Bioscience Discovery, 8(2):202-205, April - 2017

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article

In Vitro anti- inflammatory activity of *Vitex Negundo* extract by HRBC membrane stabilization

Ziyad Husain , Syed Sohail, Nazia D. Khan*, Z. H. Khan , S.M Mular

Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola
onlynazia247@gmail.com

Article Info

Received: 30-01-2017,
Revised: 01-03-2017,
Accepted: 11-03-2017

Keywords:
Vitex Negundo Anti-inflammatory, Human Red Blood Cell (HRBC), Membrane Stabilization

Abstract

Vitex Negundo – Nirgudi is a plant species of *Lamiaceae* family commonly found in India, especially in different areas of Maharashtra. It is a slender, laticiferous, semi-erect endangered shrub; specifically known for its immense medicinal values; for example-anticancerous, antiarthritic, antimicrobial, antiulcer, antivenom, antileprotic, immunomodulatory, hepatoprotective, wound healing activity etc. Its immense medicinal values can bring *Vitex Negundo* as a royal source of herbal medicine in India. Phytochemical constituents Literature indicates the presence of Alkaloids, steroids, terpenoids, flavonoids, saponins, phenolic compounds, tannins and lignins, inulins, cardiac glycosides, protein, carbohydrates etc., in aqueous and ethanolic hemidesmus indicus root extract. The percentage of membrane stabilisation for methanolic extracts and Diclofenac sodium were done at different concentrations. The maximum membrane stabilization of extracts *Vitex Negundo* was found to be 72.59 % at a dose of 50 µg/ml and Standard membrane stabilization was found to be 76.67% at a dose of 500 µg/ml of methanolic extract. Therefore, our studies support the isolation and the use of active constituents from *Vitex Negundo* in treating inflammations.

INTRODUCTION

Herbal Medicines use different alkaloids of medicinal plants for prevention and treatment of disease. It varies in large range; from traditional medicines of ancient times to present day standardized herbal drugs. In the age of clinical medicines, main blockage to use clinical drugs is drug resistance. Use of herbal medicine is cheaper for its easy availability. Modern day medicine already accepted herbalism as a form of alternative medicine. Clinical medicines however use many plant-derived metabolites in pharmaceutical drugs, for example- opium, aspirin, digitalis, quinine etc; but scope of using herbal medicine is more extended as it consists of many more unexplored herbs, minerals, fungal and algal products (Chartterjee *et al.*, 2014).

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 red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an in vitro measure of anti inflammatory activity of the plant root extracts (Chou, 1997). *Vitex Negundo* – nirgudi, is a plant species of Apocynaceae family commonly found in India. It is a slender, laticiferous, semi-erect endangered shrub; specifically known for its immense medicinal values; for example-anticancerous, antiarthritic, antimicrobial, antiulcer, antivenom, antileprotic, immunomodulatory, hepatoprotective, wound healing activity etc. Its immense medicinal values

can bring *V. negundo* as a royal source of herbal medicine in India. Therefore the present study was carried out to investigate *in Vitro* anti-inflammatory activity of *Vitex Negundo* extract by HRBC membrane stabilization

MATERIALS AND METHODS

Collection of Plant Material

The plant roots of *Vitex Negundo* were collected from Nagarjuna Medicinal Plant Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.) India. Identification of plant was done at the Botany Department of College. All the other chemicals and reagents were of pure analytical grade and obtained from local supplier.

Extraction and Preparation of Extract

The roots were dried under shade and powdered. The 10 g of dried powdered root of the plant materials were extracted separately with methanol, ethanol using soxhlet apparatus for 48 hrs. The solvent was distilled at lower temperature under reduced pressure and concentrated on water bath to get the crude extract which is stored in desiccator for further use. For aqueous extraction the 10 gm powder was taken in 100 ml distilled water for 48 hrs with continuous shaking after 15 min. intervals and filtered by Whatman's filter paper No. 1. The filtrate was seperated and store for further use.

Preparation of Human Red Blood Cells (HRBC) Suspension

According to the Seema Chaitanya *et al*, fresh whole human 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 in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline (Chaitanya *et al.*, 2011).

Assay of Membrane Stabilizing Activity

The HRBC membrane stabilizing activity assay was carried out as reported by (Sadique, J., *et al.*, 1989; Oyedapo O., *et al.*, 2010). The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

The percentage of hemolysis of HRBC membrane can be calculated as follows:

$$\% \text{ Hemolysis} = \frac{\text{Optical density of Test sample}}{\text{Optical density of Control}} \times 100$$

The percentage of HRBC membrane stabilisation can be calculated as follows:

$$\% \text{ Protection} = 100 - \left(\frac{\text{Optical density of Test sample}}{\text{Optical density of Control}} \times 100\right)$$

RESULTS AND DISCUSSION:

Table-1: Effect of *Vitex Negundo* methanolic extract on HRBC membrane hemolysis and membrane protection

Conc. (µg/ml)	% Hemolysis of Diclofenac sodium	% Protection of Diclofenac sodium	% Hemolysis of *Vitex Negundo*	% Protection of *Vitex Negundo*
50	45.00	55.00	27.41	72.59
100	36.61	63.34	33.87	63.13
250	28.33	71.67	40.32	59.68
500	23.33	76.67	50.00	50.00

Table-2: Effect of *Vitex Negundo* ethanolic extract on HRBC membrane hemolysis and membrane Protection

Conc. (µg/ml)	% Hemolysis of Diclofenac sodium	% Protection of Diclofenac sodium	% Hemolysis of *Vitex Negundo*	% Protection of *Vitex Negundo*
50	61.01	38.99	38.59	61.41
100	55.93	40.07	45.61	54.39
250	47.45	52.55	50.87	49.13
500	38.98	61.02	56.14	43.86

Table-3: Effect of *Vitex Negundo* aqueous extract on HRBC membrane hemolysis and membrane Protection

Conc. (µg/ml)	% Hemolysis of Diclofenac sodium	% Protection of Diclofenac sodium	% Hemolysis of *Vitex Negundo*	% Protection of *Vitex Negundo*
50	73.07	26.93	33.96	66.04
100	67.30	32.70	43.39	56.61
250	61.53	38.47	52.83	47.178
500	55.76	44.24	64.15	35.85

The inhibition of hypotonicity induced HRBC membrane lysis i.e, stabilisation of HRBC membrane was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilisation for methanolic, ethanolic and aqueous extracts were done at 50, 100, 250, 500, µg/ml. It was observed that of 50 µl of plant in methanol was found to be most effective as compared to ethanolic and aqueous extract of plant. % protection of membrane was found to be 72.59 % at 50 µl/ml of plant in methanolic extract. And increasing activity was observed at low concentration level, but decreased activity with higher concentration in all type of above plant extract.

In case of standard the % protection was found to increases with increasing concentration of standard. Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyses and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. *G. vulgaris* Nees of the same family Apocynacea is a common plant of North Kerala. Here anti-inflammatory activity was performed based on the folk lore information using two methods. HRBC method was selected for the in vitro evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane (Shenoy S., *et. al*., 2010) 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. The result indicted that the leaf extract of *Vitex Negundo* at various concentrations has significant anti-inflammatory property. Leaf extract of *G. vulgaris* Nees showed significant anti inflamatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids (Rosa MD., *et al*., 1971) present in the extract. Our result also indicates the efficacy *Vitex Negundo* as an effective therapeutic agent in the treatment of acute inflammations. Further and detailed studies are in process for the isolation of active constituent responsible for this property and to idendification of the possible mechanism of its anti inflamatory property.

Present *in vitro* studies on *Vitex Negundo* extracts demonstrate the depression of inflammation. Due to the presence of active principles such as flavonoids and triterpenoids (asiaticoside, madecassoside etc) and related polyphenols may act as responsible components for this activity. Hence, *Vitex Negundo* can be used as a potent anti inflammatory agent.

REFERENCES:

Chatterjee S, Banerjee A and Chandra I, **2014.** Hemidesmus indicus: A Rich Source of Herbal Medicine. *Med. Aromat. Plants*, **3**(4):3 – 4.

Chippada SC, Volluri SS, Bammidi SR and Vangalapati M, 2011. *In-vitro* anti inflammatory activity of methanolic extract of *centella asiatica* by hrbc membrane stabilisation. *Rasayan J.Chem*., **4**(2): 457 – 460.

Chou CT, 1997. The anti inflammatory effect of an extract of *Tripterygium wilfordii hook F* on adjuvant induced paw oedeme in rats. *Phytother. Res.*, **11:** 152-154.

Oyedapo O. and Akinpelu A, 2010. Red blood cell membrane stabilizing potentials of extracts of Lantana camra and its fraction, *International Journal of Plant Physicology and Biochemistry*, **2**(4): 46-51

Rajendran V. and Lakshmi K.S. 2008. *In vitro* and *in vivo* anti inflammatory activity of leaves of *Symplocos cochinchnensis moore ssp laurina*. *Bangladesh J. Pharmacol.*, **3:** 121

Rosa MD, Giround JP, Willoghby DA. 1971. Studies of the acute inflammatory response induced in rats in different sites by Carrageenan and turpentine. *J Pathol.*, **104**:15–29.

Sadique, J, W.A. Al-Rqobah, M.F. Bughaith and AR El-Gindy, 1989. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. *Fitoterapia*, **60**: 525-532.

Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. 2010. Evaluation of antiinflammatory activity of *Tephrosia purpurea* in rats. *Asian Pac J Trop Med.*, **3**(3):193–195.

How to Cite this Article:

Ziyad Husain, Syed Sohail, Nazia D. Khan, Z. H. Khan , S.M Mular, 2017. *In Vitro* anti- inflammatory activity of *Vitex Negundo* extract by HRBC membrane stabilization. *Bioscience Discovery,* **8**(2):202-205.