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Research Article

IN VITRO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *PYRENACANTHA VOLUBILIS* (EEPV)

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ABSTRACT

Objectives: The study was undertaken to evaluate the *in vitro* anti-inflammatory and anti-arthritic activity of the ethanolic extract of leaves of *Pyrenacantha volubilis* (EEPV) using human red blood cells (HRBCs) membrane stabilization and protein denaturation methods.

Methods: In the present study, the *in vitro* anti-inflammatory and anti-arthritic activity of EEPV was carried out using HRBC membrane stabilization by hypotonicity-induced hemolysis and protein denaturation using egg albumin methods at various concentrations (100, 200, 400, 800, and 1000) of EEPV. Diclofenac sodium was used as reference standard.

Results: *P. volubilis* was effective in inhibiting HRBC membrane stabilization and protein denaturation in a dose-dependent manner and was comparable to the standard drug diclofenac sodium.

Conclusion: The study suggests that *P. volubilis* has potential anti-inflammatory and anti-arthritic activity.

Keywords: *Pyrenacantha volubilis*, Anti-inflammatory, Anti-arthritic activity.

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INTRODUCTION

Inflammation is the body's immune system response to any noxious stimuli and is of two types, namely, acute and chronic inflammation. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease which occurs mainly due to the formation of anti-citrullinated protein auto-antibodies. Matrix metalloproteinases (MMPs) are generated by the activation of nuclear factor kappa B (NFκB) and mitogen-activated protein kinase (MAPK) [1]. Structural rigidity of joints is destructed when MMPs along with disintegrin and thrombospondin degrade the components of extracellular matrix. As a result, synovial proliferation takes place leading to the formation of "pannus" that sequels RA.

Available nonsteroidal anti-inflammatory drugs, glucocorticoids, and disease-modifying anti-rheumatic drugs provide only symptomatic reliefs and are associated with severe side effects. Hence, there is an urge to develop drugs with negligible risks. Herbal medicine contains many phytoconstituents and has potential medicinal values with fewer side effects.

Pyrenacantha volubilis is a climber and member of Icacinaceae family. Literature study reveals that *P. volubilis* possess richest source of camptothecin and its derivatives that have been isolated from the cotyledons, ripened whole fruit, and root, followed by seed coat, fruit coat, and young and mature leaves [2]. *In silico* docking studies reveal that camptothecin possesses good binding energy with inflammatory mediators such as MAPK, NFκB, tumor necrosis factor (TNF)-α, and cyclooxygenases 1 and 2 [3] and *in vitro* study results suggest that camptothecin inhibits angiogenesis, synoviocyte proliferation, and metalloproteinase expression [4]. Hence, the aim of our study was to determine the *in vitro* anti-inflammatory and anti-arthritic activity of ethanolic extract of leaves of *P. volubilis* (EEPV) by human red blood cell (HRBC) membrane stabilization and protein denaturation method.

METHODS

Collection of plant material and authentication

P. volubilis was collected based on good collection practice during the month of July 2019 from Poothurai region – Puducherry. The collected leaves were authenticated by Dr. N. Ayyappan, Researcher, French Institute of Pondicherry.

Drying

The collected leaves were cleaned, dried in room temperature. It was periodically checked for the presence of microbial growth. The leaves which are free from brown or yellow spots were selected and then minced to coarse powder.

Extraction of plant material

About 50 g of coarse dry powder was taken in a Soxhlet apparatus and extracted with 400 ml ethanol placed on a heating mantle and continuously percolated by hot percolation method which was followed for 3 days. The extract obtained was filtered and concentrated under reduced pressure to a semisolid mass.

Preliminary phytochemical screening

Preliminary phytochemical screening of plant extracts was carried out to determine the presence of phytoconstituents in it. Gas chromatography and mass spectrometry (GC-MS) was carried out to identify compounds present in the extracts.

In vitro anti-inflammatory activity

In vitro anti-inflammatory activity was determined by HRBC membrane stabilization using hypotonicity-induced hemolysis method. The method was carried based on steps followed by Leelaprakash and Dass [5] with some modifications. The control solution contains 1 ml of phosphate buffer, 2 ml of distilled water, and 0.5 ml of HRBC suspension. The standard solution contains 1 ml of phosphate buffer, 2 ml of hypo saline, 1 ml of different concentration of diclofenac sodium (100, 200, 400, 800, and 1000 µg/ml), and 0.5 ml of HRBC

suspension. The test solution contains 1 ml of phosphate buffer, 2 ml of hyposaline, 1 ml of different concentrations of test extract (100, 200, 400, 800, and 1000 µg/ml), and 0.5 ml of HRBC suspension. All the samples are incubated at 37°C for 30 min and centrifuged. The clear, supernatant solution was measured using colorimeter at 540 nm. Each group consists of quadruplicates for statistical analysis. IC_{50} value was determined for both standard and test treated groups.

% inhibition of hemolysis=100–(OD of sample ÷ OD of control) × 100

OD - Optical density

***In vitro* anti-arthritic activity**

In vitro anti-arthritic activity of EEPV was performed by protein denaturation using egg albumin taking diclofenac sodium as standard drug. The method was carried out based on steps followed by Vaijayanthimala *et al* [6]. The control solution (5 ml) contains of 0.2 ml of hen's egg albumin, 2.8 ml of freshly prepared phosphate buffer saline (pH 6.3), and 2 ml of distilled water. Standard solution (5 ml) contains of 0.2 ml of hen's egg albumin, 2.8 ml of freshly prepared phosphate buffer saline (pH 6.3), and 2 ml of various concentrations of diclofenac sodium (100, 200, 400, 800, and1000 µg/ml). Test solution (5 ml) consists of 0.2 ml of hen's egg albumin, 2.8 ml of freshly prepared phosphate buffer saline (pH 6.3), and 2 ml of varying concentration of test extract (100, 200, 400, 800, and1000 µg/ml).

The pH of the all the samples was adjusted to 6.3 using 1 N HCl. The samples were incubated at room temperature for 15–30 min followed by heating at 70°C for 5 min. Then, the samples are cooled and the turbidity was measured spectrometrically at 660 nm. The control represents 100% protein denaturation. Each group consists of triplicates number of samples for statistical analysis. IC_{50} value was determined for both standard and test treated groups. The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition of protein denaturation} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}}$$

Statistical analysis

Results are expressed as mean±standard error mean. The test group was compared with the standard group by Student's t-test (unpaired and two tailed) using GraphPad Prism software version 8.4.0 San Diego, California.

RESULTS

Phytochemical screening

Presence of camptothecin and its derivatives has been isolated from various parts of *P. volubilis* such as cotyledons, ripened whole fruit, and root, followed by seed coat, fruit coat, and young and mature leaves using liquid chromatography and MS analysis [2]. Preliminary phytochemical screening reveals the presences of alkaloids, phenolic compounds, saponins, glycosides, carbohydrates, proteins, gums, mucilage, fixed oils, fats, and resins. GC–MS analysis shows the presence of derivatives of decanoic acid, 1-naphthalene propanol, alpha-ethyldecahydro-5-(hydroxymethyl)-alpha, 5,8A-trimethyl-2-methyl-, 4, 22 stigmastadiene-3-one, and carbonate derivatives of coumarin.

Fig. 1 shows that EEPV inhibited hypotonicity-induced hemolysis and the percentage inhibition was more when compared to that of standard at doses 200, 400, and 800 µg/ml. There was a dose-dependent increase in the percentage inhibition of hypotonicity-induced hemolysis for both EEPV and diclofenac. The IC_{50} value for standard was found to be 197.5 µg/ml and for the test is 150.7 µg/ml.

From Fig. 2, it was seen that EEPV inhibited denaturation of proteins in a dose-dependent manner and was comparable to that of diclofenac sodium. The IC_{50} value of standard was found to be 320.9 µg/ml and test 513.04 µg/ml.

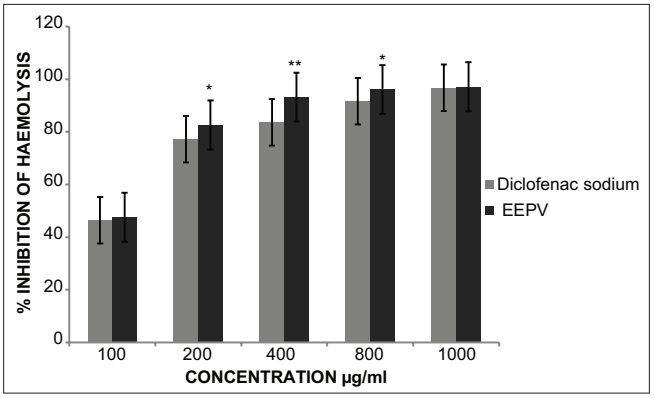

Fig. 1: Percentage inhibition of hemolysis by ethanolic extract of leaves of *Pyrenacantha volubilis* and diclofenac sodium using HRBC membrane stabilization method. Each value represents mean±standard error mean (n=4). The test group was compared with the standard group. *p<0.01 and **p<0.0001 were considered significant when compared to standard drug

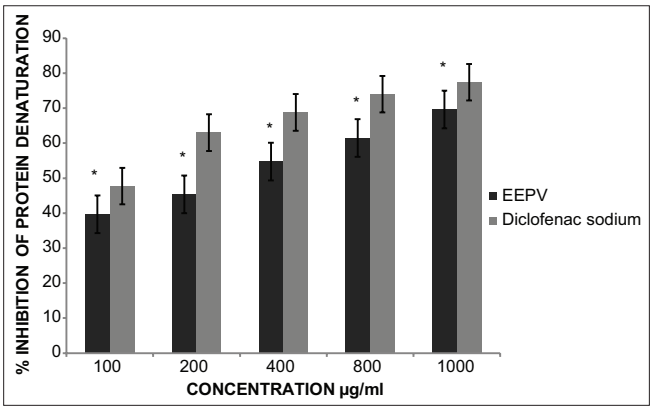

Fig. 2: Percentage inhibition of protein denaturation by ethanolic extract of leaves of *Pyrenacantha volubilis* and diclofenac sodium using egg albumin method. Each value represents mean±standard error mean (n=3). The test group was compared with the standard group. *p<0.0001 was considered to be significant when compared to standard

DISCUSSION

Inflammation occurs due to unknown stimuli with complex pathophysiological process that involves increase in vascular permeability, protein denaturation, and membrane alteration [5]. If RBC is exposed to hypotonic medium, it results in hemolysis. Since RBC membrane resembles lysosomal membrane, hypotonicity-induced hemolysis method will be considered for anti-inflammatory activity [7]. In our study, EEPV inhibited hypotonicity-induced hemolysis. This effect was more when compared to standard drug, suggesting that it has better anti-inflammatory activity than diclofenac sodium.

Chronic inflammatory disease such as arthritis occurs due to the production of auto-antigens [8]. Protein denaturation is one of the main causes for the inflammatory conditions and involves alteration in electrostatic, hydrogen, hydrophobic, and disulfide bonding [9]. EEPV found to be effective in inhibiting protein denaturation, suggesting that it has good anti-arthritic activity like diclofenac sodium. *In vitro* anti-arthritic activity of *P. volubilis* may be due to the presence of camptothecin, a quinolone alkaloid had been found to inhibit angiogenesis, synoviocyte proliferation, and metalloproteinases [4].

In silico docking studies of camptothecin exhibited better binding energy with various mediators such as MAPK, NFκB, TNF-α, and

MMPs (3, 7, 8, 10, and 13) and with enzyme of arachidonic acid pathway such as cyclooxygenases. [3] Studies reveal the presence of camptothecin and its derivatives from various parts of *P. volubilis* such as cotyledons, ripened whole fruit, and root, followed by seed coat, fruit coat, and young and mature leaves. The presence of alkaloids and saponins found to inhibit articular swelling decreased arthritic index and downregulated the contents of interleukin 1 beta and TNF-α in the inflammatory tissues of arthritic rats [10,11]. Phenolic compounds inhibit the pro-inflammatory mediators in inflammatory responses [12]. Some coumarins could inhibit both the lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism [13]. All these properties together put forth may account for the anti-inflammatory and anti-arthritic activity of EEPV.

CONCLUSION

The anti-inflammatory and anti-arthritic activity of the EEPV had been performed. It shows that *P. volubilis* is more effective than diclofenac sodium in inhibiting HRBC membrane stabilization and comparable activity in inhibiting protein denaturation. It may be due to the presence of phytoconstituents present in it. However, *in vivo* studies are required to further establish anti-inflammatory and anti-arthritic activity of *P. volubilis*.

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AUTHORS' CONTRIBUTIONS

1. Ms. Anjali P, prepared the plant extract, performed the study, and prepared the manuscript
2. Dr. Vimalavathini R, designed the project, provided guidance, and reviewed the manuscript
3. Prof. Kavimani S, supervised the work.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' FUNDING

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