



## Evaluation of anti-inflammatory effect of aqueous extract of *Boerhaavia diffusa* leaves in rats

A. Sudhamadhuri<sup>1</sup>, Vishal Kalasker<sup>2</sup>

Navodaya Medical College, Raichur, Karnataka. 1- Assistant Professor of Pharmacology, 2- Associate Professor of Biochemistry

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#### Corresponding Author:

Dr. A Sudhamadhuri, Assistant Professor, Navodaya Medical College, Mantralayam Road, Raichur.

E-mail: [allellisudhamadhuri@gmail.com](mailto:allellisudhamadhuri@gmail.com)

#### Abstract:

**Aim:** The aim of the study was to evaluate anti inflammatory activity of aqueous extract of *Boerhaavia diffusa* leaves.

**Material & Methods:** The anti inflammatory activity was evaluated by carrageenan induced rat paw edema to determine the activity on acute inflammation and cotton pellet induced granuloma to determine activity on sub acute inflammation. Rats were divided into 4 groups, 6 rats in each group. Group I (control) received 0.1ml of 4% gum acacia, Group II & III (test) received *Boerhaavia diffusa* extract at doses of 200mg/kg and 400mg/kg bodyweight, Group IV (standard) received Diclofenac 10mg/kg bodyweight, all the above drugs were administered one hour prior to sub plantar injection of 0.1ml of 1% carrageenan. Anti inflammatory activity was estimated by measuring paw volume before and 1 hr, 2 hr, 3 hr and 4hr after carrageenan administration and percentage inhibition was calculated. To determine activity on sub acute inflammation, Cotton pellet granuloma model was used. The animals were divided and treated same as in acute inflammation model for seven consecutive days. On eighth day the implanted cotton pellets were removed, dried and weighed. The percentage inhibition of granuloma formation was calculated. **Results:** *Boerhaavia diffusa* aqueous leaves extract treated groups with 200mg/kg and 400mg/kg bodyweight exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan-induced rat paw edema) and sub acute inflammation (cotton pellet induced granuloma). **Conclusion:** *Boerhaavia diffusa* extract showed significant anti inflammatory action in acute & sub acute experimental models and the activity was dose dependent.

**Key words:** Anti inflammatory activity, *Boerhaavia diffusa*, Carrageenan, Diclofenac

#### Introduction

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an

inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. Many drugs including steroidal

and non steroidal anti-inflammatory drugs are employed to control the inflammatory reaction and pain associated with it. Nonsteroidal anti-inflammatory drugs are preferred and commonly used in clinical practice. Currently available NSAIDs are associated with unwanted side effects and have their own limitations. About 34-46% of the users of NSAIDs usually sustain some gastrointestinal damage due to inhibition of the protective cyclooxygenase enzyme in gastric mucosa [2]. For this reason there is a need for anti-inflammatory drugs having less adverse effects. In recent times more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

Boerhaavia diffusa commonly known as punarnava, pigweed and hogweed in English belongs to the family nyctaginacea. A perennial diffuse herb with stout root stock and many proclimbent branches [3]. It is of two kinds, one with white flowers called "shwethapurna" and the other with red flowers called "ractapurna", the former is used in medicine. In Tibbi literature a third variety with blue flowers has also been described [4]. Pharmacological studies have demonstrated that punarnava exhibits a wide range of properties – anticonvulsant, antibacterial, analgesic and anti-inflammatory [5-7]. The major constituents of this plant are retinoid boeravinone (A-J) and two alkaloids punarnavine-1 and punarnavine-2 which belong to the group of quinolizidine [8,9]. The studies evaluating the anti-inflammatory activity of boerhaavia diffusa leaves are limited. The main purpose of the study is to evaluate anti inflammatory activity of boerhaavia diffusa leaves.

## Material & Methods

### Plant material:

Boerhaavia diffusa leaves were obtained from Osmania university campus, Hyderabad, Andhrapradesh, where it grows in plenty. Identification and Authentication were done.

### Preparation of Extract:

The shade dried leaves of Boerhaavia diffusa were made into a fine powder and passed through a sieve to make it a further fine powder. 40gm of powder was packed in soxlet apparatus, 10gm of active content was extracted with distilled water. The yield of the aqueous extract was 25% (w/v).

### Chemicals:

Diclofenac, carrageenan (sigma) and all other chemicals were of analytical grade.

### Animal:

Adult albino rats of either sex weight between 150-200gm were used in this study. Animals were

obtained from animal house of Gandhi Medical College, Secunderabad. The animals were stabilized for 1 week and maintained under standard conditions at temperature of  $25\pm 1^{\circ}\text{C}$ ,  $60\pm 5\%$  relative humidity and 12 hrs dark light cycles. They have been given a standard and pellet diet and water ad libitum.

**Ethics:** Experiments were conducted with prior permission taken from Institutional Animal Ethics Committee (IAEC) CPCSEA Regd. No. 428/1/C/ (20-6-2001).

Experimental procedure (anti-inflammatory activity):

The rats were randomly allocated into 4 groups (n=6).

Group I served as control received the vehicle only (0.1ml 4% gum acacia suspension).

Group II and Group III served as test received Boerhaavia diffusa extract at doses of 200 mg/kg bodyweight respectively. Group IV served as standard received diclofenac 10 mg/kg bodyweight. All drugs were administered orally.

### Carrageen an-induced rat paw Edema (acute inflammatory model):

The anti-inflammatory effect of Boerhaavia diffusa was evaluated Using Carrageenan induced paw edema in rats [10]. The animals pretreated with extract of Boerhaavia diffusa leaves (200mg/kg and 400mg/kg) and diclofenac sodium (10mg/kg) one hour before were injected with 0.1ml 1% carrageenan solution into subplantar region of the right hind paw. The rat paw volume up to the ankle joint was measured at 0 hr (30 min before carrageenan injection), 1hr, 2hr, 3hr, and 4hr after the injection of carrageenan using plethysmometer. Increase in the paw edema volume was considered as the difference between initial at 0 hr and of 1hr, 2hr, 3hr, or 4hr. Percent inhibition of paw volume between treated and control groups were calculated as follows.

$$\text{Percent inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where  $V_c$  and  $V_t$  represent the mean increase in paw volume in control and treated groups respectively.

Cotton pellet-induced granuloma in rat (sub acute inflammatory model):

The method is based on measuring the amount of granulation tissue formed around at implanted foreign body like cotton pellet. The animals were anesthetized under light ether. Sterile pre-weighed cotton pellets were implanted in axilla

region of each rat through a single needle incision [11].

All the animals received either extract or diclofenac sodium or vehicle orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion. On the eighth day the animals were anesthetized and cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 hr and dried at 60°C to constant weight. The increment in the dry weight of the pellet was regarded as measure of granuloma formation.

The percentage inhibition was calculated by using formula.

$$\text{Percentage inhibition} = \frac{W_c - W_t}{W_c} \times 100$$

Where  $W_t$  is granulation weight in treated groups and  $W_c$  is granulation weight in control groups.

**Statistics:** The data obtained in the studies were subjected to one way analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnett's test.  $P$  Value < 0.05 was considered to be significant. All the values we expressed as mean ± SD.

### Results:

Anti-inflammatory activity of aqueous extract of Boerhaavia diffusa against carrageenan induced paw edema is shown in table.1

In carrageenan induced rat paw edema test, the two doses of plant extract (200mg/kg and 400mg/kg) showed statistically significant ( $P < 0.001$ ) inhibitory effect on "mean increase in paw volume" at all the time intervals (1hr, 2hr, 3hr and 4hr). After 4hrs of carrageenan administration Boerhaavia extract exhibited maximum % inhibition of paw volume. The extract at test doses of 200mg/kg and 400mg/kg bodyweight reduced the edema induced by carrageenan by 45.56% & 51.97% respectively. The standard drug showed 69.70% of inhibition as compared to control group.

Anti-inflammatory activity of aqueous extract of Boerhaavia diffusa on cotton pellet induced granuloma formation is shown in table.2

In cotton pellet induced granuloma test, the two doses of plant extract (200mg/kg and 400mg/kg) showed statistically significant ( $P < 0.001$ ) reduction in granuloma formation. The rat exhibited 24.45% and 31.03% inhibition of granuloma formation at doses of 200mg/kg and 400mg/kg bodyweight

respectively. The standard drug diclofenac showed 45.45% inhibition of granuloma formation when compared to control.

### Discussion

Carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators [12]. In the first phase (during the first 2hrs after carrageenan injection) chemical mediators such as histamine and serotonin play important role, while in second phase (3-4hrs after carrageenan injection) kinin and prostaglandins are involved. Our results revealed that administration of Boerhaavia diffusa extract inhibit edema starting from the first hour and all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

Cotton pellet granuloma model was used to evaluate the anti-inflammatory activity of Boerhaavia diffusa extract in sub acute inflammation. Three phases of the inflammatory response to a subcutaneously implanted cotton pellet in rats have been described. A transudative phase that occurs during first 3hrs, an exudative phase occurring between 3-72 hrs after implanting the pellet and proliferative phase measured as the increase in dry weight of granuloma that occurs between 3 and 6 days after implantation [13]. The suppression of proliferative phase of sub acute inflammation could result in decrease in weight of granuloma formation [14]. The dry weight of cotton pellet granuloma was significantly reduced ( $p < 0.001$ ) by 200mg/kg and 400mg/kg doses of Boerhaavia diffusa extract however antiproliferative effect of Boerhaavia diffusa extract was lesser than that of standard drug.

### Conclusion

Boerhaavia diffusa leaves extract showed significant anti inflammatory action in acute & sub acute experimental models and the activity was dose dependent.

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Table 1: Anti-inflammatory effect of aqueous extract of Boerhaavia diffusa in carrageenan induced paw edema

| Treatments                           | Increase in paw volume in ml<br>(% inhibition of paw edema) |                         |                         |                          |
|--------------------------------------|---|-------------------------|-------------------------|--------------------------|
|                                      | 1hr   | 2hr                     | 3hr                     | 4hr                      |
| Control                              | 0.305±0.021   | 0.380±0.017             | 0.461±0.014             | 0.406±0.025              |
| Boerhaavia diffusa extract(200mg/kg) | 0.208±0.019*<br>(31.80)                                     | 0.228±0.017*<br>(40)    | 0.255±0.015*<br>(44.68) | 0.2217±0.014*<br>(45.56) |
| Boerhaavia diffusa extract(400mg/kg) | 0.221±0.014*<br>(27.54)                                     | 0.225±0.016*<br>(40.78) | 0.241±0.014*<br>(47.72) | 0.195±0.016*<br>(51.97)  |
| Diclofenac (10mg/kg)                 | 0.155±0.010*<br>(49.18)                                     | 0.191±0.014*<br>(49.73) | 0.176±0.013*<br>(61.82) | 0.123±0.013*<br>(69.70)  |

Results expressed as mean ± SD. P<0.001\* Compared with control  
Figures in parenthesis indicate Percentage inhibition

Table 2: Anti-inflammatory effect of aqueous extract of Boerhaavia diffusa on cotton pellet induced granuloma formation in rats.

| Treatments         | Dose     | Weight of dry cotton pellet granuloma (mg) | % inhibition of granuloma formation |
|--------------------|----------|--|-------------------------------------|
| Control            | -        | 53.17±4.16                                 | -                                   |
| Boerhaavia diffusa | 200mg/kg | 40.17±2.13*                                | 24.45                               |
| Boerhaavia diffusa | 400mg/kg | 36.67±1.21*                                | 31.03                               |
| Diclofenac         | 10mg/kg  | 29.00±2.09*                                | 45.45                               |

Results expressed as mean ±SD .P<0.001\* compared with control

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