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ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES BRYOPHYLLUM PINNATUM LINN

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ABSTRACT

The present study is carried out to investigate the anti-inflammatory potential of Ethanolic extract of Bryophyllum pinnatum. Anti-inflammatory activity was evaluated by using Egg Albumin, Turpentine Oil & Formaldehyde as phlogistic agents. The animals were treated with doses 250mg/kg and 500mg/kg of extract and Diclofenac Sodium at a dose of 10mg/kg is used as a standard drug. The Bryophyllum Pinnatum showed a significant anti-inflammatory activity in a dose dependent manner in all the models when compared with the standard treatment. The extract (500mg/kg) exhibited maximum antiinflammatory activity i.e., 46.25%, 44.30%, 46.40% (P<0.001) like standard Diclofenac 47.02%, 46.50%, 48.57% in Egg albumin, Turpentine oil and Formaldehyde induced methods respectively. Based on the above results, we conclude that the Bryophyllum pinnatumhas significant anti-inflammatory activity and might prove efficacious for further design and development of agents with significant biological activity.

KEYWORDS: Bryophyllum pinnatum, anti-inflammatory activity, phlogistic agents.

INTRODUCTION

Herbal therapy is also known as herbalism, which play a major role in treatment of so many diseases in so many countries & in traditions.^[1] Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When tissue cells become injured they release kinins, prostroglandins and histamine.^[2] Rheumatoid arthritis a ravaging disease is a major public health burden in about 1% of the population worldwide. As the currently used drugs are associated with severe side effects, the urge to develop new chemical entities with potent biological activity from natural sources with lesser side effects has become mandatory. Traditional medicine using plant extracts continue to provide health coverage for over 80% of the world's population especially in developing countries.^[3] Many medicinal plants have been investigated for novel drugs or templates for the development of new therapeutic agents.^[4] Various species from the genus alba have been reported to possess anti-inflammatory activity.

Bryophyllum pinnatumplant belongs to family Crassulaceae, commonly used as traditional medicines. Bryophyllum pinnatumis derived from greek word Bryo means to sprout and phyllon means leaf. The secondary metabolites which are obtained from different parts of plant such as alkaloid, flavanoid, tannin, glycoside, phenolic compounds, which have therapeutic value. The plant are used in different pharmacological activities such as antidiabetic antihypertensive, antileishmanial, antimicrobial, analgesic, anticancer. These are also use in bleeding disorder and ulcer and diarrhea.^[11] The present study was under taken to evaluate anti inflammatory activities of ethanolic leaf extract of Bryophyllum pinnatum

MATERIALS AND METHODS

Collection of plant material & Extraction: The leaves of Bryophyllum pinnatum were collected from surrounding villages of A.P. The plant authentication was done by Dr. A.Srinivasa Rao, Dept. of Botany, P.R Degree College, Kakinada, East Godavari District, Andhra Pradesh, India & the voucher was preserved. The plant material was thoroughly cleaned, shade dried at room temp. for 23 days & then pulverized to a coarse powder and shifted. 95% ethanol was added to coarsely powered (2kg) plant material & extracted by using soxhlet apparatus. The extract was concentrated by distillation under reduced pressure & evaporated to dryness. **Experimental animals**: Healthy adult albino rats of wistar strains weighing 150-250gm of either sex were used in this study. The animals were kept properly in polypropylene cages under standard laboratory conditions (12/12hr light/dark cycle at 25 ± 5^{0} c). The rats were fed a commercial diet & water ad libitum & were divided into 5 groups.

Methodology

Egg albumin induced rat paw edema: Five groups of adult rats (n=6) were used in this study. Animals were fasted over night with free access to water before the experiment. On the day of experiment, base line paw volume was recorded by using a plethysmometer (UGO Basile, 7140 Italy). Thereafter group-I (normal rats) received the vehicle (Distilled water 5ml/kg). Group-II (control rats) received the inducing agent & vehicle. Group-III (standard rats) received diclofenac sodium (10mg/kg) along with inducing agent. Group-IV & V received extract at doses of 250mg/kg & 500mg/kg respectively along with inducing agent. 1hr after administration of vehicle/drugs, edema was induced by administration of 0.1ml of fresh undiluted egg albumin solution into the subplantar region of right hind paw.^[7] Paw volume of each rat from all groups was measured at 0, 1, 3, 6, 12 & 24hr after phlogistic agent administration. From the mean edema volume, the percent inhibition of edema was calculated by using following formula:

% Inhibition of edema = 100 (VC-VT/VC)Where, VC = Mean paw edema volume of control group VT = Mean paw edema volume of treated group

Turpentine oil induced rat paw edema: Grouping of animals & drug treatments was same as above. 30min after administration of the vehicle/drug, edema was induced by administration of 0.05ml turpentine oil into the subplantar region of right hind paw of animal.^[8] Paw volume of each rat from all groups was measured on 0, 1, 3 & 7th day after phlogistic agent administration. From the mean edema volume, the percentage inhibition of edema was calculated.

Turpentine oil induced rat paw edema: Grouping of animals & drug treatments was same as above. 30min after administration of the vehicle/drug, edema was induced by administration of 0.05ml turpentine oil into the subplantar region of right hind paw of animal. Paw volume of each rat from all groups was measured on 0, 1, 3 & 7th day after phlogistic agent administration. From the mean edema volume, the percent inhibition of edema was calculated.

Formaldehyde induced rat paw edema: Grouping of animals & drug treatments was same as above. Drugs/vehicles were administered for a duration of 10days. 30min after administration of the drug/vehicle, edema was induced by administration of 0.1ml of 2% v/v Formaldehyde into the subplantar region of right hind paw of all animals on days 1 and 3.^[8] Increase in paw edema volume was measured on 0, 1, 3, 7 and 10th day, 30min after administration of the respective vehicle/drug. From the mean edema volume, the percent inhibition of edema was calculated.

Statistical Analysis The statistical significance was measured by using one way analysis of variance (ANOVA) & followed by Dunnett's comparison test. All the data are presented as mean \pm SEM & p < 0.001 was considered as significant.

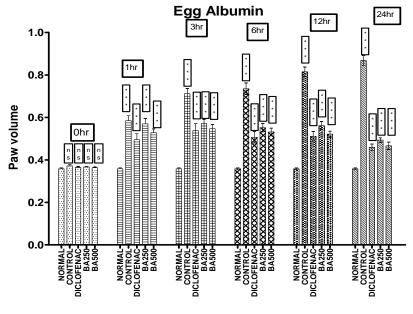
RESULTS

The phlogistic agents induced inflammation was significantly inhibited by the treatment given when compared with the standard drug. Bryophyllum Pinnatum exhibited significant anti-inflammatory activity in a dose dependent manner.

Egg albumin induced paw edema: The effect of Bryophyllum Pinnatum on egg albumin induced paw edema was depicted in the table 1. The Bryophyllum Pinnatum at a dose of 500mg/kg showed significantly greater inhibitory activity (46.25%) against standard diclofenac sodium (47.02%).

 Table 1: Anti-inflammatory activity of Bryophyllum Pinnatum on Egg albumin induced paw edema Percentage inhibition of paw edema.

| Group | Dose | 0hr | 1hr | 3hr | 6hr | 12hr | 24r |
|-------------|----------|------|--------|--------|--------|--------|--------|
| Diclofenac | 10mg/kg | 2.1% | 15.38% | 24.35% | 31.06% | 37.21% | 47.02% |
| BRYOPHYLLUM | 250mg/kg | 1.3% | 2.56% | 19.67% | 24.94% | 31.08% | 43.18% |
| PINNATUM | 500mg/kg | 1.6% | 9.68% | 23.18% | 27.66% | 35.99% | 46.25% |



Treatment

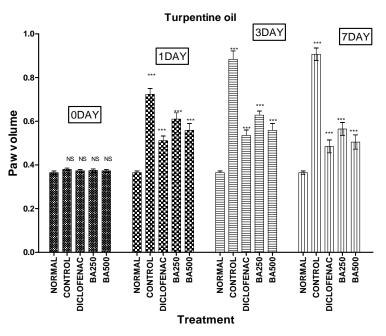
Graph 1: Concentration Vs Paw volume.

All values are expressed as mean \pm SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; ***p<0.001 as compared to control group; ns=non-significant. **Turpentine oil induced paw edema:** The inhibitory activity on turpentine oil induced paw edema are shown in table 2. The Bryophyllum Pinnatum at a dose of 500mg/kg showed inhibitory activity of 44.30% against standard (46.50%).

 Table 2: Anti-inflammatory activity of Bryophyllum Pinnatum on Turpentine oil induced paw edema

 Percentage inhibition of paw edema.

| Group | Dose | 0 DAY | 1 DAY | 3 DAY | 6 DAY |
|-------------|----------|-------|--------|--------|--------|
| Diclofenac | 10mg/kg | 1.75% | 29.26% | 39.43% | 46.50% |
| BRYOPHYLLUM | 250mg/kg | 1.31% | 15.66% | 28.86% | 37.68% |
| PINNATUM | 500mg/kg | 1.75% | 22.81% | 36.79% | 44.30% |



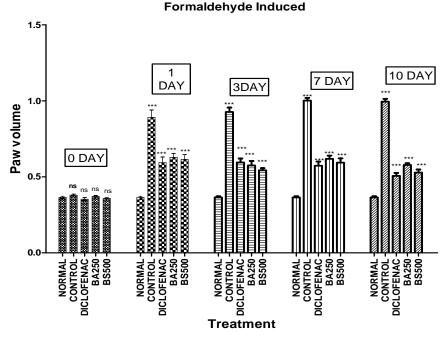
Graph 2: Concentration Vs Paw volume.

All values are expressed as mean \pm SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; ***p<0.001 as compared to control group; ns=non-significant. **Formaldehyde induced paw edema:** As shown in table 3 the BRYOPHYLLUM PINNATUM at a dose of 500mg/kg showed greater inhibitory activity (46.40%) against standard (48.57%).

 Table 3: Anti-inflammatory activity of BRYOPHYLLUM PINNATUM on Formaldehyde induced paw edema

 Percentage inhibition of paw edema.

| Group | Dose | 0 DAY | 1 DAY | 3 DAY | 7 DAY | 10 DAY |
|-------------|----------|-------|--------|--------|--------|---------------|
| Diclofenac | 10mg/kg | 7.01% | 33.45% | 35.79% | 42.92% | 48.57% |
| BRYOPHYLLUM | 250mg/kg | 2.1% | 29.53% | 30.61% | 38.43% | 41.37% |
| PINNATUM | 500mg/kg | 5.70% | 31.02% | 34.03% | 40.93% | 46.40% |



Graph 3: Concentration Vs Paw volume.

All values are expressed as mean \pm SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; ***p<0.001 as compared to control group; ns=non-significant.

DISCUSSION

The phlogistic agents induced inflammation was significantly inhibited by the treatment given when compared with the standard drug. Bryophyllum Pinnatum exhibited significant anti-inflammatory activity in a dose dependent manner. Egg albumin induced paw edema: The effect of Bryophyllum Pinnatum on egg albumin induced paw edema was depicted in the table 1. The Bryophyllum Pinnatum at a dose of 500mg/kg showed significantly greater inhibitory activity (46.25%) against standard diclofenac sodium (47.02%). Turpentine oil induced paw edema: The inhibitory activity on turpentine oil induced paw edema are shown in table 2. The Bryophyllum Pinnatum at a dose of 500mg/kg showed inhibitory activity of 68.75% against standard (65.28%). Formaldehyde induced paw edema: As shown in table 3 the Bryophyllum Pinnatum

at a dose of 400mg/kg showed greater inhibitory activity (44.30%) against standard (46.50%).

CONCLUSION

Interestingly, the test compound showed potential anti inflammatory activity like standard Diclofenac sodium and justified the traditional use of Bryophyllum pinnatumin the treatment of various types of pain & inflammation in all experimental models. Based on the above results, we conclude that the Ethanolic extract of Bryophyllum pinnatum shows significant antiinflammatory activity and might prove efficacious for further design and development of agents with significant biological activities. Further, studies are required to elucidate the detail mechanism of action of these agents at molecular level to explore the therapeutic benefits.

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