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# IN VITRO ANTI- INFLAMMATORY ACTIVITY OF HEMIDESMUS INDICUS (ROOT) EXTRACT BY HRBC MEMBRANE STABILIZATION

**Syed Sohail, Sangita Kunjwani, Zia. H. Khan and Shankar Mular**

Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola.

***Corresponding Author: Zia H. Khan**
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola.

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## ABSTRACT
Hemidesmus indicus – Anantamool, is a plant species of Apocynaceae family commonly found in India, specially 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, anti-inflammatory antimicrobial, antiulcer, antivenom, antileprotic, immunomodulatory, hepatoprotective, wound healing activity etc. Its immense medicinal values can bring H. indicus 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. Since triterpenoids and flavonoids have remarkable anti inflammatory activity, so our present work aims at evaluating the in vitro anti inflammatory activity of Hemidesmus indicus by HRBC membrane stabilization. The inhibition of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilisation for methanolic extracts and Diclofenac sodium were done at different concentrations. The maximum membrane stabilization of Hemidesmus indicus extracts was found to be 79.64% at a dose of 50 µg/ml and Standard membrane stabilization was found to be 81.04% at a dose of 500 µg/ml of methanolic extract. Therefore, our studies support the isolation and the use of active constituents from Hemidesmus indicus in treating inflammations.

**KEYWORDS:** Hemidesmus Indicus, Anti-inflammatory,Human Red Blood Cell (HRBC), Membrane Stabilization.

## 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. So, multidrug resistance somehow hampers random use of clinical medicines anymore; putting forward more use of herbal medicine. 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 S. et al., 2014).

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 peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular 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 (Rajendran V., and Lakshmi K. S., 2008). HRBC or erythrocyte membrane is analogous to the lysosomal membrane (Chou C. T., 1997) 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. Hemidesmus indicus – Anantamool, is a plant species of Apocynaceae family commonly found in India, especially in different areas of West Bengal. 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 H. indicus as a royal source of herbal medicine in India.

## MATERIAL AND METHODS

### Collection of Plant Material

The plant roots of Hemidesmus indicus was 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

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 (Seema 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 principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts and standard drug diclofenac sodium of various concentrations 50 µl, 100 µl, 250 µl and 500 µl. and control (distilled water) instead of hypo saline to produce 100% hemolysis. Incubate at 37°C for 30 min and centrifuged respectively. 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:

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

## RESULT AND DISCUSSION

**Table-1: Effect of Hemidesmus indicus methenolic extract on HRBC membrane hemolysis and membrane protection**

| Conc. (µg/ml) | % Hemolysis of Diclofenac sodium | % Protection of Diclofenac sodium | % Hemolysis of Hemidesmus indicus | % Protection of Hemidesmus indicus |
|---|---|---|---|---|
| 50 | 32.75 | 67.25 | 20.31 | 79.68 |
| 100 | 27.58 | 72.42 | 21.87 | 78.12 |
| 250 | 22.41 | 77.59 | 29.68 | 70.31 |
| 500 | 18.96 | 81.04 | 37.51 | 62.50 |

**Table-2: Effect of Hemidesmus indicus ethanolic extract on HRBC membrane hemolysis and membrane Protection**

| Conc. (µg/ml) | % Hemolysis of Diclofenac sodium | % Protection of Diclofenac sodium | % Hemolysis of Hemidesmus indicus | % Protection of Hemidesmus indicus |
|---|---|---|---|---|
| 50 | 46.0 | 54.0 | 32.07 | 67.92 |
| 100 | 42.0 | 58.0 | 22.64 | 77.35 |
| 250 | 36.0 | 64.0 | 30.18 | 69.82 |
| 500 | 30.0 | 70.0 | 45.28 | 64.72 |

**Table-3: Effect of Hemidesmus indicus aqueous extract on HRBC membrane hemolysis and membrane Protection**

| Conc. (µg/ml) | % Hemolysis of Diclofenac sodium | % Protection of Diclofenac sodium | % Hemolysis of Hemidesmus indicus | % Protection of Hemidesmus indicus |
|---|---|---|---|---|
| 50 | 53.84 | 46.16 | 24.52 | 75.48 |
| 100 | 46.15 | 53.85 | 32.07 | 67.93 |
| 250 | 40.38 | 59.62 | 35.84 | 64.16 |
| 500 | 36.53 | 63.47 | 43.39 | 56.61 |

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 79.68% at 50 µl/ml of plant in methanolic extract. And increasing activity was observed at low concentration level, but decrease activity with higher concentration in all type of above plant extract.

In case of standard the % protection was found to increase with increasing concentration of standard.

## CONCLUSION

Stabilization of the HRBCs membrane by hypo tonicity induced membrane lysis was studied to establish the mechanism of anti-inflammatory action of Hemidesmus indicus. Therefore, our present in vitro studies on Hemidesmus indicus extracts demonstrate the depression of inflammation. Due to the presence of active principles such as flavonoids and tritrepenoids (asiaticoside, madecassoside etc) and related polyphenols may act as responsible components for this activity. Hence, Hemidesmus indicus can be used as a potent anti inflammatory agent.

## REFERENCES

1. Chatterjee S, Banerjee A, Chandra I: Hemidesmus indicus: A Rich Source of Herbal Medicine. Med. Aromat. Plants, 2014; 3(4): 3 – 4.
2. Chou C. T.: The anti-inflammatory effect of Tripterygium wilfordii Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. Phytother. Res., 1997; 11: 152-54.
3. Oyedapo O., Akinpelu A. et al.,: Red blood cell membrane stabilizing potentials of extracts of Lantana camra and its fraction, International Journal of Plant Physicology and Biochemistry, 2010; 2(4) : 46-51.
4. Rajendran V., and Lakshmi K. S.: In vitro and in vivo anti inflammatory activity of leaves of Symplocos cochinchinensis (Lour) Moore ssp Laurina. Bangladesh J. Pharmacol., 2008; 3: 121.
5. Sadique, J., W.A. Al-Rqobah, M.F. Bughaith and A.R. El-Gindy: The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia, 1989; 60: 525-532.
6. Seema Chaitanya Chippada, Sharan Suresh Volluri, Srinivasa Rao Bammidi and Meena Vangalapati: In-vitro anti inflammatory activity of methanolic extract of centella asiatica by hrbc membrane stabilisation. Rasayan J. Chem., 2011; 4(2): 457–460.