RJC
http://www.rasayanjournal.com

RASĀYAN *J. Chem.*
Vol.4, No.2 (2011), 457-460
ISSN: 0974-1496
CODEN: RJCABP

IN VITRO ANTI INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *CENTELLA ASIATICA* BY HRBC MEMBRANE STABILISATION

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ABSTRACT

Centella asiatica is a valuable medicinal herbaceous aromatic creeper which has been valued for centuries in ayurvedic medicine. Phytochemical analysis of *Centella asiatica* plant extracts revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides, triterpenoids and saponins etc. Since triterpenoids and flavonoids have remarkable anti inflammatory activity, so our present work aims at evaluating the in vitro anti inflammatory activity of *Centella asiatica* 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 C. asiatica extracts was found to be 94.97 % at a dose of 2000 µg/ml. Therefore, our studies support the isolation and the use of active constituents from *Centella asiatica* in treating inflammations.

Keywords: Anti-inflammatory, *Centella asiatica*, Diclofenac sodium, Human Red Blood Cell (HRBC), Membrane stabilization.

INTRODUCTION

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[1]. HRBC or erythrocyte membrane is analogous to the lysosomal membrane[2] 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 drugs or plant extracts.

Centella asiatica is a perennial creeper, faintly aromatic and a valuable medicinal herb of which is distributed throughout tropical and subtropical regions of World such as India, China, Nepal, Madagascar, Srilanka, Indonesia and Eastern South America[3]. Traditionally, *Centella asiatica* has been valued for centuries in ayurvedic medicine for the treatment of leprosy, ulcer, asthma, bronchitis, elephantiasis, eczemas, anxiety, urethritis[4] cataract, eye troubles, diarrhoea among children, skin diseases, wound healing[5] and for revitalizing the nerves and brain cells, hence primarily known as a "Brain food" or "Memory enchancer" [6] in India. Phytochemical analysis of *Centella asiatica* plant extracts revealed the presence of various biochemical compounds such as alkaloids[7], flavonoids[8], glycosides, triterpenoids, saponins, amino acids[9], inorganic acids[10], vitamins[11], sterols and lipid compounds[12]. Since triterpenoids

and flavonoids have remarkable anti inflammatory activity, so our present work aims at evaluating the in vitro anti inflammatory activity of *Centella asiatica* by HRBC membrane stabilization.

EXPERIMENTAL

Collection of Plant Material

The fresh whole plant of *Centella asiatica* was collected from in and around the premises of Andhra University, City of Visakhapatnam, Andhra Pradesh, India. All the other chemicals and reagents were of pure analytical grade and obtained from local supplier.

Table-1: Effect of *Centella asiatica* and Standard on HRBC membrane hemolysis and membrane stabilization

Conc. (µg/ml)	% Hemolysis of *C. asiatica*	% Stabilisation of *C. asiatica*	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	32.25	67.74	47.18	52.81
100	20.77	79.22	23.47	76.54
250	16.05	84.05	18.68	81.32
500	12.43	87.56	14.34	85.67
1000	8.45	91.54	7.43	92.58
2000	5.02	94.97	1.24	98.76

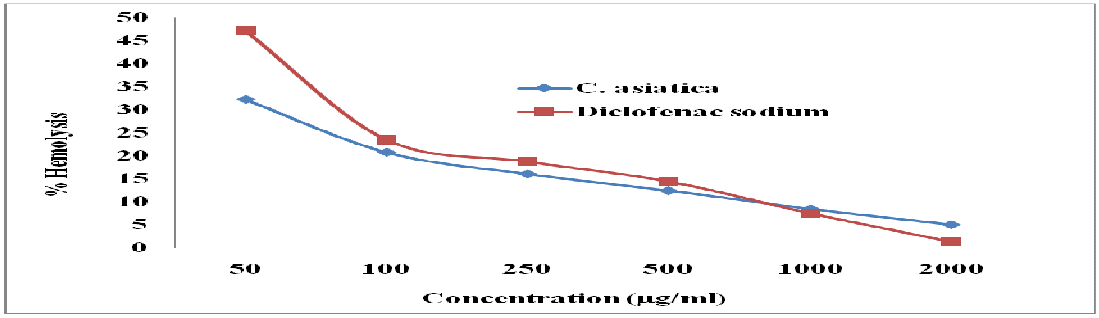

Fig.-1: Effect of *Centella asiatica* on HRBC membrane hemolysis

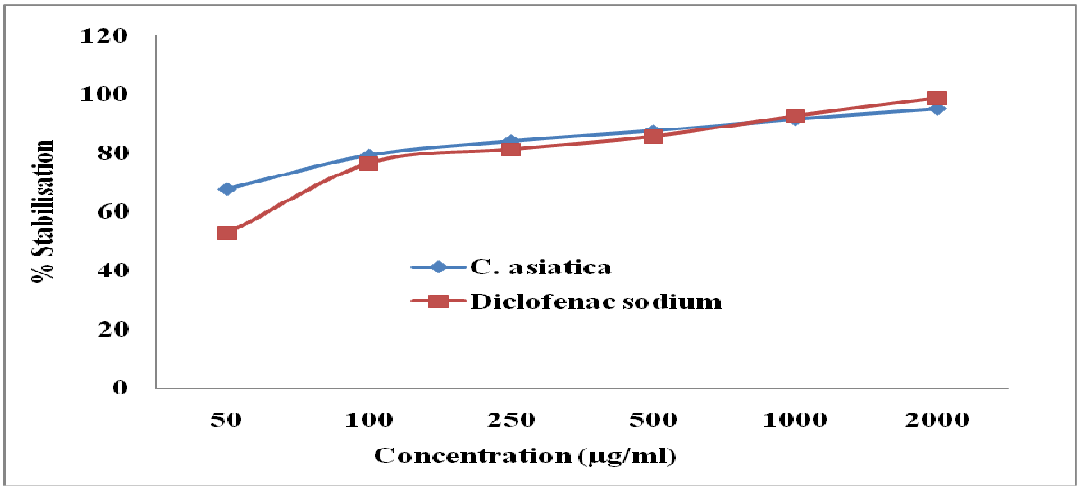

Fig.-2: Effect of *Centella asiatica* on HRBC membrane stabilisation

Extraction and Preparation of Extract

The leaves were garbled and dried under shade and powdered. The 10 g of dried powdered leaves of the plant materials were extracted separately with methanol 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 future use. The % of yield is 13.37 % respectively.

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.

Heat Induced Hemolysis

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, 100, 250, 500, 1000, 2000 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37^{o}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:
% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100
The percentage of HRBC membrane stabilisation can be calculated as follows:
% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

RESULTS AND DISCUSSION

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 extracts and Diclofenac sodium were done at 50, 100, 250, 500, 1000, 2000 µg/ml. Methanolic extracts of *C.asiatica* are effective in inhibiting the heat induced hemolysis of HRBC at different concentrations (50-2000µg/ml) as shown in Table 2. It showed the maximum inhibition 94.97% at 2000µg/ml. With the increasing concentration the membrane hemolysis is decreased as shown in Fig. 1 and membrane stabilisation / protection is increased as shown in Fig. 2. Hence anti inflammatory activity of the extracts was concentration dependent.

CONCLUSION

Stabilization of the HRBCs membrane by hypo tonicity induced membrane lysis was studied to establish the mechanism of anti-inflammatory action of *C. asiatica.* Therefore, our present in vitro studies on *C. asiatica* 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 responsible for this activity. Hence, *C.asiatica* can be used as a potent anti inflammatory agent.

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[RJC-733/2011]