% Protection
Ethanolic extract of flower of *Callicarpa macrophylla*	50	59.47
	100	64.10
	200	88.64
	400	98.21
	800	130.52
Effect of Diclofenac Sodium (Std. drugs)	50	107.24
	100	112.68
	200	147.46
	400	202.14
	800	238.96

Table 2: *In vitro* Anti-arthritic activity by Membrane stabilization method

Test Sample	Conc. ((µg/ml)	% Protection
Ethanolic extract of flower of *Callicarpa macrophylla*	50	12.8
	100	19.82
	200	30.72
	400	42.81
	800	54.84
Effect of Diclofenac Sodium (Std. drugs)	50	73.86
	100	84.06
	200	87.72
	400	90.03
	800	96.94

DISCSSUSION

There are certain problems associated with animal use in experimental pharmacological research such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in vitro assessment of anti-arthritic property of ethanolic extract of *Callicarpa macrophylla*.

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. [20,21] Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. [22] From the results of the present study it can be stated that *Callicarpa macrophylla* is capable of controlling the production of auto-antigens due to in vivo denaturation of proteins in rheumatic diseases.

Protective effect on heat and hypotonic saline-induced erythrocyte lysis is known to be a very good index of anti-arthritic activity of any agent. [23] Since the membrane of RBC is structurally similar to the lysosomal membrane, the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane.[23]

Further studies are needed to elucidate other mechanisms of the in-vitro Anti- arthritic activity of the *Callicarpa macrophylla* extract and to identify the active constituents responsible for the anti-arthritic effect.

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