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Research Article

Anti-Arthritic Activity of Roots Extract of *Boerhaavia Diffusa* in Adjuvant Induced Arthritis Rats

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Abstract: The extract of the root of the plant *Boerhaavia diffusa* was studied for their Anti rheumatoid activity in Freunds Adjuvant Induced Arthritis Rats with the dose of 500 and 1000 mg/kg p. o. The administration of extract reported significant reduction in paw swelling on 4th, 8th, 14th and 21st day after sub-plenter administration of Complete Freunds adjuvant. The paw swelling was measured as a volume displacement using digital Plethysmometer. From the results observed in the present investigation, it may be concluded that the extract of *Boerhaavia diffusa* possesses potentially useful anti-arthritic activity in Complete Freunds Adjuvant model.
Keywords: Anti-rheumatoid activity, Boerhaavia diffusa, Freunds Adjuant, Plethysmometer.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of multiple joints leading to the destruction of joint cartilage. Although the etiology underlying RA remains unknown, it is clear that inflammatory cytokine circuits are established in the synovial cells lining the joint in genetically susceptible individuals. The disease is often progressive and results in pain, stiffness and swelling of joints. In late stage deformity and ankylosis develop [1]. Symptoms include inflammation of joints, swelling, difficulty in moving and pain. Involvement of small joints of hands,wrists and the feet. The elbows, shoulders, hips, knees, and ankles may also be affected. Other symptoms include, loss of appetite, fever, loss of energy (weakness), anemia, myalgia and joint stiffness typically is worse in the morning and usually lasts at least 1 hour before maximal improvement is seen for the day. Chronic joint deformities commonly involves subluxations of the wrists, metocarpophalangeal (MCP) and proximal inter phalangeal (PIP) joints. Extra articular involvement may include subcutaneous nodules, vasculitis, pleural effusion, pulmonary fibrosis, ocular manifestations, pericarditis, cardiac conduction abnormalities and bone marrow suppression. Stiffness and myalgias may precede development of synovitis [2]

Boerhaavia diffusa (*Nyctaginaceae*) commonly known as Raktapunarnava, Shothaghni, Kathillaka, Kshudra, Varshabhu, Raktapushpa, Varshaketu, Shilatika [3, 4] is an herbaceous plant species growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds. The plant was named in honor of Hermann Boerhaave, a famous Dutch physician of the 18th century [5].

MATERIAL AND METHODS

Plant Material

The dried roots of *Boerhaavia diffusa* were collected from the local area of Jabalpur and authenticated by Prof. (Mrs) Karuna S. Verma, Senior Botanist, Department of Post Graduate Studies & Research in Biological Sciences, Rani Durgawati Vishwavidyalaya, Jabalpur.

Preparation of Crude Extract

The dried roots were coarsely powdered with the help of a hand-grinding mill and the powdered was passed through sieve no. 40, 100 gm of dried powder was extracted in petroleum ether. The extract was filtered using a Whatman filter paper no. 4 and concentrated at 40°C, dried extract was refrigerated at 4°C until use.

Animals

Wister albino rats of either sex and of approximately same age, weighing about 150-200 g were used for the study. They were housed in metal cages and fed with standard diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethical Committee.

Acute Toxicity Studies

The acute toxicity study of extract was performed using OECD guidelines. The animals were

fasted overnight prior to the experiment and fixed dose was adopted for toxicity studies (OECD Guideline No. 423). The extracts were administered in doses of 500 and 1000 mg/kg p.o.

Freund's adjuvant Induced Arthritis

The method according to Pearson and Wood [6] has been adopted for evaluation of anti-arthritic property. Freund's adjuvant induced adjuvant model was used to assess the anti-arthritic activity in albino rats. Animals were randomly divided into four groups of six animals each (n=6). First group received 1ml of normal saline, second group received indomethacin (100 mg/kg), third and fourth group received root extract of *B. diffusa* 500 mg/kg and 1000mg/kg respectively. Arthritis was induced by injecting a 0.1 ml suspension of killed Mycobacterium tuberculosis homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0 day), 30 minutes before adjuvant injection and continued till 21st day. Paw volume was measured on 4th, 8th, 14th, and 21st day by using Plethismometer.

The percentage inhibition of paw volume of injected paw over vehicle control at day 4th, 8, 14 and 21 was evaluated by using following formula,

$$i=[1 - (\Delta V_{Treated} / \Delta V_{Untreated})] \times 100$$

Where,

i = percent inhibition of paw edema,

$\Delta V_{Treated}$ = Mean change in paw volume rat,

$\Delta V_{Untreated}$ = Mean change in paw volume of untreated rat.

Statistical Analysis

The experimental results are represented as Mean ± SEM. The data statistical evaluated by ANOVA followed by Dunnet's test [7].

RESULTS AND DISCUSSION

Complete Freund's adjuvant induced paw edema

CFA induced a significant paw edema on 4th day in control group animal, which persisted till 21st day. However, the treatment with 1000 mg/kg root extract of *B. diffusa* was more significant in reducing the paw swelling gradually till 21st as compared to control treated animals. 500 mg/kg extract treatment was less significant as compared to control. Whereas, Indomethacin showed significant decrease in paw swelling on 21st day as compared to control group (Fig.4.1). On 21st day % inhibition of paw edema exhibited by 500 mg/kg extract and 1000 mg/kg extract were 32.31 %, and 62.88 %, respectively. While Indomethacin treated animal showed maximum percentage of inhibition of paw edema 77.07% on 21st day (Fig.1).

Table 1: Effects of petroleum ether extract of roots of *B. diffusa* in arthritis induced rat paw edema

Groups	Means Change in paw edema (Mean ± SEM)				% inhibition of paw edema on 21st day
	4th day	8th day	14th day	21st day	
Control	4.35±0.04	4.38±0.06	4.45±0.05	4.60±0.04	0
Standard p.o.	3.81±0.03*	3.05±0.02**	1.98±0.03**	1.05±0.02**	77.07
Extract (500 mg/kg) p.o.	4.15±0.04	3.91±0.03	3.45±0.02	3.10±0.03*	32.31
Extract (1000 mg/kg) p.o.	4.03±0.03	3.33±0.02	2.35±0.02	1.70±0.03**	62.88

Data represent in mean ± SEM (n=6) analysed by one way ANOVA followed by Dunnett's post hoc test. *p<0.05 significant, **p<0.01 most significant.

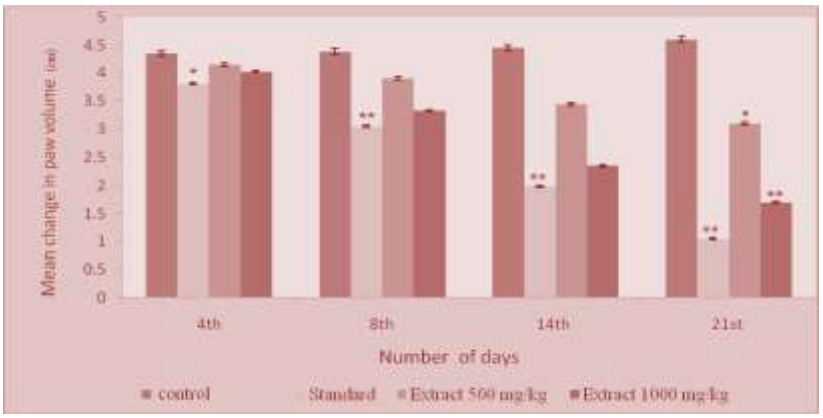

Fig.1: Mean changes in adjuvant induced arthritis

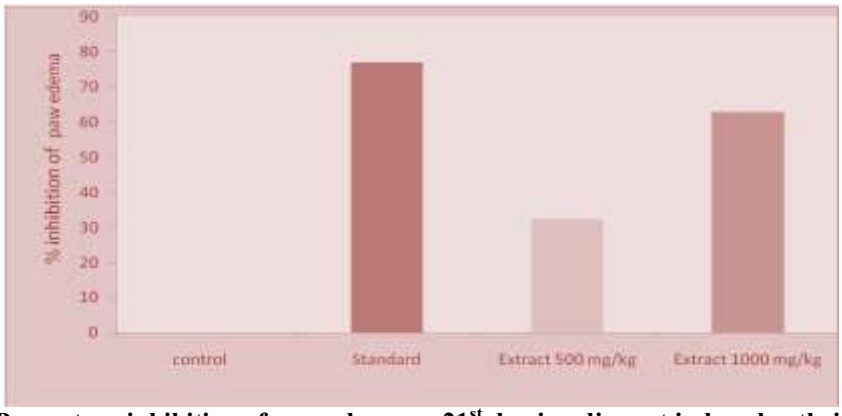

Fig.2: Percentage inhibition of paw edema on 21st day in adjuvant induced arthritic rats

CFA induced arthritis in rats is probably the best and most widely used model since it has a close similarity to human rheumatoid disease. The determination of magnitude of swelling of the injected paw is the most objective measurement that can be made to assess the antiarthritic activity. The change in the paw volume in the 21st days after the induction of CFA into the paw was illustrated. In the antiarthritc groups the injected paw showed an immediate acute inflammatory response reaching a maximam on the 4th day and persisted at up to 21st days.

In adjuvant-induced arthritic model rats developed a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint destruction and remolding which have close similar to human rheumatoid disease [8,9].

The result of present study has shown that 1000 mg petroleum ether extract of roots of *B. diffusa* shown 81.58 % response as comparable to standard drug (Indomethcin) and 500 mg petroleum ether extract of roots of *B. diffusa* shown 41.92 % shown as comparable to standard.

CONCLUSION

Thus, this study concludes that extract of roots of *B. diffusa* has significantly posses' anti-arthritis property in rats. Further study is involving the purification of the chemical of *B.diffusa* roots and investigation on the biochemical pathways may result in the development of a potent anti-arthritic agent with a low toxicity and higher therapeutic index.

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