

SHORT COMMUNICATION

Anti-inflammatory studies on *Acalypha indica* L. leaves by membrane stabilization

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The study was designed to evaluate the phytochemical screening and anti-inflammatory activity of methanolic extract of *Acalypha indica* L. leaves in HRBC membrane stabilization. The methanolic extract showed significant inhibition by using Diclofenac sodium as a standard drug at doses of 125, 250, 500 and 1000 µg/mL and showed a dose dependent inhibition hemolysis of erythrocyte induced by hypotonic solution.

Keywords: *Acalypha indica*, Anti-inflammatory, AIME, Methanolic extract, Diclofenac Sodium Drug, HRBC membrane stabilization

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Introduction

The inflammatory response begins when growth factors, chemoattractant mediators and chemoactivators are released during platelet degranulation and initiate chemotaxis of inflammatory cells to the site of injury and proliferation of inflammatory cells locally. A short period of local vasoconstriction at the site of injury is followed by vasodilatation, which increases local blood flow to the area. Vascular permeability is increased through activation of the complement pathways and coagulation cascade. There is an influx of cells and substrates necessary for healing, including early neutrophil scavengers, plasma proteins and activated complement fragments. A predominance of neutrophils within the first 24 h act to sterilize the wound.

Inflammatory response involves a complex array of enzyme activation, mediator release fluid extravasations, cell migration, tissue brake down and repair which are aimed at host defense and usually

activated in most disease conditions¹. Many mediators' co-ordinate inflammatory and allergic reactions and some are produced in response to specific stimuli, there is considerable redundancy and each facet of the response vasodilatation, increased vascular permeability, cell accumulation, etc can be produced by several separate mechanisms².

Acalypha indica L. known as *Kuppaimani* in Tamil is an annual weed. It belongs to the family Euphorbiaceae. It is a common weed in many parts of Asia. It grows in the common farmlands gardens, roadside waste lands. Parts used are leaves, root, stalk and flowers. Plants are emetic expectorant, laxative and diuretic, useful in bronchitis, pneumonia, asthma and pulmonary tuberculosis. Leaves are laxative and antiparasiticide, ground with common salt or quicklime or lime juice applied externally in scabies. Leaf paste prepared with lime juice is used for ringworm and the paste is used as emetic for children. A decoction of the leaves is given in earache. Powder of the dry leaves is given to children to expel worms; also given in the form of decoction with little garlic. In homoeopathy, the plant is used in severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis. The plant contains kaempferol, a cyanogenetic glucoside, a base, triacetanamine and an alkaloid, acalyphine. The major phytochemical constituents are alkaloids acalypus and aclyphine³. This plant is used as diuretic, antihelmintic and for respiratory problems such as bronchitis, asthma and pneumonia.

Materials and Methods

Chemicals

All the chemicals were purchased from Sigma Chemical Co., USA. All other chemicals used were of Good Quality and Analytical Grade.

The shade dried powdered leaves of *A. indica* got as a gift from the Siddha Maruthuva Salai Vellore, Tamil Nadu and identified taxonomically by Dr. N. P. M. Mohammed Tariq (Botanist), Assistant Professor, PG & Research Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi.

Preparation of methanolic extract

500g leaves were exhaustively extracted by using 95% methanol in a Soxhlet apparatus. The extract was

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concentrated *in vacuo* to a syrupy consistency. The percentage yield was found to be 9.8%. The methanolic extract of *A. indica* is abbreviated as AIME.

Phytochemical analysis and screening

Phytochemical tests were done to find out the presence of active chemical constituents such as alkaloid, glycosides, terpenoids, steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins.

The presence of alkaloids was tested as per Mayer's test procedure. The extract of *A. indica* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid (HCl). After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent⁴. The samples were then observed for the presence of turbidity or yellow precipitation⁵. For testing glycosides, Glacial Acetic Acid and few drops of Ferric chloride, concentrated sulphuric acid were added to the extract and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer⁴.

Terpenoid and steroids were tested by adding 1mL of acetic anhydride and 1mL of chloroform to the extract. Then concentrated solution of sulphuric acid (H₂SO₄) was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids⁴.

To the extract 2 mL of 50 % methanol solution was added, warmed and magnesium metal was added along with 8-10 drops of concentrated hydrochloric acid (HCl) for testing flavonoids. A red colour was observed for flavonoids and orange colour for flavones⁴.

To the aqueous extract, 9-10 drops of Fehling's solution were added and kept in boiling water bath, a brick red coloured precipitate was obtained, revealing presence of reducing sugars.

Triterpenes presence was tested by adding 10 mL of chloroform to the extract and warmed it at 80°C for 30 minutes. Few drops of concentrated sulphuric acid (H₂SO₄) was added and mixed well and observed for formation of red colour.

The extract was diluted to 10 mL of distilled water, filtered, to the filtrate 5% Ferric chloride (FeCl₃) was added and dark green colour was formed, showing the presence of phenolic compounds (Ferric chloride)

To the extract, 2 mL of water and 6 drops of ferric chloride (FeCl₃) solution were added to know the presence of tannins. Blue colour was observed for gallic tannins and green black for catecholic tannins⁶.

The membrane stabilization study

The HRBC membrane stabilization was used as a method to study the anti-inflammatory activity⁷. Blood was collected from our healthy students itself who were not taken any NSAIDS for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%, pH 7.2) and a 10 % (v/v) suspension was made with isosaline. The assay mixture containing the Diclofenac sodium and 1 mL of 0.15M (pH 7.4) phosphate buffer 2 mL of 0.36% hyposaline, 0.5 mL of HRBC suspension. Diclofenac sodium was used as reference drug. Instead of hyposaline, 4 mL of distilled water act as control. All the assay mixture were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using Spectrophotometer at 560 nm.

The percentage hemolysis was calculated by following equation:

$$\% \text{ inhibition of hemolysis} = 100 \times \frac{OD_1 - OD_2}{OD_1}$$

Where,

Optical Density₁ (OD₁) = Optical density of hypotonic buffered saline solution alone (control)

Optical Density₂ (OD₂) = Optical density of test sample in hypotonic solution.

Results and Discussion

The methanolic extracts of *A. indica* was studied, *in-vitro* anti-inflammatory activity by HRBC membrane stabilization method. Phytochemical investigation revealed that *A. indica* methanolic extract contains alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins. The results obtained demonstrate that the extract can significantly, dose dependently inhibits RBC hemolysis. The percentage protection of lysis for standard Diclofenac sodium 60 mcg/mL is 83%, at a concentration of 1000 µg/mL is 77 % (Table 1). The extracts exhibited membrane stabilization effects by inhibiting hypotonicity induced lysis of erythrocyte membrane⁸. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting

Table 1—*In vitro* anti-inflammatory activity of methanolic extract of *Acalypha indica*

Treatment	Conc (µg/mL)	% Inhibition
Control	0	0
<i>Acalypha indica</i> leaves methanolic extract	125	64.32±66.34
	250	78.72±80.74
	500	72.13±74.15
	1000	67.45±70.47
Diclofenac Sodium (Drug)	25	73.09±76.12
	50	83.18±85.22

the inflammatory responses by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which further causes tissue inflammation and damage up on extra cellular release⁹. The possible anti-inflammatory activity of *A. indica* may due to inhibitory effect on release of inflammation mediators or by membrane stabilizing activity which may play a significant role in its anti-inflammatory activity and may be due to the presence of antioxidants as well as rich in phytochemicals (Table 1). Further work is in progress to find out its exact mechanism of action. The present investigation is part of continuing programme related to the biochemical screening of local plants and their anti-inflammatory effect.

Conclusion

It is suggested that using the extracts are effective and economic. Herbal drugs may be prepared for various diseases, ailments, wounds, inflammations and pathogenic infections, etc. The preliminary phytochemical screening also supported the anti-inflammatory activity and the broad spectrum of anti-inflammatory activity is highly promising for evaluating presence of bioactive compounds.

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