

STUDY OF *PARMELIA PERLATA* FOR ITS POTENTIAL AS ANTI-INFLAMMATORY AND ANTIARTHRITIC AGENT USING *IN VITRO* MODEL

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ABSTRACT

Objective: The objective of this study was to evaluate the anti-inflammatory and antiarthritic potential of *Parmelia perlata*.

Methods: The relative study is based on *in vitro* anti-inflammatory and antiarthritic activity using hydroalcoholic extract of *P. perlata* (HAEPP). The preliminary phytochemical tests showed the presence of various phytochemical compounds such as alkaloids, flavonoids, and glycosides since the lichen species of *P. perlata* has the folklore claim of anti-inflammatory activity, thus it was studied by human red blood cells membrane stabilization method, and arthritic activity was carried using protein denaturation method using diclofenac as a standard.

Results: The results showed eminent anti-inflammatory and antiarthritic activity in a dose-dependent manner. The membrane stabilization showed the maximum effect at 78.54% at the concentration of 1000 µg/, and the protein denaturation was also found maximum at 1000 µg/ml concentration at 79.43%. Thus, our research states the potent anti-inflammatory activity and antiarthritic effect in *P. perlata*.

Conclusion: The HAEPP has a potent anti-inflammatory activity and antiarthritic activity. A further study has to be conducted to establish the pharmacological evidence behind the compound and the mechanism of action of the HAEPP on the inhibition of the inflammation process.

Keywords: *Parmelia perlata*, Anti-inflammatory activity, Human red blood cells membrane stabilization, Antiarthritic activity, Protein denaturation.

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INTRODUCTION

Inflammation is the reaction in living tissues caused due to injury, infection, or irritation which causes a complex array of enzyme activation or mediator release or fluid extravasations, cell migration, tissues breakdown, and repair, which are aimed to host defense and mostly activated in disease condition. Inflammation releases the lysosomal enzyme which produces a variety of disorders, leading to the tissue injury by damaging the macromolecule and lipid peroxidation of membrane [1,2]. The mechanism of inflammation is attributed in part to release of reactive oxygen species (ROS) from activated neutrophils and macrophages. Free radicals are important mediators that prove or sustain inflammatory process, and radical scavengers can attenuate the inflammation [3]. In addition, ROS propagate inflammation by stimulating the release of cytokines such as interleukins-1 (ILs-1), tumor necrosis factor- α (TNF- α), and interferon- γ , which stimulate recruitment of additional neutrophils and macrophages [4]. Prolonged inflammation leads to rheumatoid arthritis, hay fever, and atherosclerosis and also the development of infectious disease such as leprosy, tuberculosis, syphilis, asthma, inflammatory bowel disease, and autoimmune disease [5]. Rheumatoid arthritis is a chronic inflammatory disease of joints that result in joint pain swelling and destruction. It affects an estimated 1% of adult population throughout the world [7]. It is an autoimmune disorder characterized by synovial proliferation, inflammation, and subsequent destruction such as deformity of joints or destruction of cartilage and bone [6]. The various mediators involved in the process include receptor activator of NF- κ B (RANK) ligand and its receptor RANK, pro-inflammatory cytokines, for example, TNF- α , IL-1, IL-6, IL-17, and IL-18 and matrix-degrading enzyme, for example, matrix metalloproteases and cathepsin K [9]. The pathophysiology of exaggerated synovial tissues involves hyperplasia and subintimal infiltration of T and B lymphocyte; this results in pannus tissues that irreversibly destroy the cartilage and bone in the affected joints. Polymorphonuclear leukocytes and macrophages are also stimulated

which result in the production of inflammatory mediators including large amount of superoxide and hydrogen peroxide that can cause significant impairment and destruction of synovial fluid, cartilage, and other articular constituents [8].

As of now, there are four sorts of medication recommended for the treatment of rheumatoid joint pain which are Non-steroidal anti-inflammatory drugs (NSAIDs) e.g.: diclofenac, ibuprofen., Steroidal hormones e.g. prednisone, dexamethasone. Disease modifying anti-rheumatic drugs (DMARDs) e.g.: methotrexate, hydroxychloroquine, immunosuppressant (e.g.: azathioprine, cyclosporine) [10].

Parmelia perlata (Huds.) Ach. is a perennial lichen species belonging to family Parmeliaceae. They are found on the rocks or dead wood and also cultivated in temperate Himalayas and Kashmir hills. They are commonly known as Patthar phool, Kalpashee, Chhadila, Stone flower, Dagad phool, and Charela [11]. In traditional medicine, it was used for the treatment of edema, arthritis, for counteracting poisons, toxicosis, fever, obesity, eczema, cardiac tonic, refrigerant, and gout. The folklore claim states it to have the activity for the treatment of dyspepsia, diarrhea, dysentery, antiulcer, spermatorrhea, amenorrhoea, astringent, diuretic, and also in promoting wound healing [12,13]. The early investigation resulted in the isolation of various chemicals such as parmelandone, parmelandone, tridecyl myristate, 3-ketooleanane, icosan-1-ol, and usnic acid. [14,15]. It has been reported to possess antioxidant, hypolipidemic and cytotoxic potential [16], antibacterial [17], hepatoprotective [18], antimicrobial [19,20], antidiabetic [21], antiulcer [22], and antiviral activity [23]. The objective of the study was to establish the potential of *P. perlata* for the anti-inflammatory and antiarthritic activity.

METHODS

The lichens species of *P. perlata* was collected from local herbal market in Chennai. It was authenticated by Professor P Jayaraman of Plant Anatomy Research Centre (PARC), Herbal PARC unit,

Tambaram, Chennai. The authentication number on the certificate is PARC/2016/3228.

Extraction

The dried *P. perlata* was coarsely grounded and was defatted using petroleum ether and successive extraction was done using 70% alcohol by maceration process for 72 h at room temperature with intermittent shaking. The extract was concentrated under reduced pressure and concentrated. The extract was kept in air-tight container and stored in cool temperature [24,25].

Preliminary phytochemical analysis

A small quantity of the powdered lichen was subjected to phytochemical tests using established chemical procedure. Standard procedures for the determination of alkaloids, tannins, saponins, glycosides, flavonoids, carbohydrates, fats and oil was performed and reported.

THE IN VITRO ANTI-INFLAMMATORY ACTIVITY

Human RBC membrane stabilization method

The *in vitro* anti-inflammatory activity of hydroalcoholic extract of *P. perlata* (HAEPP) extract of *P. perlata* was evaluated by human red blood cell membrane stabilization method. The blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks before the experiment, and the blood was mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared, namely 50, 100, 250, 500, and 1000 mcg/ml using distilled water and to each concentration of 1 ml of phosphate buffer, 2 ml hyposaline, and 0.5 ml of human red blood cells (HRBC) suspension were added. It is incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated using UV-visible spectrophotometer at 560 nm. The diclofenac sodium was used as standard.

THE IN VITRO ANTIARTHRITIC ACTIVITY

Egg albumin denaturation method

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, pH6.4), and 2 ml of varying concentrations (50, 100, 250, 500, and 1000 µg/ml) of drug. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at 37°C ± 2°C in a BOD incubator for 15 min and then heated at 70°C for 5 min. After cooling, the absorbance was measured at 660 nm using the vehicle as a blank. Diclofenac sodium in the concentrations of 50, 100, 250, 500, and 1000 µg/mL was used as the reference drug and treated similarly for the determination of absorbance.

The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times [Vt/VC - 1]$$

Where,

Vt=Absorbance of the test sample,

Vc=Absorbance of control.

Statistical analysis

All data were presented as the mean ± standard deviation. Statistical analysis was performed by one-way analysis of variance to evaluate significant differences between groups using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). p<0.05 was considered statistically significant.

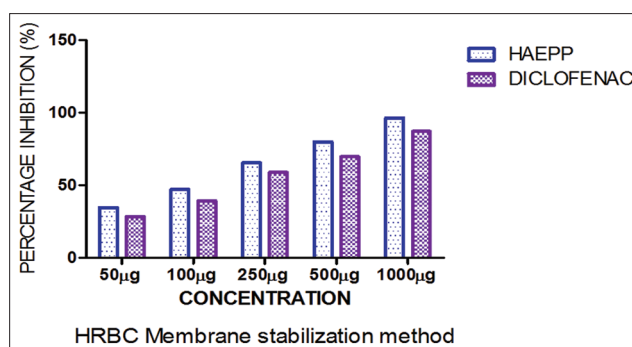


Fig. 1: Anti-inflammatory activity using HRBC membrane stabilisation method

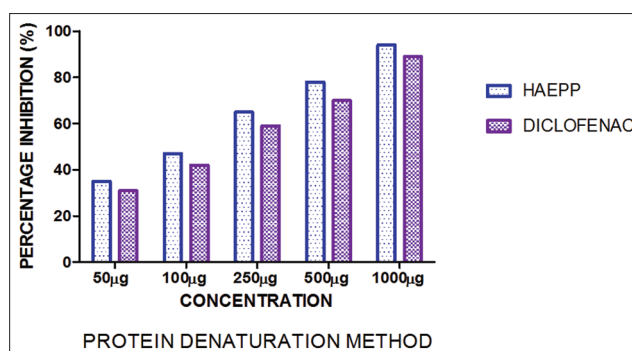


Fig. 2: Anti-arthritis activity using egg protein denaturation method

RESULTS

HRBC membrane stabilization method

The HAEPP showed anti-inflammatory activity (Fig. 1) by inhibiting the hemolysis of HRBC. The HAEPP and diclofenac response were on dose concentration as preparation showed the increase in activity as the concentration of the solution increased from 50 µg to 1000 µg/ml. The minimum activity was seen at 50 µg as 34.6% and 30.87%, respectively, and as the dose was increased to 100 µg, the activity was 47.33%. The 250 µg concentration showed the activity as 65.22% and the 500 µg concentration had 79.86% response. The maximum activity was seen at 1000 µg as it inhibited the 96.25% and 87.70%, respectively, of the hemolysis.

Egg protein denaturation method

The HAEPP showed antiarthritic (Fig. 2) activity by inhibiting the protein denaturation. The minimum activity was seen as 35.14% at 50 µg concentration for HAEPP and 31.68% for diclofenac. At 100 µg concentration, the response was seen as 47.58%, and at 250 µg concentration, the response was seen as 67.25%. 78.14% activity was seen at 500 µg concentration. The maximum activity for 1000 µg was seen as 94.20% and 89.66%, respectively, for both HAEPP and diclofenac.

DISCUSSION

The *in vitro* anti-inflammatory activity has been studied using the HRBC membrane stabilization method as the erythrocyte membrane and lysosomal membrane are analogous and stabilization of erythrocyte implies that the HAEPP extract may well stabilize lysosomal membranes as it is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as proteases and bacterial enzymes, which further causes inflammation of tissues and damage on extracellular release. The extracellular activity of these enzymes is noted to be associated with acute or chronic inflammation. The nonsteroidal drugs act either by inhibiting these

lysosomal enzymes or by stabilizing the lysosomal membrane [26].

Rheumatoid arthritis is the most common of autoimmune diseases which is prominently manifested by the inflammation and joint pain. There has been large number of mortality and morbidity associated with RA and thereby it has left substantial socioeconomic impact. The currently synthetic drugs are mainly used for symptomatic relief and side effect associated [27].

Thus, it has become essential to find an alternative method for the treatment of arthritis, thus resulting in the use of CAM therapies for the treatment of arthritis. The current researches are focused on the identification and isolation of active principle(s) from crude extracts of known medicinal plants/herbs, it's to be overlooked that the strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound, and this may also help to nullify the toxic effects of individual constituents [28].

CONCLUSION

The *P. perlata*, a potent unexplored lichen species, claimed to have an anti-inflammatory activity showed the significant response using *in vitro* model of HRBC membrane stabilization method for anti-inflammatory activity. The response increased gradually as the dose potentiated. The antiarthritic activity was also studied based on the results of the inflammatory action because the prolonged phase of inflammation leads to arthritis in bones and tendons. The antiarthritic activity was also eminent in the protein denaturation method. Thus, stating HEAPP has a potent anti-inflammatory activity and antiarthritic activity. A further study has to be conducted to establish the pharmacological evidence behind the compound and the mechanism of action of the HAEP on the inhibition of inflammation process.

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AUTHORS' CONTRIBUTION

Dr. Chitra V - Guide. Yogesh Diwakar - Performed the studies. Evelyn Sharon S - Helped in performing the statistical analysis.

CONFLICTS OF INTEREST

The authors whose name listed below certify that we have no conflicts of interest.

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