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**Preclinical Evaluation and Antiasthmatic Activity of *Euphorbia hirta* Linn.**

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**ABSTRACT**

The present study designed to evaluate the Antiasthmatic activity of extract of *Euphorbia hirta* on in vitro and in vivo animal model. Ethanolic extract of *Euphorbia hirta* was evaluated using in vitro goat tracheal chain preparation model and Clonidine induced catalepsy in mice model. The extract was subjected to phytochemical screening and found to contain essential oils, volatile oils, tannins, phenolics, flavonoids, carbohydrates and resinous matters. Histamine induced contraction in isolated goat tracheal chain shows that Ethanolic extract of *Euphorbia hirta* inhibited the contractile effect of histamine. Antiasthmatic Activity was increased with the increasing concentration of extract. Presence of Phytochemical like flavonoids, Glycosides, Saponine might contribute to the observed Antiasthmatic activity.

**Keywords:** *Euphorbia hirta*; Antiasthmatic Activity; Clonidine induced catalepsy; goat tracheal chain preparations.

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**INTRODUCTION**

Asthma affects over 20 million individuals in the US and over 60 million individuals worldwide. It affects over 5-10% of the population in industrialized countries. It is a condition in which the bronchial tubes becomes swollen and clogged resulting in difficulty in breathing. Among several of the respiratory diseases affecting man, bronchial asthma is most common disabling syndrome. The present day medicinal management of asthma includes various techniques. Unfortunately, these techniques possess some side effect. Many plants are available in India are used in traditional medicine for the treatment of asthma. *Euphorbia hirta* belongs to the plant family *Euphorbiaceae* and genus *Euphorbia*. It is a slender-stemmed, annual hairy plant with many branches from the base to top, spreading up to 40 cm in height, reddish or purplish in color. It mainly contain Afzelin (I), Quercitrin (II), and Myricitrin (III), kaempferol, gallic acid, and Protocatechuic acid<sup>2</sup>. The present work is designed to find out the safe and effective remedies for asthma from natural source. From literature review of *Euphorbia hirta* plant it has been found that this plant having various activities<sup>3</sup> and plant is mentioned in Ayurveda. *Euphorbia hirta* is distributed throughout the hotter parts of India and Australia, often found in waste places along the road sides. In given work The plant extract were subjected to preliminary phytochemical screening for the detection of various plant constituents present<sup>4,10</sup>. It has been observed that ethanol extract contains chemical constituents like alkaloid, glycosides, Saponine, flavonoids respectively. (table-2) Histamine contracts the Tracheo-bronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is easier to handle and to prepare. It is also more sensitive than guinea pig tracheal chain. In present study the isolated goat tracheal chain preparation was used. There is right side shift of dose response Curve (DRC) of histamine in the presence of *Euphorbia hirta* Ethanolic extract in indicating Antiasthmatic action. The extract also significantly inhibited the Clonidine induce catalepsy. The inhibition of Clonidine induced catalepsy of *Euphorbia hirta* may be due to the potential to antagonize H1 receptor or inhibition of mast cell Degranulation induced by Clonidine.

## MATERIALS AND METHODS

### Collection of plant Material:

The fresh plant *Euphorbia hirta* was collected around the surroundings of main campus of The Sitabai Thite College Of Pharmacy, Shirur Dist;-Pune. Taxonomical Identification of the plant was confirmed by Mr. Acharya sir, head of dept of botany, C T. Bora College, Shirur, Dist: Pune.

### Drugs and reagents:

These included histamine diphosphat, sodium chloride, sodium hydroxide, petroleum ether, chlorpheniramine maleate, ethanol, methanol, Stop watch, Organ bath, Carbogen, Krebs solution and all other chemicals and reagents used in this study were of analytical grade.

### Preparation of plant material:

The fresh plant was harvested, rinsed with tap water and air dried under shade for 14 days and reduced to coarse powder using pestle and mortar and then grinded to fine powder using the Kenwood electric blender. The powder was stored in an airtight bottle until needed for use.

### Extraction method:

The resulted Powder Material Were extracted with petroleum ether by using Soxhlet apparatus of extraction. Greenish residue was obtained as extract. Successive extraction with ethanol gives slight red colored sticky and semisolid extract.

### Animal:

Isolated adult goat trachea tissue, Albino mice and albino rats of either sex weighing of 20-25g and 150-200g respectively, were used for studies. Isolated adult goat trachea tissue was obtained immediately after slaughter of the animal. Pieces of the trachea were collected in ice cold oxygenated Krebs solution. The albino mice were obtained from animal house of national chemical laboratory, Pune.

### Antiasthmatic activity:

#### Isolated goat trachea preparation<sup>1,16,17,6</sup>

Isolated adult goat trachea was obtained immediately after slaughter of the animal. Trachea was cut into small individual rings and tied together series to form a chain. Trachea was suspended in bath of Krebs solution and was continuously aerator at 37°c .DRC of histamine in plane Krebs solution and 80ug/ml *Euphorbia hirta* extract in Krebs solution was taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of drug extract.

#### Clonidine induced catalepsy in mice

Albino mice were divided in to five groups, each group contains five animals. Control group received saline (10 ml /kg) and other groups received single dose of extract (25, 50, 100mg/kg p .o. body weight) respectively. Chorpheniramine Maleat (10mg/kg i. p.) was used as standard. All the groups were received Clonidine (1mg/kg s.c) one hour after the drug administration and the duration of Catalepsy was measured at 15, 30, 60, 90, 120, 150, and 180min.

### Statistical analysis

The results of various studies were expressed as mean ± SEM and analyzed statistically using one-way ANOVA,followed by Dunnet's Multiple Comparison Test to find out the level of significance. P < 0.05 was considered statistically significant. The analysis was performed using Graph pad Prism software.

## RESULTS AND DISCUSSION

### Physicocemical and phytochemical screening

In the present study *Euphorbia hirta* linn was used to study antiasthmatic activity. Preliminary qualitative phytochemical screening of ethanolic extract of leaves of euphorbia hirta linn showed the presence of alkaloid, glycoside, tannin, Saponin, flavanoids [table no.2]. the physical constant /physicochemical parameters were also studied [table no.1]

**Table 1: Result of Physicochemical Parameters**

| S. No. | Parameters               | Inference |
|--------|--------------------------|-----------|
| 1      | Loss on drying           | 15%       |
| 2      | Total Ash value          | 14%       |
| 3      | Water soluble ash value  | 7%        |
| 4      | Acid insoluble ash value | 4%        |
| 5      | Sulphated ash value      | 13%       |

**Table 2: Preliminary phytochemical screening of the entire plant powder of *Euphorbia hirta* Linn.**

| S. No. | Test                           | Petroleum ether extract | Ethanol Extract |
|--------|--------------------------------|-------------------------|-----------------|
| 1      | Alkaloid                       | -                       | +               |
| 2      | Glycoside                      | -                       | +               |
| 3      | Saponine                       | -                       | +               |
| 4      | Flavonoid                      | -                       | +               |
| 5      | Tannins and phenolic compound. | -                       | +               |
| 6      | Fixed oil                      | -                       | +               |
| 7      | Carbohydrate                   | -                       | -               |
| 8      | Protein                        | -                       | -               |
| 9      | Steroids                       | +                       | -               |

**1) Isolated goat tracheal chain preparation:**

It is observed that *Euphorbia hirta* extract inhibit the contraction produced by histamine in these tissue preparation. Histamine (50ug/ml) was taken in different dose level and DRC was plotted. Study revealed that *euphorbia hirta* extract inhibit significant percentage decreased contraction at concentration 80ug /ml in goat tracheal chain preparation dose dependent response was seen [table no.3]

**Table 3: Isolated goat tracheal chain preparation result**

| Group | Dose of histamine (50ug/ml) | Control group<br>Contraction in mm.<br>(without extract) | Test group<br>Contraction in mm.<br>(with extract) |
|-------|-----------------------------|--|--|
| 1)    | 0.1                         | 2.233±0.1202   | 1.1±0.0577*  |
| 2)    | 0.2                         | 3.033±0.0333   | 1.667 ±0.1856*                                     |
| 3)    | 0.4                         | 5.033±0.0334   | 1.667±0.066*                                       |
| 4)    | 0.8                         | 7.467±0.0335   | 2.667±0.1856*                                      |
| 5)    | 1.6                         | 8.033±0.0332   | 3.33±0.1764*                                       |
| 6)    | 3.2                         | 9.069±0.0666   | 4.167±0.0333*                                      |

All values are represented as Mean ± SEM. P<0.05 as compared to control by t-test (unpaired)

**Fig. 1 Effect of *Euphorbia hirta* extract on histamine induced contraction of Isolated Goat Tracheal chain Preparation**

## 2) Clonidine induced catalepsy in mice

Clonidine(1mg/kg,s.c.) produced catalepsy in mice, which remained for 3 hours.the vehicle treated group has shown maximum duration of catalepsy at 180min after the administration of clonidine There was significant inhibition of clonidine in induced catalepsy in the animal pretreated with *Euphorbia hirta* and the duration of catalepsy found to be Chlorpheniramine maleate treated group significantly reversed the clonidine induced catalepsy in mice [table no.4]

**Table 4: Clonidine induced catalepsy in mice result**

| Group | Duration of catalepsy (sec.) at |              |              |               |              |              |               |
|-------|---------------------------------|--------------|--------------|---------------|--------------|--------------|---------------|
|       | 15min.                          | 30min.       | 60min.       | 90min.        | 120min.      | 150min.      | 180min.       |
| I     | 46.87±0.29                      | 52.53±0.90   | 50.77±0.38   | 87.17±0.61    | 85.43±0.97   | 88.87±0.29   | 110.8±1.70    |
| II    | 28.57±0.26***                   | 30.7±0.36*** | 32.2±0.55*** | 31.47±0.87*** | 34.8±1.73*** | 36.3±0.90*** | 51.9±1.222*** |
| III   | 44.6±1.73                       | 47.27±0.60   | 46.07±1.44   | 77.1±0.55     | 81.27±0.53   | 86.13±0.60   | 89.97±0.85    |
| IV    | 40.23±0.50                      | 41±0.36      | 46.1±0.56    | 67.57±1.16    | 69.83±0.31   | 72.17±0.57   | 81.87±1.78    |
| V     | 31.0±0.36**                     | 30.6±0.85**  | 32.77±0.93** | 33±0.41**     | 35.83±0.66** | 40.47±0.91** | 59.7±1.60**   |

Group -I = vehicle

Group-II = chlorpheniramine maleate (10mg/kg).

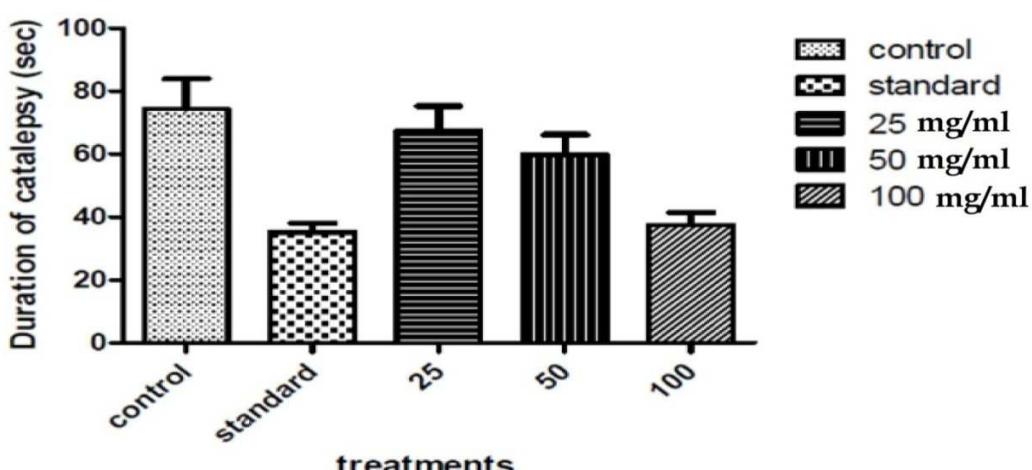
Group-III = *E. hirta* extract (25mg/kg).

Group-IV= *E. hirta* extract (50mg/kg).

Group-V =*E. hirta* extract (100mg/kg).

**Fig 2.Clonidine induced catalepsy in mice**

**Data 1**



### Statistical Analysis:

Each value represents mean ±SEM (n=3) (p<0.01)\*\* The duration of catalepsy was significantly reduced in test 100 ug/ml as compared to control group by ANOVA followed by Dunnett's test. Duration of catalepsy was also reduced significantly in standard group (p<0.001) \*\*\* as compared to control group.

### CONCLUSION

In conclusion the present study confirmed that ethanolic extract of *Euphorbia. hirta* exhibit significant dose dependent antiasthmatic activity in various *in-vitro* and *in-vivo* animal model and further support the traditional claim of plant in the treatment of asthma. Further studies are in fact underway to isolate and characterize the active principle responsible for the Antiasthmatic Activity. The plant show Antiasthmatic activity it might be because of presence of flavonide so this experiment will be helpful in future for scientific evaluation of constituents which show Antiasthmatic activity.

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