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IN VITRO ANTI-INFLAMMATORY ACTIVITY OF THE STEM BARK OF *Nyctanthes arbor-tristis* (L.)

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ABSTRACT

Nyctanthes arbor-tristis is popularly known as Night jasmine used extensively in the Ayurvedic system of medicine. To evaluate the anti-inflammatory potential of ethanolic bark extract of Nyctanthes arbor-tristis. Preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, glycosides, tannin. Different concentrations viz 50, 100, 200, 400 and 800 µg/ml. Nyctanthes arbor-tristis were used to study the in vitro anti-inflammatory activities albumin denaturation and HRBC membrane stabilization activity. Aspirin was used as reference drug. It was found that ethanolic bark extract of Nyctanthes arbor-tristis was dose dependent RBC membrane stabilization and protein denaturation activity. The results suggested that anti-inflammatory activity of Nyctanthes arbor-tristis was reasonably due to the presence of flavonoid, quercetin. So, it can be used as a potential source of anti-inflammatory agent.

KEY WORDS

Nyctanthes arbor-tristis, anti-inflammatory, aspirin, HRBC membrane, stabilization.

INTRODUCTION

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat and swelling and loss of function in the injured area. Loss of function occurs depending on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation and bacterial or viral invasion. Plants have been the base for the development of a new medicine, or they may be used as phytomedicine for the treatment of disease in different formulations since a long time [1].

Nyctanthes arbor-tristis is small sacred ornamental shrub known across the country for its fragment white flowers. The plant is commonly known as night jasmine or 'parijata'. It belongs to the family Verbenaceae. It is native of India, distributed widely in the Sub Himalayan regions and Southward to Godavari. It grows in Indo-Malayan region and distributed across Terai tracts as well as Burma and Ceylon. Traditionally the powdered stem bark is given in rheumatic joint pain, in treatment of malaria and also used as an expectorant [2]. The bark is used for the treatment of snakebite and bronchitis. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic [3].

MATERIALS AND METHODS

Collection of plant materials

Bark of *Nyctanthes arbor- tristis* Linn was collected from in and around Mannargudi, Thiruvarur district, Tamilnadu, India. The collected materials were cleaned, shade dried and coarsely powdered. The plant material was powdered and used for further studies. The plant was identified with the help of The Flora of Presidency of Madras as authenticated by Dr.S.John Britto, RAPINAT Herbarium and Centre for Molecular Systematic. St. Joseph's college, Tiruchirappalli.

Preparation of ethanol extract

The powder was extracted with ethanol in 1:5 ratio using soxhlet apparatus for 6-8 hours and then centrifuged. From the centrifuged extract the supernatant was filtered through Whatman No. 1 filter paper. The filtrate was then subjected to dryness under reduced pressure at 37°C (not exceeding 40°C), until usage DMSO was added and stored. All the extracts were stored in a dessicator for further evaluation.

Qualitative analysis of phytochemical and screening

Ethanolic extract of *Nyctanthes arbor-tristis* was subjected to preliminary screening of phytochemical constituents [4].

In vitro anti-inflammatory activity

Albumin Denaturation

The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, and after cooling the samples the turbidity was measured at 660nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated [5].

Percentage inhibition (%) =

(Abs Control –Abs Sample) / Abs control X 100

HRBC membrane stabilization method

The anti-inflammatory activity of plant extract was assessed by *in vitro* HRBC membrane stabilization method. Fresh blood was collected and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride) was stored at 4°C and used within 5 hrs. Saline at two different concentrations (0.85% isosaline and 0.25% hyposaline) were prepared with 0.5 M phosphate buffer pH 7.4. 19ml of 0.5 M sodium dihydrogen phosphate solution was added to 81 ml of 0.15 M sodium hydrogen phosphate solution. The pH was checked and adjusted with monobasic or dibasic solutions as required and stored at room temperature for 4 weeks. RBC suspension: The blood samples were centrifuged at 3000 rpm at room temperature for 10 minutes and the packed cells obtained were washed with isosaline (pH 7.2) 3 times and 10% (v/v) suspension was made with isosaline. The assay mixture contained different concentration of extract viz 50, 100, 200, 400 and 800 µg/ml, the standard aspirin was at 200 µg/ml, 1 ml of phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline and 0.5 ml of 10% RBC suspension. In another tube 2ml of distilled water was taken and this served as the control. All the tubes were incubated at 37°C for 30 min. Then it was centrifuged and the haemoglobin content in the supernatant was estimated using UV-spectrophotometer at 560 nm [6].

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula

Percentage protection (%)

= 100-OD of Test/ OD of Control X 100

Statistical Analysis

Results are expressed as mean ± SD

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The bark extract of *Nyctanthes arbor- tristis* L. bark extract revealed the presence of medicinally active metabolites (**Table 1).** The phytochemical evaluation of the bark extract showed the presence of alkaloids, flavonoids, glycosides, tannins and absence of steroids, terpenoids, saponin, carbohydrate, protein, aminoacids.

Table 1: Qualitative phytochemical analysis of *Nyctanthes arbor – tristis*

S.No	Constituents	Ethanol extract
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Steroids	-
5	Tannins	+
6	Terpenoids	-
7	Saponin	-
8	Carbohydrate	-
9	Protein	-
10	Amino acids	-

+ indicates present; - indicates absent

***In vitro* Anti-inflammatory Activity**

Albumin Denaturation

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. The Inhibitory effect of different concentration of *Nyctanthes arbor - tristis* on protein denaturation is shown in **Table 2.** Nyctanthes *arbor-tristis* at a concentration range of 50,100,200,400 and 800µg/ml showed significant inhibition of denaturation of egg albumin in concentration dependent manner. Maximum inhibition of 73.40 ± 2.9% was observed at the concentration of 800µg/ml. The minimum inhibition of 18.14 ± 0.99% was showed at the concentration of 50µg/ml. Aspirin, a standard anti-inflammatory drug showed the inhibition of 80.12 ± 3.5% at the concentration of 200 µg/ml. It showed a significant concentration dependent inhibition of protein denaturation providing us an IC_{50} of 413.12 µg/ml.

Table 2: *In vitro* anti- inflammatory activity of ethanolic extract of *Nyctanthes arbor- tristis* stem bark and aspirin of Albumin denaturation method

Treatments	Concentration (µg/ml)	Absorbance at 560 nm	% inhibition of Albumin Denaturation
Control	-	0.25 ± 0.06	-
N.A	50	0.21 ± 0.09	18.14 ± 0.99
N.A	100	0.19 ± 0.06	23.76 ± 1.1
N.A	200	0.14 ± 0.06	45.58 ± 1.8
N.A	400	0.10 ± 0.07	58.16 ± 2.2
N.A	800	0.27 ± 0.06	73.40 ± 2.9
Aspirin	200	0.05 ± 0.06	80.12 ± 3.5
IC_{50} value(ug/ml)	-	-	413.12

Protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory activity of aqueous extract of flowers of *Nerium oleander*. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain inflammatory diseases could be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Agents that can prevent protein denaturation, therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by *Nerium oleander* and reference drug diclofenac sodium. *Nerium oleander* contains alkaloids, flavonoids, tannins and a phenolic acid are known to promote anti-inflammatory activity [7].

HRBC Membrane Stabilization Method

The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The extracellular activity of these

enzymes is said to be related to acute or chronic inflammation. The main action of anti-inflammatory agents is the inhibition of cyclooxygnase enzymes which is responsible for conversion of arachidonic acid to prostaglandins (PG)[8].The non-steroidal drugs (NASIDs) act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membranes by mean of inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes (cyclooxygenase) and proteases which cause further tissue inflammation and damage upon extra cellular release or stabilizing the lysosomal membranes[9].

The inhibition of hypotonicity induced HRBC membrane lysis stabilization of HRBC membrane was taken as a measure of the anti-inflammatory activity. The percentage of membrane stabilization for ethanolic extracts was done at 50, 100, 200, 400 and 800 µg/ml. It showed the maximum inhibition of 82.56 ± 0.43 % at 800µg/ml. The minimum inhibition of 21.40 ± 0.98% was showed at the concentration of 50µg/ml. Aspirin, a standard anti-inflammatory drug, showed the maximum inhibition 88.76 ± .43% at the concentration of 200 µg/ml with the increasing concentration the membrane hemolysis was decreased as shown in **Table 3.** Hence anti-inflammatory activity of the extracts was concentration dependent. It provides an IC_{50} of 280.76 µg/ml.

Table 3: *In vitro* anti- inflammatory activity of ethanolic extract of *Nyctanthes arbor- tristis* stem bark and aspirin of HRBC Membrane stabilization

Treatments	Concentration (µg/ml)	Absorbance at 560 nm	% inhibition of Membrane stabilization
Control	-	0.24 ± 0.12	-
N.A	50	0.19 ± 0.09	21.40 ± 0.98
N.A	100	0.15 ± 0.009	35.61 ± 0.85
N.A	200	0.103 ± 0.001	57.86 ± 0.63
N.A	400	0.07 ± 0.006	70.43 ± 0.57
N.A	800	0.04 ± 0.006	82.56 ± 0.43
Aspirin	200	0.02 ± 0.001	88.76 ± 0.43
IC_{50} value(ug/ml)	-	-	280.76

Anti-inflammatory refers to the property of a substance that reduces inflammation or swelling. The inflammatory response coordinated by a large range of mediators that form complex regulatory networks. They activate specialized sensors which then elicit the production of specific step of mediators. The mediators in turn, alter the functional states of tissues and organs (which are the effectors of inflammation) in a way that allows them to adapt to the condition indicated by the particular inducer adopt to the conditions of inflammation. Membranes damage refers to the damage of cell membranes which disturb the state of cell electrolytes, which when constantly increased, induces apoptosis [10].

RBC is essentially a bag of hemoglobin (Hb). The RBC is unique among eukaryotic cells in that it is a-nuclear, has no cytoplasmic structures organelles. Structural properties are linked to the membranes. RBCs take up oxygen in the lungs and release in into tissues while squeezing through the body's capillaries. Anesthetics tranquilisers and nonsteroidal anti-inflammatory drug stabilize erythrocytes against hypotonic hemolysis at low concentration. When RBC is subjected to hypotonic stress the release of Hb from RBC is prevented by anti-inflammatory agent because of membrane stabilization, and the results in stabilization of HRBC membrane. So, the stabilization of HRBC membrane by drug against hypotonicity –induced hemolysis serves as a useful tool. *In vitro* method for assessing the anti-inflammatory activity of plant extracts showed that maximum level of anti-inflammatory activity was seen in *A. marmelos* (87.49 ± 0.215%), which is followed by dasamula preparation (32.56 ± 0.043%) and *G.arborea* (20.36 ± 0.043%). Similarly, the membranes stabilizing profile of various extracts fractions of *L. camara* of bovine RBC exposed to both heat and hypotonic showed as significant amount of anti-inflammatory activity and exhibited a maximum activity of 27.95% [11].

CONCLUSION

It is concluded on the basis of the results that the ethanol extract of *Nyctanthes arbor-tristis* possess anti-inflammatory activity in a concentration dependent manner. This could due to the presence of flavonoid,

Quercetin. The data provide a basis for further investigation to isolate the active constituents and drug development against diseases related to inflammation.

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