

In-Vitro Anti-Arthritic Activity of Methanolic Extract of *Bacopa Monniera*

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Article History:
Received: 7 June 2011
Accepted: 30 June 2011

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, bacosides, flavonoids, glycosides, triterpenoids and saponins etc. Our present work aims at investigating the in-vitro anti-arthritic effect of *B. monniera* at various concentrations. The inhibition of protein denaturation and membrane stabilisation were taken as a measure of the in-vitro anti-arthritic activity. The maximum percentage inhibition of protein denaturation and membrane stabilisation for *B. monnieri* extracts were found to be $90.34 \pm 0.83\%$ and $93.67 \pm 1.34\%$ respectively at a dose of 2000 μ g/ml. When compared to standard Diclofenac sodium was found out to be $96.52 \pm 1.25\%$ and $98.76 \pm 1.67\%$ respectively at a dose of 2000 μ g/ml. Therefore, our studies support the isolation and the use of active constituents from *B. monnieri* in treating arthritis.

Keywords: *Bacopa monniera*, anti-arthritic activity, Diclofenac sodium, triterpenoids, bacosides.

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INTRODUCTION

About 1% of the world's population is afflicted by rheumatoid arthritis, women three times more often than men. Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attack synovial joints [1, 2]. The production of auto antigens in certain arthritic diseases may be due to in vivo denaturation of proteins [3]. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [4]. So, by controlling the production of auto antigen and inhibiting denaturation of protein and membrane lysis in rheumatic disease leads to anti-arthritic activity. Hence, inhibition of protein denaturation and membrane lysis were taken as a measure of the in vitro anti-arthritic activity. A larger number of herbal extracts are in vogue for the treatment of various types of arthritis.

Bacopa monniera (Family-Scrophulariaceae) is a creeping, succulent herb, rooting at nodes, distributed throughout India in all plain districts, ascending to an altitude of 1,320m [5, 6]. *Brahmi* is also known as "Medhya Rasayana" in Ayurveda as it increases mental clarity and brain stimulating action [7]. It also possesses anti-inflammatory, analgesic, antipyretic, epilepsy, anticancer and antioxidant activities [8, 9]. The medicinal properties of *B. monnieri* responsible for improving memory-related functions have been attributed to the presence of different types of saponins such as bacosides A, B, C and D which are the active triterpenoid principles and known as "memory chemicals" [10]. Our present work aims at evaluating the anti-arthritic effect of *B. monniera* at various concentrations by using *in-vitro* pharmacological models.

MATERIALS 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 %.

Inhibition of Protein Denaturation

The following procedure was followed for evaluating the percentage of inhibition of protein denaturation-

1. Test solution (0.5ml) consist of 0.45ml of Bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of test solution.
2. Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of distilled water.
3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.
4. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml Of Diclofenac sodium.

Various concentrations (50, 100, 250, 500, 1000, 2000 $\mu\text{g}/\text{ml}$) of plant extracts (test solution) and diclofenac sodium (standard) of were taken respectively. All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm.

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation of different concentrations was tabulated in Table 1 & Figure 1. The percentage inhibition of protein denaturation can be calculated as-

$$\% \text{ of Inhibition} = \frac{[100 - (\text{O.D of test solution} - \text{O.D of product control})]}{(\text{O.D of test control})} \times 100.$$

The control represents 100% protein denaturation .The results were compared with Diclofenac sodium.

Effect on membrane stabilisation / Inhibition of membrane lysis

The principle involved here is stabilization of human red blood cell (HRBC) 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 $\mu\text{g}/\text{ml}$) 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 membrane stabilisation at different concentrations was tabulated in Table 1 and shown in Figure 2.

The percentage inhibition of membrane stabilisation can be calculated as-

$$\% \text{ of Inhibition} = 100 - [(\text{O.D of test solution}) \div (\text{O.D of control}) \times 100].$$

Table-1: Effect of *B. monniera* and Diclofenac sodium on inhibition of protein denaturation and membrane stabilisation.

Concentration ($\mu\text{g}/\text{ml}$)	% Inhibition of protein denaturation by <i>B. monniera</i>	% Inhibition of protein denaturation by Diclofenac sodium	% of membrane stabilisation of <i>B. monniera</i>	% of membrane stabilisation of Diclofenac sodium
50	60.34 \pm 1.08	64.81 \pm 1.47	64.54 \pm 1.77	52.81 \pm 1.75
100	65.23 \pm 1.22	70.54 \pm 1.58	74.37 \pm 0.85	76.54 \pm 1.94
250	73.16 \pm 2.02	81.32 \pm 1.83	80.32 \pm 1.83	81.32 \pm 0.89
500	82.95 \pm 1.99	85.67 \pm 0.63	83.94 \pm 1.37	85.67 \pm 1.56
1000	85.66 \pm 1.34	92.78 \pm 1.69	90.84 \pm 0.77	92.58 \pm 1.34
2000	90.34 \pm 0.83	96.52 \pm 1.25	93.67 \pm 1.34	98.76 \pm 1.67

Each value represents means \pm SD (n=3).

RESULTS AND DISCUSSIONS

The methanolic extract of *B. monniera* has showed significant activity at various concentrations and its effect was compared with the standard drug Diclofenac sodium. The maximum percentage inhibition of protein denaturation and membrane stabilisation of *B. monniera* was observed as 90.34 \pm 0.83% and 93.67 \pm 1.34% at 2000 $\mu\text{g}/\text{ml}$ respectively as shown in Table 1. When compared to standard Diclofenac sodium was found out to be

$96.52 \pm 1.25\%$ and $98.76 \pm 1.67\%$ respectively at a dose of $2000 \mu\text{g/ml}$. The production of auto antigen in certain arthritic disease may be due to denaturation of protein and membrane lysis. From the results (Figure 1 and 2) of our present study, it can be stated that methanolic extracts are capable of controlling the production of auto antigen and inhibits denaturation of protein and membrane lysis in rheumatic disease.

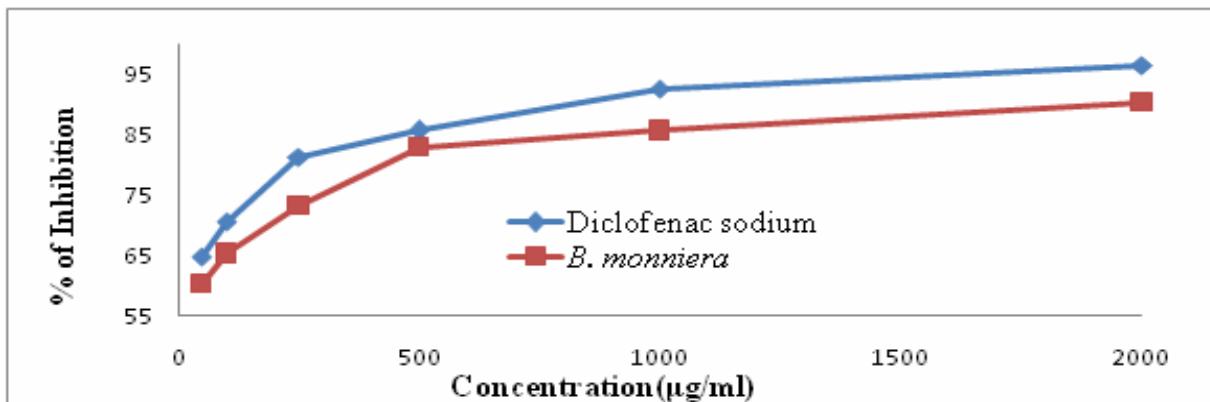


Fig.-1: Effect of *B. monniera* and diclofenac sodium on percentage inhibition of protein denaturation

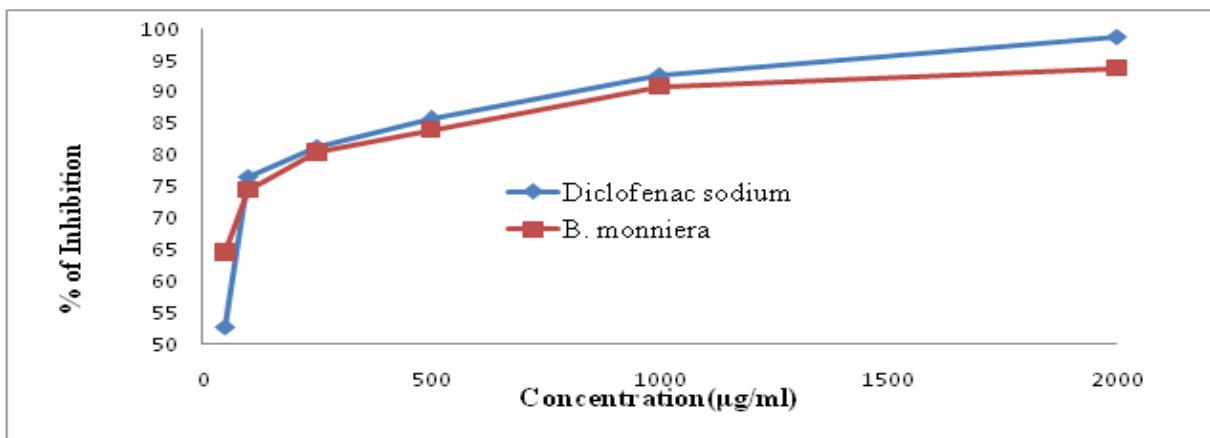


Fig.-2: Effect of *B. monniera* and diclofenac sodium on percentage of membrane stabilisation

CONCLUSION

Inhibition of protein denaturation and membrane stabilisation was studied to establish the mechanism of anti-arthritic effect of *B. monnierii*. Therefore, our present *in-vitro* studies on *B. monnierii* extracts demonstrated the significant anti-arthritic activity. Due to the presence of active principles such as flavonoids, bacosides and triterpenoids and related polyphenols may responsible for this activity. Hence, *B. monnierii* can be used as a potent anti-arthritic agent.

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[IJCEPR-156/2011]

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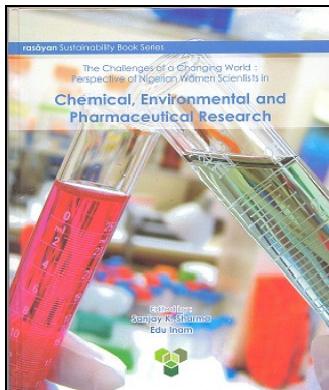
ISSN: 2229-3892(Print); ISSN: 2229-5283(Online)

[Abstracted in : Chemical Abstracts Service , American Chemical Society, USA and CAB(I) , UK]

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