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ANTIASTHMATIC STUDIES ON LEAF EXTRACT OF *TRAGIA PLUKENETII* R. SMITH AND *T. INVOLUCRATA* L.

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ABSTRACT
Asthma is a common, chronic inflammatory disease of the airways that affects people of all ages and imposes a substantial burden on patients, their families, and the community. Among India's 1.31 billion people, about 6% of children and 2% of adults have asthma. Many medicinal plants are used to treat asthma in Ayurveda and Siddha system of medicine. The present study was undertaken to investigate the effect of the leaf extracts of *Tragia plukenetii* and *Tragia involucrata* for its antiasthmatic activity. The anti-asthmatic activity was carried out on isolated guinea pig ileum preparation (*in-vitro*) and histamine induced bronchospasm in guinea pigs. The present observations showed that the ethanolic extract of leaves of *T. plukenetii* and *T. involucrata* significantly inhibited ($p < 0.05$) the contractile effect of histamine. Ethanolic extracts of both medicinal plants were found to inhibit ($p<0.05$) bronchospasm induced by histamine in guinea pigs by varying degree. Although these results provide a support for the traditional uses of *T. plukenetii* and *T. involucrata* as anti-asthmatic action, further studies are necessary to better evaluate its safety and modes of action.

KEYWORDS: Anti-asthmatic, Bronchospasm, Guinea Pig, Histamine *Tragia plukenetii, Tragia involucrate.*

INTRODUCTION
Asthma is one of the major non-communicable diseases. It is a chronic disease of the air passages of the lungs which inflames and narrows them. It was estimated that more than 339 million people had Asthma globally in 2016.[1] It is a common disease among children. Most asthma-related deaths occur in low- and lower-middle income countries. According to WHO estimates, there were 417,918 deaths due to asthma at the global level and 24.8 million Disability Adjusted Life Years (DALYS) attributable to Asthma in 2016.[2] [3] The strongest risk factors for developing asthma are inhaled substances and particles that may provoke allergic reactions or irritate the airways. Medication can control asthma. Avoiding asthma triggers can also reduce the severity of asthma. Appropriate management of asthma can enable people to enjoy a good quality of life.

Tragia, a genus of perennial, usually climbing or twining herbs, with stinging hairs, found in the tropical and sub-tropical parts of the world. *Tragia plukenetii* R. Smith (Tamil name: Karunkanchori) the root is diaphoretic and alterative and is given for fevers to cause perspiration.[4] The root of *Tragia involucrata* L. (Tamil name: Chenthatti) is popular for various medicinal uses in the indigenous system of medicine. The plant used in treating pruritic skin eruptions, venereal diseases, hemorrhoids, gastropathy, guinea worms, blood impurities, dipsia, vomiting giddiness, vitiated conditions of pitta, melalgia and brachialgia.[5] The root also forms the basis of an external application in leprosy.[6] [7] [8] The root is also used in old venereal complaints and a blood purifier. The root system forms the official part in Ayurveda.[9] The important formulations using the drug are Duralabharistam, Dasamularistam and Rasnadikasayam.[10] The drug is also found to be useful in siddha system of medicine (Cirukanchuri ver).[4] The present study was undertaken to investigate the effect of the leaf extracts of *T. Plukenetii* and *T. involucrata* for its antiasthmatic activity.

MATERIALS AND METHODS
Mature and healthy plants of *Tragia plukenetii* and *Tragia involucrata* belonging to the family Euphorbiaceae were collected from Southern Western Ghats in the district of Tirunelveli, South India. The specimens were identified, comparing the characteristics of floral and vegetative characters in the *'Flora of the Presidency of Madras'*[11] and *Flora of Tamilnadu Carnatic'*.[12]

Preparation of Drug

The plant material was shade dried and pulverized. Ethanol extract of the coarsely powdered leaf material was prepared by employing Soxhlet method. The extract was concentrated and stored in brown bottles for future use.

Animals

Guinea Pig (600-700 g) were procured from lab animal house, Dept. of Pharmacology, Govt. Siddha Medical College, Palayamkottai, Tamil Nadu. The animals were housed in microlan boxes in a controlled environment (temperature 25^{o}C and 12 hrs dark and light cycle) with standard diet and water *ad libitum*.

Antiasthmatic studies

Isolation of Guinea pig ileum preparation (*in-vitro*)

Overnight fasted Guinea pig was sacrificed and ileum was mounted in an organ bath containing tyrode solution. The tyrode solution was continuously aerated and maintained at 37^{o}C. The tissue was allowed to equilibrate for 30 min under a load of 500 mg of plasticine. The response of histamine was recorded by 5 min time cycle after contact 30 seconds. After obtaining a dose response curve of histamine (10 µg/ml) on ileum, ethanolic extract (100 µg/ml) was added to the presence of plant extract. This procedure was repeated for standard drug Chlorpheniramine maleate (CPM 10 µg/ml) as ethanolic extract. A dose response was based on maximum contractile response of histamine, in absence and presence of plant extract. The standard drug was recorded and tabulated.

Histamine induced bronchoconstruction in guinea pig[13]

Overnight fasted guinea pigs were divided into three groups each containing six animals. Group I was treated as control, Group II received standard drug Chlorpheniramine maleate (CPM 2 mg/kgbw). Animals belonging to Group III received extract in a dose of (100 mg/kgbw). All the doses were given orally. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. The Pre Convulsion Time (PCT) was determined from the time of to onset of convulsions. As soon as the PCT were noted, the animal were removed from the chamber and placed in air. 24 hr later, the animals of group II and group III were again subjected to histamine aerosol after 1hr of drug administration and PCT was determined. The protection offered by treatment was calculated by using the following formula.

% protection = (1-T1/T2) X 100

Where, T1 = the mean of PCT before administration of test drug.

T2 = the mean of PCT after administration of test drug.

RESULTS AND DISCUSSION

Ethanolic extracts of *T. plukenetii* and *T. involucrata* have been showed that significant ($p<0.001$) percent was decreased the contraction at 100 µg/ml in isolated guinea pig ileum preparation (Table: 1). The results of extract of *T. plukenetii* and *T. involucrata* have protected significantly the guinea pig against histamine – induced bronchospasm. The guinea pigs have been exposed to histamine aerosol, showed signs of progressive dyspnoea leading to convulsion. The extracts have been prolonged the latent period of convulsions as compared to control following the exposure of histamine aerosol after one hour of drug administration. The antihistaminic drug chlorpheniramine maleate used in the study has been produced significantly than the extracts, in the latent period of convulsion after one hour. The results of present study indicates that the utility of extracts of *T. plukenetii* and *T. involucrata* in antihistaminic study in isolated Guinea pig ileum was remarkable activity in higher dose of above extracts (Table: 1 & 2).

There are hundreds of plants used all over the world, which are used in herbal medicine as treatments for histamine attacks**.** Phytochemical investigations of the Euphorbiaceae family have demonstrated the presence of various bioactive compounds such as flavonoids, alkaloids, phenolics, lignans, terpenoids, saponins.[14] [15] All these chemical constituents contribute to multidirectional pharmacological activities.

Gas chromatographic and mass spectroscopic analysis showed the presence of Melamine, Eugenol, 2,3-Dihydro-3, 5-Dihydroxy-6-Methyl-4h-Pyran-4-One, Lauric acid, Neophytadiene, (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, palmitic acid, ethyl palmitate, phytols, Linoliec acid, (7Z,10Z,13Z)-7,10,13-Hexadecatrienal, Methyl linoleate, Piperin, Gamma-Tokoferol, Vitamin E, Methyl Comate A and Cholest-4-en-3-one Tocopheryl acetate, lupenol, linoleic acid, hexadecanoic acid, Gamma-sitosterol and stigmasterol were common in *T. plukenetii* and *T. involucrate*.[16] Melamine is a first generation antihistamine used in treating allergies, symptomatic relief of hypersensitivity reaction, and in pruritic skin disorders. In a rat study, specifically the eugenol in clove showed antihistamine and antianaphylactic activity.[17] Similarly the present study *Tragia* also reported with eugenol. From various disease-targeted animal models, these reports indicated that lupeol has anti-asthma efficiency under various routes of administration such as topical, oral, subcutaneous, intraperitoneal and intravenous.[18] The effects of lupeol were similar to those of dexamethasone, a synthetic glucocorticoid commonly used as a gold standard anti-inflammatory drug. Glucocorticoids have a myriad of effects initiated by binding to their cytosolic receptors, translocating to the nucleus, and altering the regulation of inflammatory cytokine gene expression.[19] [20] In allergic asthma, this results in the inhibition of macrophages, T-lymphocytes, eosinophils, and epithelial cells, reduced numbers of airway mast cells, reduced numbers of circulating and airway eosinophils, inhibit airway mucus secretion, and reduce histamine- and methacholineinduced airway responsiveness.[21] Lupeol

reduced the production of Prostaglandin E2 (PGE2), Tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) *in vitro* ([22], ear oedema induced by tissue plasminogen activator (TPA) in mice and paw swelling in an adjuvant arthritis model in rats.[23] The fact that lupeol does not show antinociceptive, anti-pyretic, and ulcerogenic effects indicate that this triterpene does not seem to act mainly by an inhibitory effect on Prostaglandin synthetase, suggesting that the mechanism of anti-inflammatory action of lupeol is distinct from classical non-steroidal anti-inflammatory drugs.[23] Clinical studies and animal models of asthma indicate dietary factors such as vitamin E as protective for asthma risk.[24]

The potential benefits of stigmasterol explored in the treatment of asthma, an airway disorder characterized by immune pathophysiology and with an ever-increasing worldwide prevalence. The modulatory effect of the intraperitoneal administration of stigmasterol on experimentally induced airway inflammation in guinea pigs. Stigmasterol at 10–100 mg/kg reduced proliferation of eosinophils, lymphocytes, and monocytes while reducing peribronchiolar, perivascular, and alveolar infiltration of inflammatory cells. Histopathology revealed stigmasterol maintained lung architecture and reversed collagen deposition, an index of lung remodeling. Overexpression of serum vascular cell adhesion molecule-1 (VCAM-1) and ovalbumin-specific immunoglobulin E (OVA sIgE) elicited by ovalbumin sensitization and challenge was significantly controlled with stigmasterol. Taken together, stigmasterol possessed significant antiasthmatic properties and had suppressive effects on key features of allergen-induced asthma.[25]

Table 1: Effect of Ethanolic extract of *T. plukenetii* and *T. involucrata* on histamine induced contraction in isolated Guinea Pig ileum.

Dose (ml)	Control (10 µg/ml)	Chlorpheniramine Maleate (10 µg /ml)	*T. plukenetii* (100 µg/ml)	*T. involucrata* (100 µg/ml)
0.1	47.16±2.20	19.12il.37**	19.38±0.96**	19.31±0.98**
0.2	65.41±3.02	29.21±1.73**	33.15±1.67**	34.17±1.87**
0.4	69.95±2.35	41.70±2.77**	35.25±1.17**	36.15±1.21**
0.8	81.41±2.73	45.31±2.33**	39.18±1.41**	39.15±1.52**
1.6	85.60±1.77	54.20±1.97**	45.06±0.87**	46.02±0.89**
3.2	100±1.75	65.01±2.21**	50.33±0.93**	51.13±0.91**

n = 5, values are Mean ± SEM
Control=DRC of histamine in absence of ethanolic extracts of *T. plukenetii*, *T. involucrata* (100 µg/ml)
CPM (10 µg/ml) = DRC of histamine in presence of Chlorpheniramine maleate which is standard (10 µg/ml).
p<0.05, ** p<0.01, and *** p<0.001 significantly different from control.

Table 2: Effect of Ethanolic extract of *T. plukenetii* and *T. involucrata* on histamine induced Bronchoconstriction in Guinea Pig.

S.No.	Group	Onset of convulsion in secondary	% protection
1	Control	91.45 ± 0.0093	-
2	Standard	1028.0 ± 4.553*	91.10
3	*Tragia plukenetii*	495.10 ± 0.253**	81.50
4	*Tragia involucrata*	406.20 ± 0.357**	77.48

Values are expressed as mean ± SEM (n=6) *p<0.001 when compared with control group, **p<0.001 when compared with standard group.

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