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R E S E A R C H A R T I C L E

Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of *Pergularia Daemia*

Sandeep Akkam[*1], Dr. C. Madhusudhan Chetty[2], D. Chinna Babu[3], V. Vijay Kumar[4], Y. Chaitanya Kumar[5], N. Swaroop Reddy[6]

Department of Pharmacy Practice, Santhiram college of Pharmacy, Nandyal, Andhra Pradesh, India.

A B S T R A C T

The plant *Pergularia daemia* has been traditionally used for treating various diseases. The present study was aimed to investigate the anti-inflammatory activity of aqueous extract of the whole plant of Pergularia *daemia by* human red blood cell (HRBC) membrane stabilization method. The prevention of hypo tonicity induced human red blood cells (HRBC) membrane lysis was taken as a measure of the anti-inflammatory activity. Phytochemical assay was done to find the main active constituents. Among all the concentrations 400µg/ml showed significant anti-inflammatory activity and 78.97% protection and the standard drug diclofenac showed 86.68% protection. IC50 values were calculated and found to be 217 µg/ml and 200µg/ml for plant extract and standard diclofenac respectively. The results obtained in the present study suggest that Pergularia *daemia* can be a potential source of anti-inflammatory agents.

Key words: *Pergularia daemia*, anti-inflammatory activity, HRBC, IC50.

A R T I C L E I N F O

Corresponding Author
Sandeep Akkam
Department of Pharmacy Practice,
Santhiram college of Pharmacy,
Nandyal, Andhra Pradesh, India.

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C O N T E N T S

1. Introduction

Traditional medicine, Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid per oxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc[1]. The extracellular activity of

these enzymes is said to be related to acute or chronic inflammation.

Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of Lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane[2,3]. HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its Stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human [4].Red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an in vitroMeasure of anti-inflammatory activity of the drugs or plant extract.

The plant Pergularia daemia (Family: Asclepiadaceae) is known as "Uttaravaruni" in Sanskrit and "Utranajutuka" in Hindi. In ethno medicinal practices the traditional healer use Pergularia daemia (Asclepiadaceae) as anthelmintic, emetic, thermogenic, expectorant, antipyretic and laxative. Leaves juice is given in catarrhal affections, asthma, and infantile diarrhoea and is applied to inflammatory swelling in combination lime [5]. Aerial parts of the plant used for snake bite [6]. Latex of this plant used for boils and sores [7]. Fresh roots of plant used as an abortifacient [8] and used to treat gonorrhoea [9]. The latex or a decoction of the roots were used in many countries as a medicine to treat several illnesses, such as venereal diseases, arthritis, muscular pains, asthma, rheumatism, snake-bites. The latex may also be used as a fish poison [10] and toothache [11]. Plant has been documented for presence of presence of triterpenes, saponins cardenolides and alkaloids [12]. The present study was aimed to investigate the anti-inflammatory activity of aqueous extract of the whole plant of Pergularia daemia by human red blood cell (HRBC) membrane stabilization method.

2. Materials and Methods

Collection of plant material:

The leaves of Pergularia daemia were collected from the local areas of Nellore district, identified and authenticated by Prof. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University and Tirupathi.

Extract Preparation:

The leaves were dried under shade and powdered . Powdered plant (200g) was extracted in Soxhlet apparatus using water for 18hrs. The extract was filtered using a wattman filter paper No.10. The filtered extract was then evaporated under vacuum below 45°C in a vacuum drier to give a final yield of 14.98g (7.49% w/w).

Phytochemical screening:

In order to identify the chemical components of the plant , A variety of indicators including Dragendorff, and Wagner for detection of alkaloids, vanillin sulphuric acid and vanillin phosphoric acid for terpenoids, ferric chloride for phenol components, natural product polyethylene glycol (NP/PEG) for flavonoids and kef and blood agar tests for saponins was used in this screening.

The Human Red Blood Cell (HRBC) membrane stabilization method:

Fresh human blood (5 mL) was collected and transferred to the centrifuged tubes containing Heparin or EDTA or Sodium citrate to prevent clotting. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of saline. The volume of the blood was measured and reconstituted as 10% v/v Suspension with normal saline The reaction mixture consists of 1.0 mL of test sample of different concentrations (50µg –400 µg) in normal saline and 0.5 mL of 10% HRBC suspension, 1 ml of 0.2 M phosphate buffer, 1 ml hypo saline were incubated at 37$_o$C for 30 min and centrifuged at 3,000 rpm for20 min and the haemoglobin content of the supernatant solution was estimated Spectro photo metrically at 237 nm. Diclofenac was used as standard and a control was prepared without extracts. The percentage of HRBC Hemolysis and membrane stabilization or Protection was calculated by using the following formula.

$$\textbf{\% Hemolysis} = \frac{Optical\ density\ of\ test\ sample}{Optical\ density\ of\ control} \text{ X } 100$$

$$\textbf{\% Protection} = 100 - \frac{Optical\ density\ of\ test\ sample}{Optical\ density\ of\ control} \text{X} 100$$

3. Results and Discussion

Phytochemical screening:

Phytochemical screening showed the presence of terpenoids, flavonoids and saponins in the plant extract. The results are tabulated (Table 1).

The human red blood cell (HRBC) membrane stabilization method:

The aqueous extract of Pergularia daemia was studied for in vitro anti- inflammatory activity by HRBC membrane stabilization method. The RBC membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is play an important role in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. Various concentrations of the plant extracts (50,100,200,300,400µg/ml) were used for the study.

Among all the concentrations used 400 µg/ml showed significant anti-inflammatory activity and showed 78.97% protection of HRBC in hypotonic solution compared with standard diclofenac which showed 86.68% protection. The results are tabulated (Table: 1). IC50 values was found to be 217 µg/ml and 200µg/ml for plant extract and standard diclofenac respectively. With the increasing concentration the membrane haemolysis is decreased as and membrane stabilisation / protection is increased as shown in graph:1. Hence anti-inflammatory activity was concentration dependent.

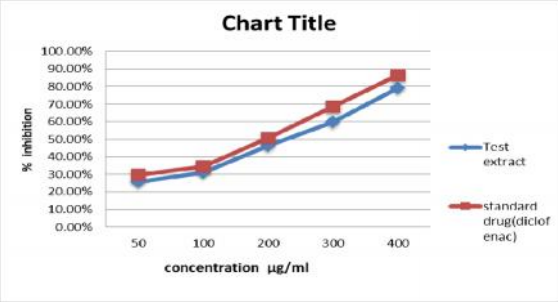

Fig 1: Detailed graph for HRBC suspension method

4. Conclusion

Stabilization of the HRBCs membrane by hypo tonicity induced membrane lysis was studied to establish the mechanism of anti- inflammatory action of Pergularia daemia. Therefore, our present in- vitro studies on Pergularia daemia extracts demonstrated the depression of inflammation. Active principles such as flavanoids, glycoside, tri terpenoids, phenol may be responsible for this activity. Hence, Pergularia daemia can be used as a potent anti-inflammatory agent.

Table 1: Phytochemical screening results of Aqueous extract of *Pergularia daemia*

S.no	Categories of compounds	Tests, reagent used	Results
1	Alkaloids	Mayer's test	+
		Dragendorff reagent	+
		Wagner's reagent	+
2	Terpenoids and phenyl propanoids	Vanillin sulphuric acid reagent	+
3	Terpenoids	Vanillin phosphoric acid	+
4	Phenolic compounds	Ferric chloride	+
5	Flavonoids	Natural product reagent	+
6	Saponins	blood agar tests	+

Table 2: Membrane Stabilization Test by (HRBC) Suspension Method

S.no.	**Concentration of plant extract µg/ml**	**Absorbance at 237 nm**	**% Haemolysis**	**% Protection of sample**	**IC_{50} VALUE**
1.	50	0.084±0.023	74.39%	25.61%	
2.	100	0.102±0.054	68.90%	31.1%	
3.	200	0.152±0.067	53.65%	46.35%	217**µg/ml**
4.	300	0.196±0.020	40.24%	59.76%	
5.	400	0.259±0.034	21.03%	78.97%	
Control	0.318	-	-	-	-
standard drug	**Diclofenac µg/ml**	**Absorbance at 237 nm**	**% Haemolysis**	**% Protection of sample**	**IC_{50} VALUE**
1.	50	0.098±0.089	70.12%	29.88%	
2.	100	0.113±0.012	65.54%	34.46%	
3.	200	0.166±0.034	49.39%	50.61%	199**µg/ml**
4.	300	0.226±0.021	31.40%	68.6%	
5.	400	0.275±0.009	13.32%	86.68%	

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