

## SCREENING OF *INVITRO* ANTI-INFLAMMATORY ACTIVITY OF *MICHELIA CHAMPACA* LINN. FLOWERS

T.ANANTHI\*, M.CHITRA

PG and Research Department of Biochemistry, S.T.E.T women's College, Mannargudi. Email: elangani576@gmail.com

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**ABSTRACT**

**Objectives:** *Michelia champaca* is an ayurvedic medicine which is used as anti-inflammatory agent. Therefore our investigation was aimed to evaluate anti-inflammatory property of the flower extract of *M.champaca* by membrane stabilization method.

**Methods:** The prevention of hypo tonicity induced human red blood cells (HRBC) membrane lysis was taken as a measure of the anti-inflammatory activity. The potency of the plant extract was compared with standard diclofenac sodium.

**Results:** *Michelia champaca* showed significant stabilizing activity of 57.4% at concentration of 300 µg/ml. The percentage membrane stability exhibited by the extract was concentration dependent.

**Conclusion:** It can conclude that the methanolic extract of *M.champaca* posses significant *invitro* anti-inflammatory activity.

**Keywords:** *Michelia champaca*, hypotonic, membrane stabilization, diclofenac sodium

**INTRODUCTION**

Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of more number of proliferative factors (like Vasoactive, chemotatic) at different stages and there are many targets for anti inflammatory activity. Their respective tissue injury is a type of inflammatory response suppressed by glucocorticoids and this is the basic clinical uses and also it interferes with several steps in the inflammatory response. Numbers of corticoids are only palliative; do not act on the inflammation instead of they favor spread of infections capacity of defensive cells to kill microorganisms is impaired at the same time interfere with healing and scar formation. The alternate use of corticoids is hazardous other than the corticosteroids the NASIDs are also used to treat inflammation. The main mechanism of action of the NASIDs is the inhibition of prostaglandin (PG) synthesis or preferential or selective COX-2 inhibition. Due to the inhibition of prostaglandin (PG) synthesis it may produce toxic effects like bleeding, inhibition of platelet function, gastric mucosal damage, asthma and anaphylactic reactions may cause some individuals [1].

The plant *Michelia champaca* (Sampige) is widely used in both Ayurveda and Homeopathic medicine. It belongs to the family Magnoliaceae. Root and bark are purgative, emmenagogue and are useful in the treatment of inflammation, constipation and dysmennorhea. The stem bark is astringent, febrifuge used in gastritis, fever and cough. Flower and flower buds, fruits are useful in ulcers, skin disease wounds [2]. In this study, it has been attempted to evaluate the anti inflammatory activity of *Michelia champaca* by membrane stabilization. Erythrocytes were chosen for the purpose because of their readily availability and relative simplicity so they are considered to be an excellent model for the study of the biomembrane oxidative damage by measuring hemoglobin leakage.

**MATERIALS AND METHODS****Collection of plant material**

The *Michelia champaca* flowers were procured from the local areas of Udumalaipettai, Coimbatore District, Tamilnadu. The collected plant material was botanically identified and confirmed by Dr.S.John Britto, The Director, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, and Tamilnadu. The herbarium specimens were preserved and submitted to Department of Biochemistry, S.T.E.T Women's College, Mannargudi, Thiruvavur District, and Tamilnadu for further reference (Voucher no.001).

**Preparation of the extract**

The flowers were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The coarse powders were then subjected to successive extraction with methanol by Soxhlet method [3]. The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents were removed in vacuo and stored at 4°C.

**HRBC Membrane Stabilization Method**

The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the *invitro* anti-inflammatory activity [4]. Blood was collected from healthy human volunteer who was not taken any non steroidal anti inflammatory drugs for 2 weeks prior to the experiment. The blood was centrifuged at 3000rpm/ min. The packed cells were washed with isosaline (0.90% NaCl) and 10% suspension was made. The reaction mixture (4.5ml) consists of 2ml of hypo saline (0.25% w/v NaCl), 1ml of 0.15M phosphate buffer (PH 7.4) and 1ml of test solution (100, 200, 300µg/ml) in isosaline, 0.5ml of 10% HRBC in isosaline was added. For test control, 1ml of distilled water was used instead of hypo saline (to produce 100% hemolysis), while product control lacked red blood cells. The mixtures were incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm/min for 20 minutes. Diclofenac sodium was used as a reference drug. The hemoglobin content in the suspension was estimated using a spectrophotometer at 560 nm.

**Calculation**

The percentage of HRBC membrane stabilization was calculated using the formula.

$$\text{Percentage protection} = \frac{100 - \text{Optical density of sample}}{\text{Optical density of control}} \times 100$$

**RESULTS AND DISCUSSION**

During inflammation, lysosomal hydrolytic enzymes are released which causes damages of the surrounding organelles and tissues with attendance variety of disorders [5]. Various methods were employed to screen and study drugs, chemicals, herbal preparations that exhibit anti-inflammatory properties or potentials. In the present study, stabilization of erythrocyte membrane exposed to hypotonic induced lyses was employed. Methanolic flower extract of

*Michelia champaca* showed significant anti-inflammatory activity in concentration dependent manner (100,200, 300µg/ml). The increased concentration of plant extract increases the activity of membrane stabilization. The maximum membrane stabilization activity was observed at highest concentration (300 µg/ml). *Michelia champaca* flowers extract showed a 57.4% inhibition at a concentration of 300 µg/ml to that of Diclofenac sodium having 60.60% inhibitory activity. The results are shown in Table.1

**Table 1: *In vitro* Anti-inflammatory activity of methanolic extract of *Michelia champaca* L. flowers**

Concentration (µg/ml)	% of Protection	
	<i>Michelia champaca</i>	Standard
100	48.3± 0.02	52.27± 0.01
200	51.2± 0.03	55.43± 0.03
300	57.4± 0.04	60.60 ± 0.01

Values are expressed as mean ± S.D

The extracts exhibited membrane stabilization effect by inhibiting hypo tonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane [6] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important 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 [7]. The mode of action of the extracts and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells [8]. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the haemolysis of red blood cells. It has been reported that certain saponins and flavonoids exerted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro*, while tannins and saponins possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules [9].

In another study, aqueous and methanolic extracts of the leaves of *Datura metel* were subjected *in vitro* screening models such as DPPH free radical activity, total phenolic content, total anti-inflammatory activity by HRBC membrane stabilization. The results suggested that leaves of *D.metel* possess considerable activity and therefore further it can establish it as a potential anti-inflammatory and anti oxidant activity [10].

## CONCLUSION

From the above study it was concluded that the methanolic flower extract of *Michelia champaca* has significant anti-inflammatory activity (membrane stabilization property) with standard drug diclofenac. The extracts exhibited membrane stabilization effect by inhibiting hypo tonicity induced lysis of erythrocyte membrane. The plant therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases.

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