Phytochemical and Anti-Inflammatory Activity of Polygonum Barbatum

***Gowri R.[1], Gnanasekar [2] and Madhavan V.[3]**

*****Corresponding Author Email: gowri.pc.ph@msruas.ac.in

Contributors:

[1]Assistant Professor,
[3] Professor and HOD, Department of Pharmacognosy,
Faculty of Pharmacy, M.S.Ramaiah University of Applied Sciences,
[2] Professor, Department of Pharmacology, Kamalakshi Pandurangan College of Pharmacy, Tiruvannamalai, Tamilnadu

Abstract

Aim: To evaluate the *in vitro* anti-inflammatory activity of methanolic and aqueous extracts of *Polygonum barbatum* leaves by human RBC membrane stabilization method. **Methods:** The methanolic and aqueous extracts of leaves of *Polygonam barbatum* were prepared by continuous hot percolation and maceration method respectively. **Results** The preliminary phytochemical screening was carried out for ethanol and aqueous extracts. The prevention of hypo tonicity- induced HRBC membrane lysis was taken as a measure to determine the anti-inflammatory activity of the extracts **Conclusion:** In HRBC membrane stabilization method, both the extracts showed significant membrane stabilization effect and their anti-inflammatory activity was comparable to that of the standard drug Diclofenac sodium.

Key words: *Polygonum Barbatum, Methanolic Extract, Aqueous Extract, HRBC Membrane Stabilization*

1. INTRODUCTION

Flavonoids constitute one of the most characteristics compounds in higher plants. These compounds occur as a yellow and white plant pigment. Flavonoids have been referred as a "nature's biological response modifiers" because of their ability to change the body's response to allergen, pathogens and carcinogens[1]. Experiments have proven the various therapeutic effects of Flavonoids such as antioxidant, antiviral, anti-inflammatory, antihypertensive and in various cardiovascular diseases[2].

There are nearly 85 species of plants under the genus Polygonum. Polygonum barbatum Linn (polygonaceae) commonly called as joint weed; knotgrass is an annual herb, distributed throughout India, particularly in marshy places. The whole plant is used in siddha medicine to treat ulcers, diarrhea, skin eruptions and abdominal disorders[3].The shoots are used in the treatment of ulcer; seeds as tonic, purgative and emetic[4].

Traditional healers use it as a component in various recepies. The preliminary chemical test showed the presence of phenolic compounds like flavonoids and tannins. Since many flavonoids have shown potent anti-inflammatory activity, we proposed to evaluate the anti-inflammatory activity of Polygonum barbatum by HRBC stabilization. Erythrocytes have been used as a model system for the study of interaction of drugs with membranes. Drugs like anesthetic tranquillizers and non-steroidal anti-inflammatory stabilize erythrocytes against hypotonic haemolysis at a low concentration. When the RBC is subjected to hypotonic stress the release of hemoglobin from RBC is prevented by anti- inflammatory agents because of membrane stabilization. So the stabilization of RBC membrane by drugs against hypotonicity induced haemolysis serves as a useful *in vitro* method for

assessing the anti-inflammatory activity of various compounds.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant *Polygonum barbatum* was collected from Tiruvannamalai District, Tamil Nadu. The plant was identified and authenticated by Dr. P Jayaraman, Taxonomist, Plant Anatomy Research Centre, and Chennai. A voucher specimen (P.COG /NO.15) was deposited in the Department of Pharmacognosy for future reference. The leaves were separated, washed, with running tap water and dried in shade. The drug material was powdered, sieved through 60 mesh and stored in an airtight container.

2.2 Preparation of the Extracts

A weighed quantity (500 g) of air-dried powdered drug was extracted with methanol by continuous hot percolation method using soxhlet apparatus. Aqueous extract was prepared by cold maceration process by treating 100 g of fresh powder with 100 ml of chloroform water. The maceration was carried out for 7 days. Both the extracts were filtered, evaporated and concentrated to dryness under reduced pressure.

2.3 Preliminary Phytochemical Screening

Alcohol and aqueous extracts were screened for the presence of various phytoconstituents by adopting standard procedures[5].

2.4 Anti-inflammatory Activity (HRBC membrane stabilization method) [6, 7]

Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alseiver solution (2 % dextrose, 0.8 % sodium citrate, 0.05 % citric acid and 0.42 % Nacl). The blood was centrifuged at 3000rpm and the pellet obtained was washed with isosaline (0.85 % PH 7.2) and a 10% HRBC suspended was mixed with a mixture containing 4 ml of 0.25 % hypo saline, 1 ml of phosphate buffer and different concentrations of drug (250 -1000 µg/ml). All the tubes were incubated at 37°C for 30 min. Then centrifuged in a centrifuge and the supernatant hemoglobin content was estimated using Calorimeter at 560 nm. A control has been done without the drug. The % hemoglobin content was calculated by assuming the hemoglobin produced in the presence of distilled water as 100 %. The % of RBC membrane stabilization or protection was calculated using the formula

$$\% \text{ Protection} = 100 - \frac{\text{O.D of the drug}}{\text{O.D of control}} 100 \quad \ldots(1)$$

3. RESULT AND DISCUSSION

Preliminary phytochemical test showed the presence of alkaloids, steroids, phenolic compounds and glycosides in alcohol extract and carbohydrates, tannins and glycosides in aqueous extract (Table 1).

Table 1. Preliminary phytochemical screening of extracts of *Polygonum barbatum*

Tested group	Methanol Extract	Aqueous Extract
Alkaloids	+	-
Saponins	-	-
Glycosides	+	+
Flavonoids	+	-
Tannins	+	+
Sterols	+	-

The methanol and aqueous extract of leaves of *Polygonum barbatum* were studied for *in vitro* activity by HRBC membrane stabilization method. Among the extracts the methanol extract showed significant anti

inflammatory activity and it is comparable with the standard drug Diclofenac sodium.

During inflammation process, lysosomal enzymes are released which produce a variety of disorders[8]. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibition of these lysosomal enzymes or by stabilizing the lysozomal membranes[9].

Since HRBC membranes behave like lysosomal membrane components, the prevention HRBC membrane lysis is measured to determine the anti-inflammatory activity of drugs. The methanolic and aqueous extracts showed significant HRBC membrane stabilization (Table 2 & 3). They have shown increased activity at low concentration and it is comparable to that of standard drug.

The extract at the concentration range of 250-1000 µg/ml protects the HRBC membrane against lysis induced by hypotonic solution. At 250 µg/ml dose, both the extracts produced 49.2 and 37.4 % inhibition of RBC haemolysis as compared with standard Diclofenac, which produced 67.50 % inhibition.

Table 2. *In vitro* anti-inflammatory activity of extracts of *polygonum barbatum*

Treatment	Concentration (mg/ml)	Absorbance (540nm)	% inhibition
Control	-	0.48 ± 0.012	-
Methanolic Extract	1000 500 250	0.21± 0.002 0.27± 0.001 0.29± 0.005	56.3 43.8 40.2
Aqueous Extract	1000 500 250	0.31± 0.001 0.33± 0.005 0.35± 0.001	35.4 31.3 27.1
Diclofenac	10	0.14± 0.002	70.8

Table 3. Variation of activity with time

Concentration (µg/ml)	% of lysis prevention		
	Methanol extract	Aqueous extract	Diclofenoc sodium
250	65.2	57.5	
500	51.4	43.5	87.0
1000	44.5	37.2	

Hence the result confirms that *Polygonum barbatum* was found to be effective in stabilizing HRBC membrane.

4. CONCLUSION

The present study confirms the anti-inflammatory activity of leaf of *Polygonam barbatum*. The methanol extract has shown better anti-inflammatory activity than the aqueous extract. Several studies have correlated the anti-inflammatory effect with the presence of alkaloids, phenolic compounds like flavonoids and tannins[10,11]. Preliminary phytochemical screening revealed the presence of phenolic compounds and alkaloids in methanol extract which may be responsible for the activity. Further studies like isolation of active constituents and elucidation of mechanism of action responsible for the activity has to be done in future.

Acknowledgement

The authors are thankful to the management of Kamalakshi Pandurangan College of pharmacy for providing the facilities to carry out the work.

REFERENCES

1. Andrew pengelly. The constituents of medicinal plants. 2nd ed. CABI Publishing: USA; 33 1996: 33p.
2. Yamamoto C., Gayner A. Therapeutic potential of inhibition of the NF-KB

pathway in the treatment of inflammation and cancer. J Clinical Investigation. 2006; 107 (2):135-140.
3. Yoganarasimhan SN. Medicinal plants of India (Tamil Nadu). Bangalore: Interline publishing private Ltd; 2000. 433p.
4. Anonymous. The Wealth of India, Raw Materials V-1. CSIR: New Delhi; 1998. 195-196.
5. C.K. Kokate, Practical Pharmacognosy. Vallabh Prakashan, 4th Edn. NewDelhi, 6-40. (1994).
6. Murugesh N, Vembar S and Damodaran C. Studies on erythrocyte membrane IV: *In vitro* haemolytic activity of oleander extract. Toxicol Lett 1981; 8**:** 33-38.
7. Gandhisan R, Thamaraichelvan A and Baburaj. Anti-inflammatory action of *Lannea coromandelica* HRBC membrane stabilization. Fitoterapia 1991; 62**:** 82-83.
8. Goodman and Gillman. The pharmacological basis of therapeutics. 6 th ed. MacMillan publishing company: New York; 1982.
9. Geissler AJ, Bekemeier H, Hirschelmann R and Bakathir HA. Pharmacology, Biochemist and immunology of inflammatory Reaction. Martin Luther University.682.
10. Mona S. Mohammed, Wadah J.A. Osman, Elrashied A.E. Garelnabi, Zuheir Osman, Bashier Osman, Hassan S. Khalid, Magdi A. Mohamed, Secondary metabolites as anti-inflammatory agents. J Phytopharm. 2014; 3(4): 275-80**.**
11. Serafini M, Peluso I, Raguzzini A. Flavonoids as anti- inflammatory agents. Proc Nutri Soc 2010; 69(3): 273-8.