



*RESEARCH ARTICLE*

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**EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF LEAVES OF  
HIPTAGE BENGHALENSIS (L) KURZZ USING VARIOUS  
EXPERIMENTAL ANIMAL MODELS.**

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**Abstract:**

**Objectives:**

To evaluate the anti-asthmatic activity *Hiptage benghalensis (L) Kurz* leaves on early and late phase of asthma in various experimental animal models.

**Methods:**

An Anti-asthmatic activity of *Hiptage benghalensis (L) Kurz* leaves was studied on Total leukocytes Counts (TLC) and Differential Leukocyte Counts (DLC) using bronchoalveolar lavaged (BAL) fluid of guinea pigs sensitized by egg albumin and PAF acether. Study was also conducted for same in ketotifen treated group which was taken as standard for comparison of anti-asthmatic activity.

**Results:**

Treatment with *Hiptage benghalensis* for 15 days resulted in significant decrease in Total Leukocytes Count (TLC) as well as Differential Leukocytes Count (DLC) in BAL fluid of guinea pigs sensitized by egg albumin and PAF acether. It was comparable to standard drug ketotifen Fumarate.

**Conclusion:**

*Hiptage benghalensis (L) Kurz* inhibits the migration of leukocytes on exposure to antigens in early stage (Egg albumin sensitized, by I.V. route) as well as in late phase (PAF acether sensitized, by aerosol route) of asthma, thus conforming its anti-allergic activity in actively as well as passively sensitized condition. So it was concluded that *Hiptage benghalensis (L) Kurz* have anti-asthmatic activity on both early as well as late stage of asthma.

**Keywords:** TLC count, Egg albumin, BAL Fluid, PAF acether

## INTRODUCTION

According to WORLD HEALTH ORGANIZATION asthma is a disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person<sup>1</sup>. This condition is characterized by a variable degree of airflow obstruction secondary to bronchial smooth muscle constriction, airway wall inflammation and edema, epithelial desquamation, mucous hyper-secretion, bronchial hyper-responsiveness, and in some but not all, airway remodeling<sup>2,3</sup>.

India alone has an estimated 15-20 millions asthmatics, where as in united states the population is around 17 million, and this is 75% increase in the last 20 years. This means that about 1 out of every 20 adults and close to 1 out of 13 children today have asthma<sup>4,5</sup>.

The currently used drugs for the treatment of this disease in modern medicine are so far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use<sup>5</sup>.

Hence, ayurveda an ancient system of Indian medicine has recommended a number of drugs from indigenous plant sources for the treatment of asthma and allergic disorders. *Hiptage benghalensis* (L.) Kurz belong to the family Malpighiaceae and also found almost throughout India and North of South America<sup>6</sup>. The bark leaves and flower of *H. benghalensis* are aromatic. They are useful in conditions of burning sensation, wounds, ulcers, inflammations, leprosy, scabies, cough, rheumatism, and asthma<sup>7,8</sup>.

The phytochemical analysis of the leaf extract revealed the presence of steroid, tannin, phenol, coumarin, flavonoid, saponin, terpene and sugar<sup>9</sup>. While alkaloid, anthraquinone and xanthoprotein compound were not found in all crude extracts. Aqueous extracts showed more potent anthelmintic Compound-2(3, 4-dihydroxyphenyl)-3(4, 6dihydroxy-3 -methoxytetrahydro-2H-pyran-2carbaldehyde)-5-hydroxy, 7methoxy-4H-chromen-4one<sup>9</sup>. The plant posses anti-inflammatory and anthelmintic activity reportedly.

Airway wall inflammation is characterized by an influx of eosinophils, neutrophils, lymphocytes, and degranulated mast cells<sup>2, 10</sup>. When egg albumin is administered to guinea pigs it acts as an antigen and activates the body's immune system and produces various pathological changes in the bronchi and bronchioles immediately.

Platelet activating factor (PAF) is among the most potent chemotactic factor for Eosinophils. Furthermore, similar to antigen inhalation, inhaled PAF induces an acute and late-phase response in dual responder animals and in addition, has been shown to increase bronchial hyper-reactivity in different species including man<sup>11, 12, 13, 14</sup>.

Most allergic and non-allergic asthmatics, including those with mild asthma, have bronchial eosinophilia and there is a significant association between Eosinophils activation and asthma severity as well as bronchial hyper responsiveness. Therefore, differential leukocytes count was carried out for *Hiptage benghalensis*.

## MATERIALS AND METHODS

### Collection and authentication of plant

Fresh leaves of *Hiptage benghalensis* (L) Kurz were collected and authenticated at

Department of Medicinal and Aromatic Plant Association of India (MAPAI), Lambhvel, Anand. The leaves were dried in shade and were ground to get a coarse powder.

### Preparation of plant extract

The coarse powder (500 g) of the dried leaves was exhaustively extracted using 95% ethanol (2,000 ml) in a Soxhlet extractor at a temperature of 60–70°C (yield 3% w/w). Cold aqueous extract of *Hiptage benghalensis* (L) Kurz was prepared by extracting 1 part of leaves powder with 10 parts of water for 7 days without heating (yield 9.3% w/w). The extracts were concentrated under reduced pressure to yield a syrupy mass and stored in air tight container and used throughout the project.

### Selection of animals

Hartley strain guinea pigs, weighing 400–700 g were selected for evaluation of anti-asthmatic activity. All animals were housed at ambient temperature (22 ± 1°C), relative humidity (55 ± 5%) and 12/12 h light/dark cycle. Rats had access to standard pellet diet and water given ad libitum. Guinea pigs had access to standard pellet diet, tomatoes, grass and carrots. The protocol (CPCSEA/IAEC/ARCP/2010-11/02) of the

study was approved by the Institutional Animal Ethics Committee (IAEC) as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

### **Studies on Bronchoalveolar lavage (BAL) fluid in Egg albumin sensitized guinea pigs**

Hartley strain guinea pigs of either sex weighing 400- 600 g were selected randomly and divided into five groups. Each group contained six animals. The suspension of drug was administered orally. The different groups of guinea pigs were treated as follows.

Group 1	Distilled water
Groups 2	Egg albumin
Groups 3	Ethanollic extract of <i>Hiptage benghalensis</i> (100 mg/kg,p.o)
Groups 3	Ethanollic extract of <i>Hiptage benghalensis</i> (200 mg/kg,p.o)
Groups 4	Ethanollic extract of <i>Hiptage benghalensis</i> (400 mg/kg,p.o)
Groups 5	Ketotifen Fumarate (1mg/kg, p.o.)

The guinea pigs of all groups except group 1 were sensitized with egg albumin (1 ml, 10 % w/v).The animals of group 3, 4, 5 were dosed once daily for 15 days with the suspension of the drug under study. Two hrs after the last dose of the drug administration on 15<sup>th</sup> day all the animals except animals of group 1 were challenged with egg albumin ( 0.5 ml , 2 % w/v) through sephenous vein. After 3 hrs of the challenge of egg albumin or just prior to death of animal, which ever was earlier, the tracheobronchial tree was lavaged with 10 ml of saline solution and the fluid so collected was centrifuged at 2000 rpm for 5 min and the pellets was resuspended in 0.5 ml saline.0.2 ml of Geisma Stain in buffer saline pH 6.5 was added to it. After 5 min each type of leucocytes in 0.5 ml fluid was determined by observing under the microscope using 45 X magnification. The TLC and DLC obtained in test group were compared with the TLC and DLC obtained in unsensitized guinea pigs.

### **Studies on Bronchoalveolar lavage (BAL) fluid in PAF acether sensitized guinea pigs**

Hartley strain guinea pigs of either sex weighing 400- 600 gms will be selected randomly and divided in to 6 groups. Each

group will contain 6 animals. The suspension of drug will be administered orally. The following schedule of treatment was being followed.

Group 1	Distilled water
Groups 2	PAF acether (10 µg/ml aerosol for 5 min.)
Groups 3	Ethanollic extract of <i>Hiptage benghalensis</i> (100 mg/kg,p.o)
Groups 4	Ethanollic extract of <i>Hiptage benghalensis</i> (200 mg/kg,p.o)
Groups 5	Ethanollic extract of <i>Hiptage benghalensis</i> (400 mg/kg,p.o)
Groups 6	Ketotifen Fumarate (1mg/kg, p.o.)

The animal of group 3, 4, 5 will be dosed once daily for 15 days with the suspension of the drugs. One hrs after the last dose of the drug administration on 15th days all the animal except group 1 animal will be challenged with PAF acether aerosol (10µg/ml) through Nebuliser for 5 minute. After 48 hrs of the challenge of PAF acether or just prior to death of animal, which ever will be earlier, the tracheobronchial tree will be lavaged with 10 ml of saline solution and the fluid so collected will be centrifuged at 2000 rpm for 5 mins and the pellets will be

resuspended in 0.5 ml saline.0.2 ml of Geisma Stain in buffer saline pH 6.5 will be added to it. After 5 mins each type of Eosinophill in 0.5 ml fluid will be determined under the microscope using 45 X magnification. The results obtained will be compared with unsensitized guinea pigs.

### Statistical analysis

Results were analyzed by One way Analysis of Variance (Tukey's test) (n=6), which were expressed as mean ± S.E.M. at the probability level of 95% and P< 0.005 was considered as significant where as p< 0.001 considered as highly significant.

## RESULTS AND DISCUSSION

### The Effect of *Hiptage benghalensis* extract on Bronchoalveolar lavage (BAL) fluid in Egg albumin sensitized guinea pigs

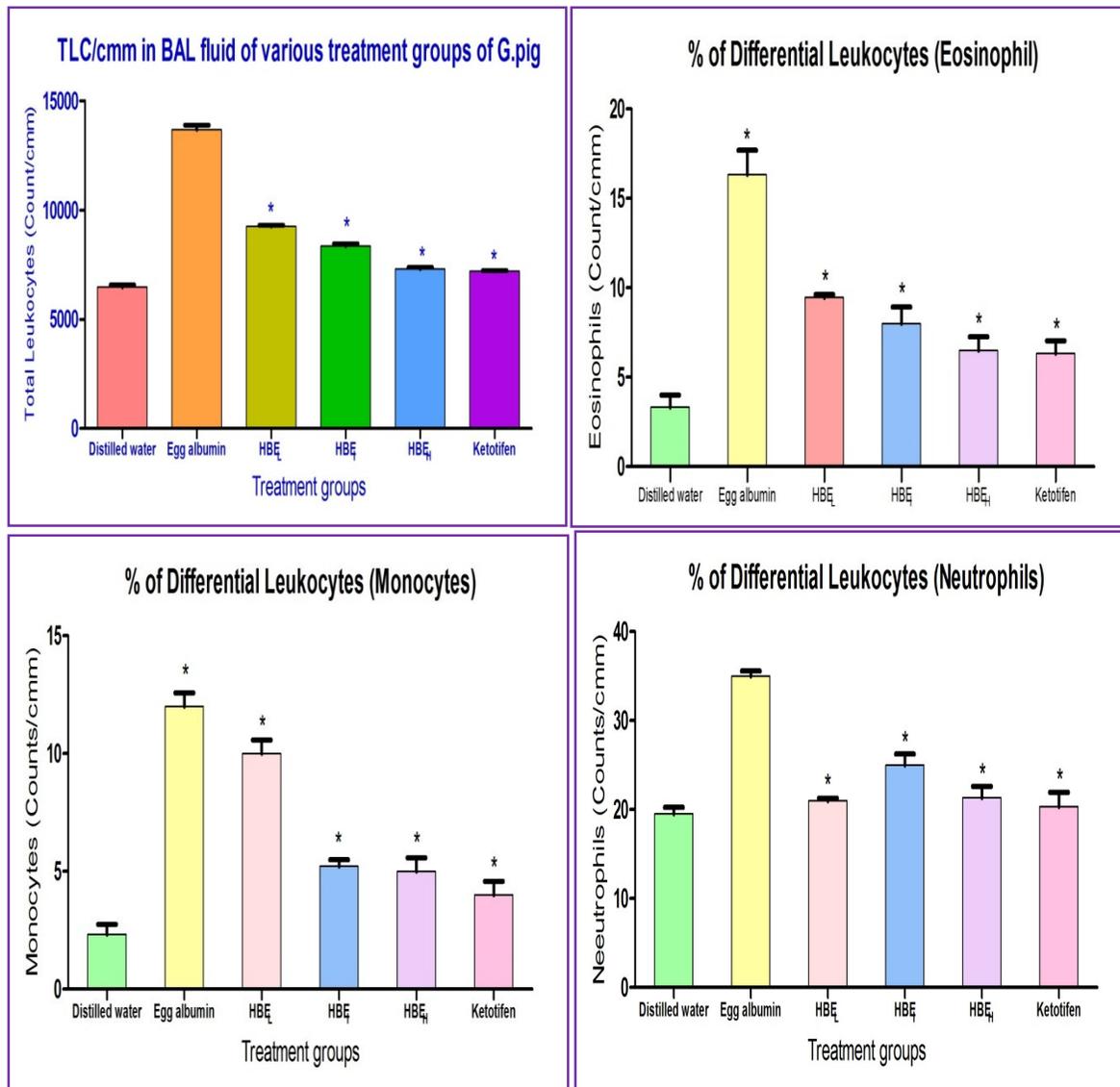
BAL fluid obtained from Egg albumin (1 mg/ml, p.o.) sensitized guinea pigs pre-treated with *Hiptage benghalensis*(100 mg/kg, p.o. 200 mg/kg, p.o. and 400 mg/kg, p.o) showed decrease in TLC as well as counts of various other leucocytes as compare to the counts observed in BAL fluid of egg albumin sensitized guinea pigs. Results of TLC and DLC in both *Hiptage*

*benghalensis* and Ketotifen Fumarate treated groups were found highly significant.

**The Effect of *Hiptage benghalensis* extract on Bronchoalveolar lavage (BAL) fluid in PAF acether sensitized guinea pigs**

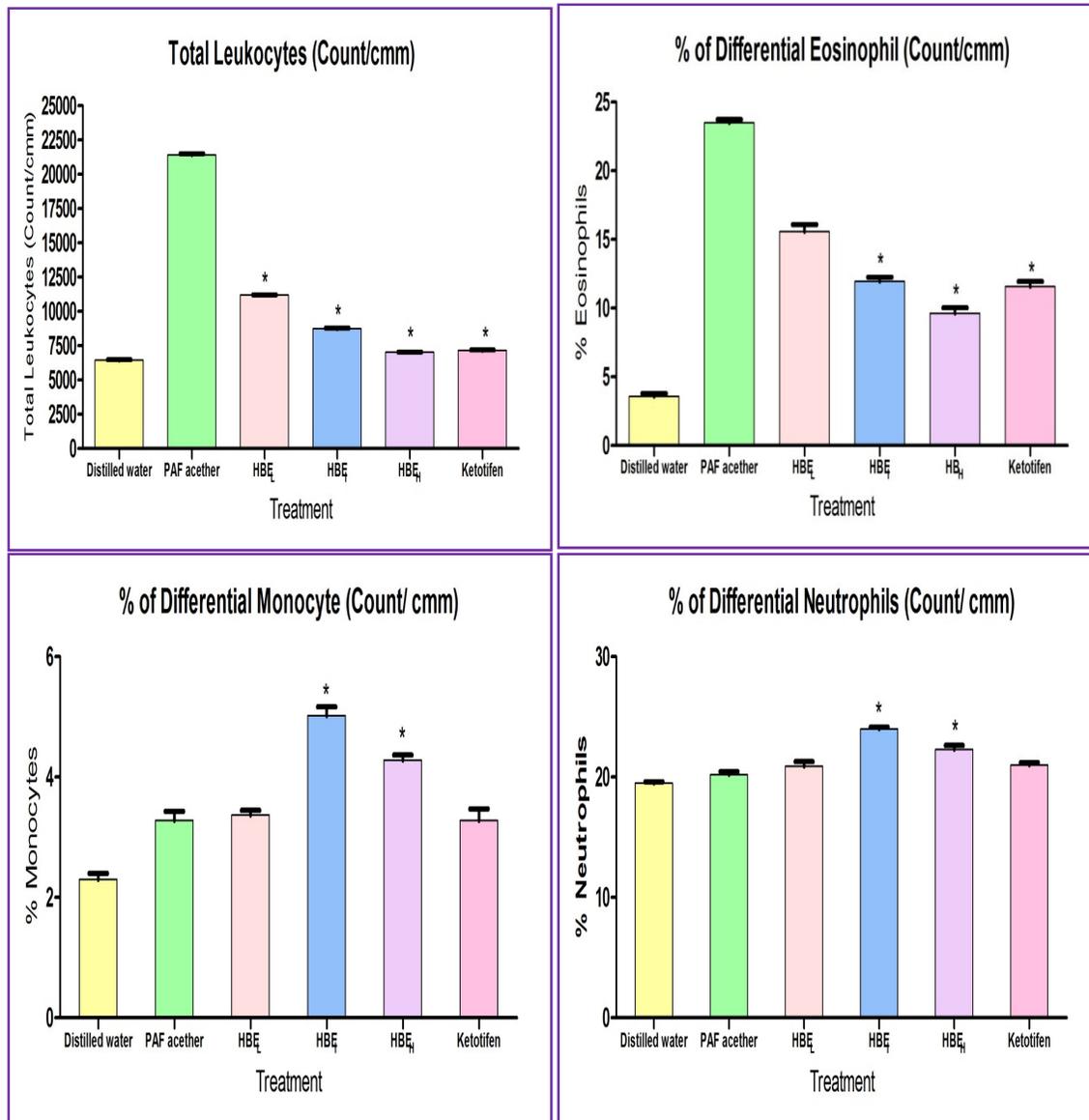
Differential leukocytes count was carried out after 48 hrs of exposure to PAF for *Hiptage benghalensis*. BAL fluid obtained

from guinea pigs pre-treated with *Hiptage benghalensis* (100 mg/kg, p.o. 200 mg/kg, p.o. and 400 mg/kg, p.o) showed decrease in TLC count compare to the counts observed in BAL fluid of PAF acether sensitized guinea pigs. Comparing TLC count in *Hiptage benghalensis* groups with Ketotifen Fumarate treated group, highly significant result were obtained.



Results are expressed as mean  $\pm$  S.E.M. values.\* $P < 0.001$ , highly significant from each group when compared in Tukey's test. (One way ANOVA test) (n=6).

Figure 1. Percentage of TLC, Eosinophils, Monocytes, Neutrophils in BAL Fluid of Various Treatment Groups of G.Pig. (Egg albumin model)



Results are expressed as mean  $\pm$  S.E.M. values.\* $P < 0.001$ , highly significant from each group when compared in Tukey's test. (One way ANOVA test) (n=6).

Figure 2. Percentage of TLC, Eosinophils, Monocytes, Neutrophils in BAL Fluid of Various Treatment Groups of G.Pig (PAF model).

## DISCUSSION

The effect of ethanolic extract of *Hiptage benghalensis* on WBC was evaluated on BAL fluid of egg albumin sensitized guinea pigs. Increased in number of TLC as well as eosinophils, monocytes and neutrophils were observed in egg albumin treated groups. Increased in the TLC and DLC were significantly decreased by *Hiptage benghalensis* in sensitized groups at low dose, intermediate as well as at high dose which is comparable with sensitized group treated with Ketotifen Fumarate. Similar results of decreased in TLC and DLC has also been reported for *Curculigo Orchioides* 3.

The effect of ethanolic extract of *Hiptage benghalensis* on WBC was evaluated on BAL fluid of PAF acether sensitized guinea pigs after 48 hrs. of sensitization. Increased in number of TLC were observed in PAF acether treated groups. Increased in the TLC was significantly decreased by *Hiptage benghalensis* in sensitized groups at low dose, intermediate as well as at high dose which is comparable with sensitized group treated with Ketotifen Fumarate. Similar results of decreased in TLC after 48 hrs of exposure to PAF has been reported for Ketotifen and AH21-132(PAF antagonist)<sup>11</sup>.

*Hiptage benghalensis* (L) Kurzz has inhibited the migration of leukocytes in egg albumin sensitized BAL fluid model (Early stage) as well as in PAF acether sensitized BAL fluid model (Late phase, after 48 hr. of aerosol) on exposure to antigens, thus confirming its anti-allergic activity. These effects are important evidence for traditional use of *Hiptage benghalensis* plant as an anti-asthmatic.

## CONCLUSION

From present study it is concluded that ethanolic extract of *Hiptage benghalensis* possess highly significant anti-asthmatic activity which is due to inhibited migration of leukocytes in early as well as in late phase (after 48 hr.) on exposure to antigens, thus confirming its anti-allergic activity. These effects are important evidence for traditional use of *Hiptage benghalensis* plant as an anti-asthmatic.

However, due to presence of multiple chemical constituents in plants it is important to isolate chemical constituents from the alcoholic extract and carry out further studies to establish the actual active constituent and probable mechanism of action by which *Hiptage benghalensis* (L) Kurzz exerts its anti-asthmatic activity.

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