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In vitro Anti Inflammatory Activity of Methanolic Extract of *Bacopa monniera* by HRBC Membrane Stabilisation

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ABSTRACT

Bacopa monniera also referred to as *Bacopa monnieri, Herpestis monniera*, water hyssop, and "Brahmi," has been used in the Ayurvedic system of medicine for centuries. Phytochemical analysis of *B. monniera* plant extracts revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides,bacosides, 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 *B. monniera* 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 percentage of membrane stabilization and hemolysis of *B. monniera* extracts were found to be 92.92±1.41% and 7.08±1.17% at a dose of 2000 ìg/ml respectively, when compared to standard Diclofenac sodium was found out to be 98.76±1.26% and 1.24±0.87% at a dose of 2000 µg/ml respectively .Therefore, our studies support the isolation and the use of active constituents from *B. monniera* in treating inflammations.

Key words: Anti-inflammatory, *Bacopa monniera*, 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.

Bacopa monnieri (Coastal Waterhyssop, Brahmi, Thyme-leafed gratiola, Water hyssop) is a perennial, creeping herb whose habitat includes wetlands and muddy shores [3]. It commonly grows

in marshy areas throughout India, Nepal, Sri Lanka, China, Taiwan, and Vietnam, and is also found in Florida, Hawaii and other southern states of the USA where it can be grown in damp conditions by the pond or bog garden. This plant has a number of uses in Ayurveda. It is a traditional treatment for epilepsy and asthma [4]. The other reported activities include sedative, antioxidant, vasoconstrictor and anti inflammatory [5, 6]. Phytochemical analysis of *B. monniera* plant extracts revealed the presence of various biochemical compounds such as alkaloids, betulic acid, stigmastarol, beta-sitosterol, bacopasaponins, tetracyclic triterpenoid saponins, hersaponin[7, 8, 9]. Since triterpenoids and flavonoids have remarkable anti inflammatory activity, so our present work aims at evaluating the in vitro anti inflammatory activity of *B. monniera* by HRBC membrane stabilization.

MATERIAL AND METHODS

Collection of Plant Material

The fresh whole plant of *Bacopa monniera* was collected from Araku valley situated near Visakhapatnam, Andhra Pradesh, India.

Chemicals

All chemicals and reagents used were of analytical grade or purest quality.

Extraction and Preparation of methanolic extracts

10 gm of powder of the plant was packed in thimble flask and 250ml of methanol was added in 1 litre round bottom flask. Then the Soxhlet assembly was set up to complete 10 to 15 cycles. The solvent was distilled at lower temperature under reduced pressure, after that the extract was filtered and filtrate was concentrated using water bath to get the crude extract which is stored in freezer for future use. The percentage yield of methanolic extract of *Bacopa monniera* is 15.47 %.

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°C for 30 min and centrifuged respectively. The haemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

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

% of Hemolysis = (O.D of Test sample / O.D of Control) X 100

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

% of Protection = 100 – [(O.D of Test sample / O.D 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 of *B. monniera* and Diclofenac sodium were done at 50, 100, 250, 500, 1000, 2000 µg/ml. Methanolic extracts of *B. monniera* are effective in inhibiting the heat induced hemolysis of HRBC at different concentrations (50 2000µg/ml) as shown in Table 1. It showed the maximum inhibition 92.92±1.41% at 2000µg/ml respectively, when compared to diclofenac sodium that is 98.76±1.26% at 2000ìg/ml respectively. With the increasing concentration the membrane hemolysis is decreased as shown in Figure 1 and membrane stabilisation / protection is increased as

Table 1: Effect of *B. monniera* and Diclofenac sodium (standard) on percentage of HRBC membrane hemolysis and membrane stabilization

Conc. (µg/ml)	% of Hemolysis of *B. monniera*	% of Stabilisation of *B. monniera*	% of Hemolysis of Diclofenac sodium	% of Stabilisation of Diclofenac sodium
50	31.34±1.02	68.66±1.13	47.18±1.31	52.81±1.73
100	27.08±0.78	72.92±0.77	23.47±0.79	76.54±1.12
250	23.46±0.45	76.54±1.06	18.68±1.65	81.32±1.08
500	18.65±1.21	81.35±0.86	14.34±0.23	85.67±0.71
1000	11.76±0.95	88.24±0.92	7.43±1.11	92.58±1.09
2000	7.08±1.17	92.92±1.41	1.24±0.87	98.76±1.26

Each value represents means ± SD (n=3).

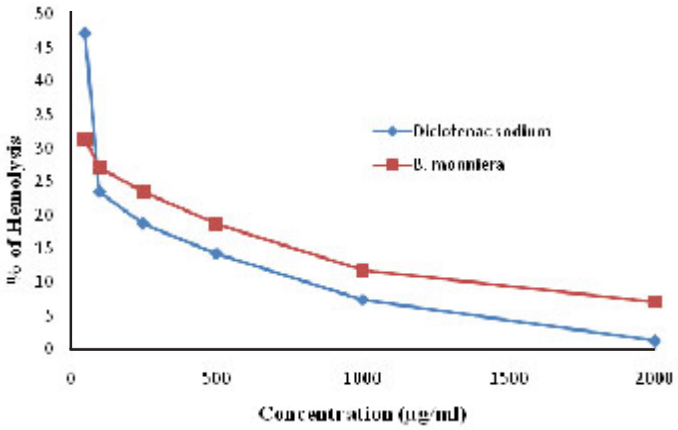

Fig. 1: Effect of *B. monniera and* Diclofenac sodium (standard) on percentage of HRBC membrane hemolysis

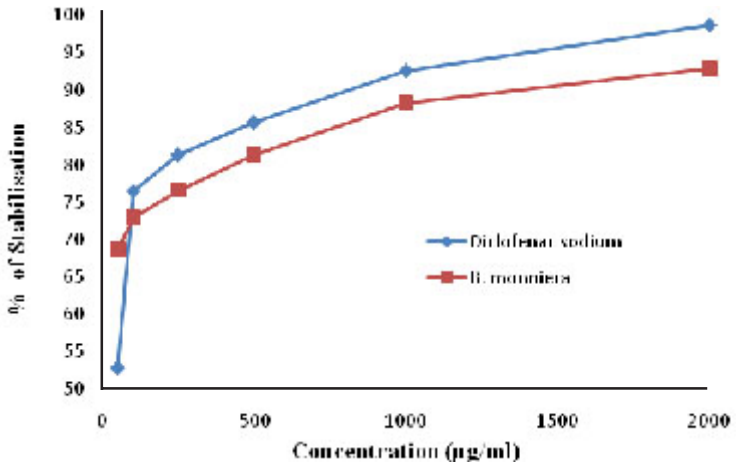

Fig. 2: Effect of *B. monniera* and Diclofenac sodium (standard) on percentage of HRBC membrane stabilisation

shown in Figure 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 *B. monniera* Therefore; our present in vitro studies on *B. monniera* extracts demonstrate the depression of inflammation. Due to the presence of active principles such as flavonoids, bacosaponins, tritrepenoids and related polyphenols may be responsible for this activity. Hence, *B. monniera* can be used as a potent anti inflammatory agent.

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