Inhibition of Formalin Induced Paw Edema in Rats by Various Fractions/Extracts of Bryophyllum pinnatum

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ABSTRACT

Traditionally, Bryophyllum pinnatum is used in the management of arthritis and inflammatory diseases. However, B. pinnatum has not been analysed previously for anti-inflammatory activity. Hence, this study is designed to determine the anti-inflammatory effects of various fractions of B. pinnatum leaf extract using rat model of formalin-induced paw edema. Treatment with various fractions showed marked decrease in formalin-induced paw volume and edema in rats. The results of BPAAF treatment were comparable to standard drug, diclofenac. These results indicate that B. pinnatum could be developed as anti-inflammatory drug after further studies.

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1. Introduction

Bryophyllum pinnatum (Syn. B. calycinum and kalanchoe pinnata) belonging to the family Crassulaceae is a native of tropical Africa but it has been naturalized throughout the tropics particularly in India and Bengal. It is popularly known as Parnabija, Patharchuur, Patharchat, Hemsaagar, Zakhm-hayat (Unani) (Kirtikar & Basu, 1935; Anonymous, 1998). Traditionally the plant is used as anti-inflammatory drug (Kirtikar & Basu, 1935; Anonymous, 1998-b). The leaves of Bryophyllum pinnatum are reported to contain bryophyllol, bryopone, bryophollenone, bryophynol, 18 α-oleanane, taraxasterol and α- and β-amyрин and their acetates (Anonymous, 1998-b; Sofowora, 1993). Till date, to the best of our knowledge, the plant is not reported previously for its anti-inflammatory effects. Therefore, the current investigation was designed to study the anti-inflammatory effects of various fractions of B. pinnatum leaf extract using formalin-induced paw edema rat model.

2. Materials and method

The Bryophyllum pinnatum leaves were collected from the herbal garden of Babu Banarsi Das National Institute of Technology and management chinut Lucknow and were identified in the taxonomy division of “National Botanical Research Institute, Lucknow”. The collected plant material was dried under shade and powdered coarsely. The methanol extract of powdered leaves was prepared by Soxhlet apparatus and obtained extract was dried under vacuum. The extract was fractionated into different solvent by liquid-liquid partitioning.

3. Animals

Sarague Dawely rats (120-150 g) were used for determining the anti-inflammatory effects of prepared fractions. All the animals were kept in polypropylene cages at room temperature and were feed with standard diet and drinking water ad libitum. Ethical clearance was obtained from “Institutional Animal Ethics Committee”.

4. Experimental protocol (Anonymous, 1998-b; Sofowora, 1993)

Subaponeurotic injection (0.1 ml) of formalin (2%) was used for induction of acute inflammation at the rat hind paw. Animals were administered with i.p. different doses of
extracts and diclofenac (5 mg/kg) in respective groups 60 min before induction of edema. The volume of paw edema was determined by plethysmometer at different 1, 2 and 4 h following the injection of formalin. The percent inhibition was determined by % inhibition = [(Paw volume of control group – paw volume of test group)/ Paw volume of control group] × 100

Group I-Control group. Animals were orally administered distilled water.
Group II-Standard. Animals were administered diclofenac at 5 mg/kg.
Group III-BPAF treated. BPAF was administered at 100mg/kg.
Group IV-BPFF treated. BPFF was administered at 100mg/kg.
Group V-BPPA treated. BPPA was administered at 100mg/kg.
Group VI-BPAAF treated. BPAAF was administered at 100mg/kg.

5. Statistical Analysis
The results are presented as mean±SD and analyzed by one-way-ANOVA. Results were considered significantly different at p≤0.05.

6. Result
Table 1: % inhibition of paw volume by various fractions of Bryophyllum pinnatum.

| Group | Dose         | Paw volume (Mean±SD) | (% inhibition) |
|-------|--------------|----------------------|----------------|
|       |              | 1h       | 2h       | 4h       |               |
| I     | Disease control | 0.52±0.04 | 0.63±0.06 | 0.70±0.06 |               |
| II    | BPAF (100 mg/kg) | 0.28±0.03** (46.16%) | 0.34±0.04** (42.74%) | 0.42±0.05** (40.00%) |
| III   | BPFF (100 mg/kg) | 0.40±0.10* (25.38%) | 0.49±0.04* (20.68%) | 0.52±0.05 (25.00%) |
| IV    | BPPA (100 mg/kg) | 0.46±0.04 (8.23%) | 0.56±0.04 (8.5%) | 0.63±0.06 (8.31%) |
| V     | BPAAF (100 mg/kg) | 0.23±0.03*** (60.53%) | 0.30±0.04*** (56.68%) | 0.30±0.06*** (56.71) |
| VI    | Diclofenac (5 mg/kg) | 0.28±0.02** (46.16%) | 0.34±0.01** (42.74%) | 0.30±0.02*** (57.14%) |

*** p<0.001, **p<0.01, *p<0.05 vs Group I. Normal control: 0.14±0.02.

7. Discussion
Rat model of formalin-induced paw edema is most commonly used method to explore newer anti-inflammatory and anti-arthritic drugs, as it produce inflammation that mimics to human arthritis (Greenwald, 1991). Thus, in the present study various fractions of Bryophyllum pinnatum were tested for anti-inflammatory potential employing formalin-induced paw edema in rat.

Treatment with all the fractions (except BPFF) of B. pinnatum showed marked reduction in formalin-induced paw volume and edema in rats (Table 1). Amongst all fractions, BPAAF exhibited marked decrease in paw edema and volume which was comparable to standard group. Formalin is documented to provoke the generation of endogenous mediators, such as, prostaglandins and histamine leading to development of paw edema (Zimmerman, 1989). Therefore in the present study, reduction of paw edema by various fractions of Bryophyllum pinnatum could be attributed to its anti-histaminic effects.

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