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EVALUATION OF *IN-VIVO*ANTI-ASTHMATIC ACTIVITY OF ETHANOLIC EXTRACT OF*Geniosporum prostratum (L) Beanth* IN GUINEAPIGS.

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ABSTRACT

In the present study, extracts of *Geniosporum prostratum (L) Beanth* was evaluated for preliminary phytochemical screening and anti-asthmatic activity using Histamine-induced bronchospasm in guinea pigs.ANOVA and Dunett's test were used for statistical analysis.The result of present investigation showed that the Ethanolic extract of *Geniosporum prostratumsignificantly* (*P*<0.001) decreased the bronchospasm induced by histamine as compared to control groups. *Geniosporum prostratum*ethanolic extract has significant bronco-dilatory activity against histamine induced bronchospasm.

Keywords: Anti-asthmatic, Antihistaminic, Histamine, *Geniosporum prostratum*

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways. It involves in complex interactions between many cells and inflammatory mediators that results in inflammation, obstruction (partially or completely reversible after treatment or resolves spontaneously), increased airway responsiveness (i.e. hyper-responsiveness) and episodic asthma symptoms .[1] Research indicates that airway hyper-responsiveness is important in the pathogenesis of asthma and that the level of airway hyper-responsiveness usually correlates with the clinical severity of asthma. [2] The available treatment options have major limitations owing to low efficacy, associated adverse events and compliance issues .[3] As a result, there is high prevalence of usage of complementary and alternative medicines for treatment of this disease. Ayurveda, an ancient system of Indian medicine, has recommended several drugs from indigenous plant sources for the treatment of bronchial asthma and allergic disorders. [4]

Geniosporum prostratum Linn (Lamiaceae) is a creeping, glabrous, succulent herb, rooting at nodes, distributed throughout India in all plain districts, ascending to an altitude of 1,320 m. The plant is reported to show sedative, antiepileptic, vasoconstrictor and anti-inflammatory activity.[5,6]. It has been reported that the plant contains tri-terpenoid saponins, β-sitosterols, glycosides, alkaloids, phenols and flavonoids.[7] The purpose of this investigation was to evaluate the effects of *Geniosporum prostratum*extracts in Bronco dilatory activity.

Material and Methods

Plant Material:

For study the plant Geniosporum prostratum (L) Benth was procured within the month of Jan. 2009, from Orakadam forest close to metropolis. The plant was known by professor. **P. Jayaraman Director, Plant Anatomy analysis (PARC)** UN agency documented the plant from accessible literature and herbarium is ready. The whole plant was dried initially under shade. It was preserved in a tightly closed container and powdered as per requirements.

Drugs and chemicals

Histaminedi-hydrochloride, Chlorpheniraminemaleate were purchased from Sigma (St. Louis, MO, USA). Histamine solution was freshly prepared in normal

saline (NaCl, 8.5 g/l). All the other chemicals were of analytical grade.

Preparations of Extracts:

Dry coarse powder of aerial part of *Geniosporum prostratum*(L) Benth was dried completely by hot air oven below 50°C and then packed into soxhlet apparatus and extracted with the ethanol until the extraction was completed. The extract was filtered when hot and the resultant extract was distilled in the vacuum under reduced pressure for removing of solvent completely and dried in a desiccator. Weighed the extract and calculated its percentage yield in terms of air dried powdered crude material.[8]The plant extracts were suspended in 1% DMSO in distilled water and administered orally. The control animals were given an equivalent volume of DMSO in normal saline as vehicle. The IV group received standard anti-asthmatic drugs.

Anti-Asthmatic Evaluation

Experimental animals

Antihistaminic study was conducted on guinea pigs (350-500 g) of either sex. They were group housed under standard conditions of temperature (22 ± 20C), relative humidity (60 ± 5%) and 12:12 light/dark cycle, where lights on at 0700 and off at 1900 h). They were divided in groups of six animals each. The saline fed group served as control and one group was treated with a standard drug. Before experimentation, the animals were kept on fast for 24 h but water was given *ad libitum*. During experiments, animals were also observed for any alteration in their general behavior.

All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), Pinnacle Biomedical Research Institute (PBRI), Bhopal, India (approval number-1283/c/09/CPCSEA). All the experiments and the care of the laboratory animals were according to current ethical guidelines by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

Pharmacological screening

There are so many models to test the anti-asthmatic activity. We have selected histamine induced bronchospasm in guinea pig.

- **Isolated goat tracheal chain preparation** [9]
- **Isolated guinea pig ileum preparation** [10]
- **Haloperidol-induced catalepsy**[11]
- **Passive paw anaphylaxis in rats** [12]
- **Milk-induced leucocytosis in mice** [13]
- **Histamine-induced bronchospasm in guinea pigs** [14]

The guinea pigs fasted for 24 h were exposed to an atomized fine mist of 2% histamine di-hydrochloride aerosol (dissolved in normal saline) using nebulizer at a pressure of 300 mm Hg in the histamine chamber (24 x 14 x 24 cm, made of perplex glass). Guinea pigs exposed to histamine aerosol showed progressive signs of difficulty in breathing leading to convulsions, asphyxia and death. The time until signs of convulsion appeared is called pre-convulsion dyspnoea (PCD). By observation experience was gained so that the pre convulsion dyspnoea can be judged accurately. As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. In the present experiments the criterion used was time for onset of dyspnoea and percent protection was calculated. [15]

Those animals which developed typical histamine asthma within 3 min were selected out three days prior to the experiment and were given habituation practice to restrain them in the histamine chamber. They were divided in groups of five animals each. Chlorpheniramine maleate (2 mg/kg) was administered orally 30 min prior to exposure. Animals, which did not develop typical asthma within 6 minutes, were taken as protected.[16]

Percentage increased in time of PCD = $(1\text{-}T_1 / T_2) \times 100$

Where T_1 = time for PCD onset on day 0, T_2 = time for PCD onset on day 7

Experimental Design

The Histamine -induced asthmatic guinea pig were randomly assigned into five groups (1-5) of five guinea pig (n=5) each as Follows, namely

Group 1- Received DMSO in normal saline 10 mg/kg of body weight, per orally.

Group2- Received *Geniosporum prostratum* ethanolic extract 100 mg/kg of body weight, perorally.

Group 3- Received *Geniosporum prostratum* ethanolic extract 200 mg/kg of body weight, perorally.

Group 4- Received Chlorpheniramine maleate 2mg/Kg of body weight, per orally.

Statistical analysis:

Each value represents the mean ± S.E.M. The data were statistically analyzed using ANOVA followed by Dennett's *t*-test; versus control group. The values of $p<0.01$ were considered as significant. [17]

Results and Discussion

The results of the present study revealed antihistaminic actions of different extracts of plant (*G. Prostratum*) that in histamine aerosol study, the control animals showed convulsion during the first 3 min of the experiment. Prior treatment of*G. prostratum*ethanolic

extract (200 mg/kg, orally) protected the animals (Table -1) to a significant extent ($P < 0.01$) from the development of asphyxia produced by histamine aerosol confirming that it has antihistaminic activity. The role of histamine in asthma is well established. [18]The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins. Although there are various model of asthma, guinea pig airways react to histamine, acetylcholine, leukotrienes, and other bronco-constrictors in a manner similar to that seen in humans. [19] Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to β-adrenergic antagonists .[20] Thus, the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release. Histamine antagonists can be conveniently recognized and assayed by their ability to protect guinea pigs against lethal effects of histamine-induced bronchospasm. [21]Chlorphenarminemaleate (CPM), a standard anti-histaminic drug, (2 mg/kg, orally.) significantly protected 90 % of animals from asphyxia ($P < 0.01$).

In the early stage of asthma, release of inflammatory mediators like histamine, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergens, irritants, cold air or exercise. [22] Some of these mediators directly cause acute bronchoconstriction. Spasmolytic drugs like β-adrenergic agonists, xanthine derivatives and anticholinergic drugs are used as quick relief medications in such acute asthmatic attacks. [23, 24] In the present study, we have used histamine in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. Chlorphenarminemaleate (2 mg/kg)) was used as reference standard against histamine induced bronchospasm respectively. [21] The bronco-dilatory effect of higher dose of*G. prostratum*ethanolic extract was found comparable to the protection (74.12%) offered by the reference standard drug Chlorphenarminemaleate (82.35%).

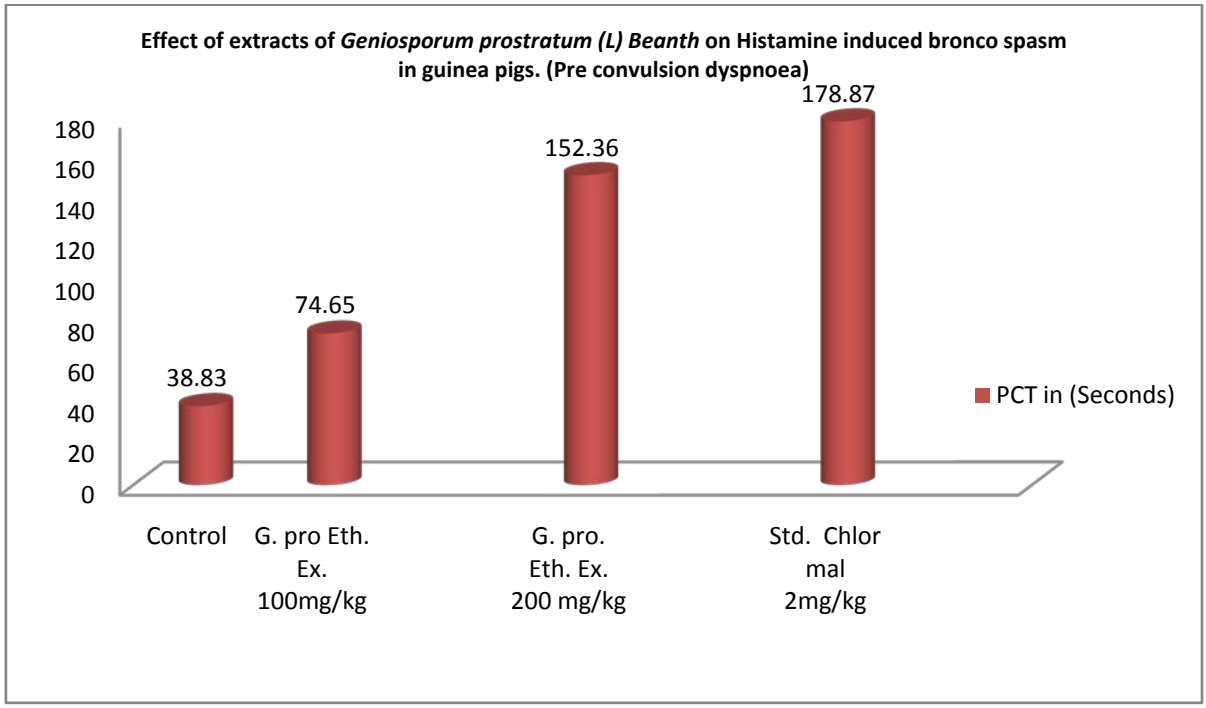
Conclusion

In conclusion, the results of present investigation suggest that, higher dose of*G. prostratum*ethanolic extract has significant bronco-dilatory activity against histamine. However, further studies are suggested to establish molecular mechanism and also to isolate and characterize the active principles responsible for the action.

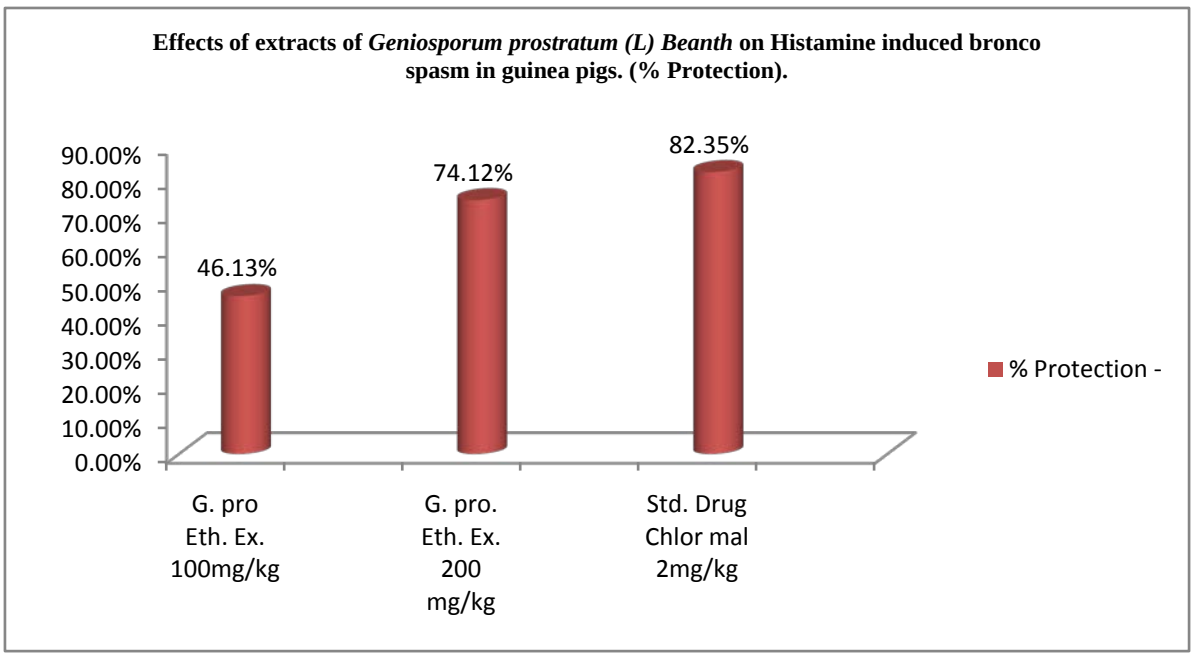
Table 1: Result of extracts of *Geniosporum prostratum (L) Beanth* on Histamine induced bronco constriction in guinea pigs.

S.No.	Groups	Dose(mg/kg)	PCT in (Seconds)	% Protection
1.	Positive Control	10 mg/kg	38.83±2.26	-
2.	*G. prostratum* Ethanolic Extract	100 mg/kg	74.65±1.29^{ns}	46.13%
3.	*G. prostratum* Ethanolic Extract	200 mg/kg	152.36±2.36**	74.12%
4.	Std. Drug Chlorpheniramine maleate	2mg/kg	178.87±2.18**	82.35%

n =6;*p<0.05,**p<0.01, ns-no significantcomparedwithcontrolgroup (ANOVAfollowedbyDunett'stest)



Graph -1: Result of extracts of *Geniosporum prostratum (L) Beanth* on Histamine induced bronco spasm in guinea pigs. (Pre convulsion dyspnoea)



Graph -2: Results of extracts of *Geniosporum prostratum (L) Beanth* on Histamine induced bronco spasm in guinea pigs. (% Protection)

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