EX-VIVO ANTI-ARTHRTIC AND MEMBRANE STABILIZING ACTIVITY OF AQUEOUS EXTRACT OF BREYNIA RETUSA

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ABSTRACT

Anti-arthritic properties of aqueous extract of Breynia retusa was evaluated for the inhibition of protein denaturation by using Bovine serum albumin. The extract exhibited 22.27% of the inhibition of protein denaturation at lower concentration (62.5 µg/ml) whereas the percentage of inhibition of protein denaturation was 56.45% at higher concentration (1000 µg/ml). HRBC membrane stabilization method was used to measure the membrane stabilizing activity of aqueous extract of Breynia retusa. Aqueous extract of Breynia retusa demonstrated 87.3% of membrane stabilization at 1000 µg/ml and 63.77% at 62.5µg/ml concentration. The results revealed that this aqueous plant extract contains moderate anti-arthritic activity and significant membrane stabilizing activity at higher concentration respectively.

KEYWORDS: Breynia retusa, Anti-arthritic, Diclofenac sodium, Protein denaturation, HRBC, membrane stabilization.

INTRODUCTION

Medicinal plants have been used as a beneficial source to treat various diseases from ancient time. Undoubtedly it is one of the primary source of plant derived medicine in modern era. According to the recent reports of World Health Organization (WHO) at least 75-95% of the populations of developing countries were mainly depend on traditional therapies involves the
use of plant extract products or their active constituents and traditional medicines because of its minimal cost.\textsuperscript{[2]} Use of traditional medicine is a common practice in developed and developing countries at initial healthcare level.\textsuperscript{[3]} Scientific evaluations of such plant-derived medicines validate the traditional knowledge along with contribution in the development of better allopathic drugs.\textsuperscript{[4, 5]} \textit{Breynia retusa} is a shrub with spreading branches. It grows abundantly in Bangladesh\textsuperscript{[6]}, India, Sri Lanka, Thailand, China, Bhutan, Cambodia, Laos, Malaysia, Nepal and Vietnam.\textsuperscript{[7]} In Bangladesh, it is widely distributed in different parts of Bangladesh such as scrub forests of Sylhet and Chittagong Hill Tracts. The plant is beneficial in inflammations and diseases of the blood. It is also effective as an astringent to the bowels. Leaf of this plant is fruitful in case of hasten suppuration. The juice of the stem is used in conjunctivitis.\textsuperscript{[6]}

**MATERIALS AND METHODS**

**Plant material**

\textit{Breynia retusa} was collected from local area of Chittagong district, Bangladesh and authenticated by the Botanist Dr. Shaikh Bokhtear Uddin, Assistant Professor, Department of Botany, University of Chittagong, Bangladesh.

**Preparation of extraction**

The leaf was sun dried and ground. The ground (500 g) were soaked in sufficient amount of water for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The aqueous extract was then preserved in a refrigerator at 4º C till further use.

**Chemicals and reagents**

The chemicals used were Bovine serum albumin (BSA), Diclofenac sodium, Sodium Chloride, sodium citrate, Sodium di-hydrogen phosphate, di-sodium hydrogen phosphate, dextrose, citric acid were purchased from Sigma-Aldrich. All chemicals in this investigation were of analytical reagent grade.

**Inhibition of protein denaturation**

Diclofenac sodium was treated as standard solution for the inhibition of protein denaturation. The test solution (0.5 ml) contains 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of aqueous extract of \textit{Breynia retusa}. The control solution (0.5 ml)
contains 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) contains 0.45 ml of distilled water and 0.05 ml of aqueous extract of *Breynia retusa*. Standard solution (0.5 ml) contains 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium. Five concentrations (62.5, 125, 250, 500, and 1000 µg/ml) of aqueous extract of *Breynia retusa* and diclofenac sodium (standard) were taken respectively. All the solutions were adjusted to pH 6.3 using 1 N HCl. Samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the previous solutions. UV-Visible spectrophotometer is used to measure the absorbance at 416 nm. The control shows 100% protein denaturation. The results were compared with diclofenac sodium.[8]

The percentage inhibition of protein denaturation of different concentrations is tabulated in Table 1. The percentage inhibition of protein denaturation can be calculated as

\[
\% \text{ inhibition} = \left[100 - \left(\text{OD of test solution} - \text{OD of product control}\right)\right] \times 100
\]

Where OD = optical density.

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

**The human red blood cell (HRBC) membrane stabilization method**

In this method human red blood cell membrane was used for the hypotonicity induced membrane lysis. Fresh blood (2ml) was collected from the healthy human volunteer who had not taken any NSAIDs for the prior to the experiment and then the blood was mixed with with equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. For the cleansing purpose of packed cells Isosaline was used. A 10% v/v suspension was made and kept at 4°C undistributed before use. Five concentrations (62.5, 125, 250, 500, 1000 µg/ml) of extracts were used and blood control (distilled water instead of hypo saline to produce 100% hemolysis) were separately mixed with 1ml (0.15M) of sodium phosphate buffer, 2ml of hyposaline and 0.5ml of 10% HRBC suspension was added to prepared. Erythrocyte suspension was absent in drug control when drugs were omitted in blood control. All the assay mixture were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min and hemoglobin content of supernatant solution was estimated by using spectrophotometer at 560nm.[9]
The percentage of HRBC membrane stabilization activity was calculated by using the following formula

\[
\% \text{ of membrane stabilization value} = \frac{100 - [(\text{Drug test value} - \text{Drug control value}) \times 100]}{\text{Blood control value}}
\]

Where, the blood control represented 100% lysis.

RESULTS

Anti-arthritic study

Different concentrations of aqueous extract of *Breynia retusa* and diclofenac sodium were tested for anti-arthritic activity and found significant percentage inhibition in protein denaturation (Table 1). Here, in lower concentration the extract of *Breynia retusa* showed 22.27% whereas the standard drug diclofenac sodium showed 61.29% of inhibition and in higher concentration, the extract of *Breynia retusa* exhibited the 56.45% of inhibition whereas the diclofenac sodium exhibited 85.49% of inhibition of protein denaturation.

Table 1. Percent inhibition of protein denaturation of *Breynia retusa*

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Percent of inhibition in protein denaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BR(Test Solution)</td>
</tr>
<tr>
<td>62.5</td>
<td>22.27%±1.03*</td>
</tr>
<tr>
<td>125</td>
<td>29.03%±1.35*</td>
</tr>
<tr>
<td>250</td>
<td>38.71%±1.71*</td>
</tr>
<tr>
<td>500</td>
<td>45.16%±1.93*</td>
</tr>
<tr>
<td>1000</td>
<td>56.45%±1.03*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM of three replicate (n=3). **P<0.01, *P<0.05

Fig.1. Inhibition of Protein denaturation (%) of *Breynia retusa* leaf and Diclofenac sodium
Membrane stabilizing activity
Aqueous extract of *Breynia retusa* was studied for *ex-vivo* membrane stabilizing activity by HRBC membrane stabilization method which is reported in Table 2. The *ex-vivo* membrane stabilizing activity of the extract was concentration dependent, with the increasing concentration, the activity is also increased. Here, aqueous extract of *Breynia retusa* showed 87.3% of membrane stabilization at 1000 µg/ml concentration and 63.77% at 62.5 µg/ml. All the results were compared with standard Diclofenac sodium which showed 94.44 % and 73.33% at 1000µg/ml and 62.5µg/ml respectively.

Table 2. Percent of stabilization of membrane of *Breynia retusa*

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Percent of membrane stabilization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>BR(Test Solution)</strong></td>
</tr>
<tr>
<td>62.5</td>
<td>63.77%±1.41*</td>
</tr>
<tr>
<td>125</td>
<td>72.51%±1.12*</td>
</tr>
<tr>
<td>250</td>
<td>78.78%±2.43*</td>
</tr>
<tr>
<td>500</td>
<td>81.23%±1.22*</td>
</tr>
<tr>
<td>1000</td>
<td>87.3%±2.21*</td>
</tr>
<tr>
<td></td>
<td><strong>Diclofenac sodium</strong></td>
</tr>
<tr>
<td></td>
<td>73.33% ±2.08</td>
</tr>
<tr>
<td></td>
<td>81.1% ±0.91</td>
</tr>
<tr>
<td></td>
<td>86.67% ±1.33</td>
</tr>
<tr>
<td></td>
<td>90.56% ±2.29</td>
</tr>
<tr>
<td></td>
<td>94.44% ±1.96</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM of three replicate (n=3). **P<0.01, *P<0.05

Fig. 2. Inhibition of hemolysis (%) of *Breynia retusa* leaf and Diclofenac sodium

DISCUSSION
Denaturation of protein is one of the causes of arthritis as documented. Production of auto antigen in certain arthritic disease may be due to denaturation of protein. The mechanism of denaturation probably involves alteration of electrostatic hydrogen, hydrophobic and disulphide bonding. Aqueous plant extract showed that the membrane stabilization effect by inhibiting hypotonicity induced lysis of the erythrocyte membrane. The erythrocyte
membrane is similar to the lysosomal membrane\[11\] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Lysosomal membrane must be stabilized to restrain the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil. This activated neutrophil is responsible for further tissue inflammation as well as damage upon extracellular release.\[12\] Hemolysis may emerge from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The process may enhance the efflux of these intracellular components which can be prevented by the extract.\[13\] This can be reduced by plant derived extract such as aqueous extract of *Breynia retusa*.

**CONCLUSION**

In conclusion, aqueous extract of *Breynia retusa* leaf possess moderate anti-arthritic and promising membrane stabilizing activity *ex-vivo*. It would be fascinating to investigate the mechanism underlying percentage of inhibition of protein denaturation as well as percentage of inhibition of hemolysis demonstrated by *Breynia retusa* extract. However, these activities might be due to the presence of bioactive or inhibitory compounds or synergism by the existence of some compounds. Because a variety of constituents, such as saponin, tannin, polyphenols, flavonoids, and alkaloids, may be present in the extracts, further extensive investigations are required to determine the specific active anti-arthritic and membrane stabilizing properties present in this leaf extract.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST STATEMENT**

Authors declare that they have no conflict of interest.

**REFERENCES**


