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Antiasthamatic Activity of Tinospora Cordifolia Leaves Extract on Ova Induced Airway Inflammation in Rats

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KEYWORDS
Antiasthmatic activity; Tinosporacordifolia; OVA albumin; Bronchoaleveolar lavage

ABSTRACT:

Introduction: There are several plants used and registered as medicine in alternative systems of medicine but their claim is not yet evaluated scientifically in laboratory animals. Present study was intended to evaluate anantiasthmatic activity of Tinosporacordifolia (TC) leaves extract in rats to confirm anethnomedical claim made by traditional health practitioners.

Objectives: Procurement of a plant TC and its authentication; preparation of extract; preparation of oral solution and its administration in rats; in-vivo activity evaluation in rats for one month; histopathology study; Results discussion and conclusion.

Methods: Plant was obtained from the botanical garden located at Pune and authenticated at Botanical Survey of India, Pune. Dried leaves of a plant were extracted in ethanol using soxhletextracter. Ethanolic extract was dried using rota evaporator and stored at cool and dry place. Extract was orally administered in rats (125mg/kg, 250mg/kg and 500mg/kg) and evaluated using OVA induced allergic airway inflammation, simultaneously taking Montelukast (10mg/kg) as a standard drug. Rats were sacrificed at the end of study and organs were fixed for the histopathology details.

Results: In OVA induced airway inflammation model, TC leaves extract significantly reduces white blood cell count in BALF. It also shows significant decrease in Emphysema, Hemorrhage, Bronchial Hyperplasia and Mononuclear cells in histopathology report. The histo-pathological examination showed milder pathology, reduced inflammatory cell infiltrates and a lower density of Eosinophils in the lungs of treated rats, which further supported our results from the BALF analysis

Conclusions: Significant antiasthmatic activity comparable with standard dose of Montelukast was observed in experimental rats. This confirms anethnomedical claim of a few workers regarding anantiasthmatic activity of this plant possibly through its antihistaminic, antispasmodic, or anti-inflammatory effects.

1. Introduction

More than 50% population in ruralIndiabelieves in the traditional alternative systems of a medicine;like Ayurveda, Siddha,Unani and Homoeopathy other than an allopathy[1]. In rural India still there are tribes which totally rely on traditional medicines where the modern health practices are not reaching adequately. Ayurveda is the branch of medicine which has maximum use of plants and traditionally processed herbal products as medicine. Ayurvedicmedicine reduces the chance of disease or they decrease the symptoms of it; or totally cure the diseases in some situations with less precision.[2]

Tinosporacordifolia, which is known by the common name Guduchi, is an herbaceous vine of the family Menispermaceae indigenous to the tropical areas of India, Myanmar and Sri Lanka. The plant is a glabrous climbing shrub found throughout India, typically growing in deciduous and dry forests. The leaves are heart shaped. The succulent bark is creamy white to grey in color, with deep clefts spotted with lenticels. It puts out long, slender aerial roots, and is often grown on mango orneem trees. Flowers are yellow, growing in lax racemes from nodes on old wood. Fruits are drupes, turning red when ripe[3].

All parts of Tinosporacordifolia plant are used for various medicinal purposes. The stem is a bitter stomachic; stimulates bile secretion; causes constipation; tonic; allays thirst, fever[4], burning sensation, vomiting; diuretic; enriches the blood; cures

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jaundice, useful in skin diseases[13]; the juice is useful in diabetes, vaginal and urethral discharges, low fevers, and enlarged spleen (In Ayurveda)[2,3].

Stem bitter; appetizer, stomachic, tonic, antipyretic[6], expectorant; good in cough, jaundice, giddiness, vomiting, piles, anaemia, chronic fever[4]; renews the blood; mixed with sesame oil it is useful for massaging the body (Yunani)[13]. The root and stem are prescribed in combination with other drugs as an antidote to snake-bite (Charaka, Sushruta, Vagbhata, Sharangdharasamhita, Vaidyavinoda, Yogaratnakara) and scorpionsting (Sushruta)[3].

An infusion of the powered stem is used as an alterative and tonic and has enjoyed the reputation among ancient Hindu writers of being an aphrodisiac. Among the Mundas of Chota Nagpur the whole plant, well ground; is applied on fractures[7,8]. In Ceylon the stems are used in fevers, skin diseases, jaundice, and syphilis. The starch obtained from the roots and stems of the plant is similar to Arrow-root in appearance and effect[9].

Table 1: Plant Profile[11,12]

Botanical Name	**Tinosporacordifolia**	**Vernacular names**	
Family	Menispermaceae	**Marathi**	Guduchi, gulvel
Synonym	Guduchi, gulvel	**Sanskrit**	Amritavali, amrta
Hindi	Giloe, guruc	**Gujarati**	Galac, garo

Botanical Description of a Plant: Tinosporacordifolia, which is known by the common name Guduchi, is an herbaceous vine of the family Menispermaceae indigenous to the tropical areas of India, Myanmar and Sri Lanka[11].Tinosporacordifolia a large, glabrous deciduous climbing shrub distributed throughout tropical and subtropical India up to an altitude of 300m. The plant is sometimes cultivated as an ornamental plant. Its leaves are cordate, membranous with a broad sinus. Flowers are small yellow or greenish yellow in axillary and terminal racemes which appears when the plant is leafless[13].Drupaceous fruits are ovoid, glossy and succulent with red colour while the seeds are curved. The dry intact stem which forms the commercial drug is rather succulent with long filiform, fleshy aerial roots from the branches. Stem is terete or sparcelyleniticellate. Tender stem is greenish with a smooth surface while the older one have a warty surface due to the presence of circular leniticels. It consists of greyish brown, warty bark. It breaks with a fibrous fracture. The drug is odourlesswith an intensely bitter taste[12].

Distribution: The plant is a glabrous climbing shrub found throughout India, typically growing in deciduous and dry forests[11].

Parts used: Bark, root, leaf, flower, rhizome, twigs etc[12].

Figure 1: Tinosporacordifolia Plant

Chemical constituents present in Tinosporacordifolia: A variety of constituents have been isolated from different parts of Tinosporacordifolia. They belong to different classes such as alkaloids, diterpenoid lactones, steroids, glycosides aliphatic compounds, polysaccharides[13]. Some constituents have been isolated from plant mainly they are tinosporone, tinosporic acid, cordifolisides A to E, syringen, berberine, giloin, gilenin, crude giloininand, arabinogalactan polysaccharide, picrotene, bergenin, gilosterol, tinosporol, tinosporidine, sitosterol, cordifol, heptacosanol, octacosonal, tinosporide, columbin, chasmanthin, palmarin, palmatosides C and F, amritosides, cordioside, tinosponone, ecdysterone, makisterone A, hydroxyecdysone, magnoflorine, tembetarine, syringine, glucan polysaccharide, syringineapiosylglycoside, isocolumbin, palmatine, tetrahydropalmaitine, jatrorrhizine respectively[13,20].

2. Objectives

1) Procurement of a TC plant sample.
2) Authentication of a Plant sample from a botany expert.
3) Presentation to and approval of a study plan by Institutional animal ethics committee.
4) Procurement of rats and their acclimatization.
5) Conduct of an in-vivo study and subsequent patho-physiology study.
7) Result discussion and conclusion.

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3. Materials and Methods

Procurement and Authentication of plant: Fresh leaves of Tinosporacordifolia were collected from the Botanical Garden of Indira College of Pharmacy, Pune; in the month of February 2013 and shade dried for the period of two weeks. Dried parts of the plant were sent to the Botanical Survey of India, Pune (Voucher Specimen. No. TICAM1) for authentication. Botanists identified the plant and authenticated the same. After authentication leaves were crushed to make powder using mixer blender and utilized for the process of extraction[5].

Preparation of Extract: About 200gm powdered Tinosporacordifolia leaves were packed into a thimble and transferred into soxhlet extractor (Borosil) with 1L of 90% ethanol for about 72hrs until there was no color change in the ethanol, indicating the end point of extraction[14].The extract was harvested and concentrated in a rotary evaporator (Spacelab) separating the ethanol from the real extract. However, the remnant ethanol in the extract was removed by placing the extract in porcelain dishes in the oven at 60oC until the weight remained constant. The extract weighed 5.48g and was then collected in air tight plastic container and stored in the refrigerator at 4oC ready for future use[14].

Acute toxicity studies: Acute toxicity studies were not conducted for this extract as safety of this plant and its various extracts is sufficiently investigated by other researchers[18,19].

Animals: Sprague Dawley rats8 weighing 200-300gm were obtained from Haffkine Institute Parel, Mumbai, India. The animals were maintained under standard laboratory conditions at temperature 24 ± 2˚C and relative humidity (50-65%) with a 12:12 hr light: dark cycle throughout all the experiments. The animals were fed with standard pellet diet and free access of water. The animals were shifted to the laboratory one hour prior to the experiment. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) with approval no: ICP/IAEC/12-13/09 for OVA induced airway inflammation[15].

In-vivo study: Rats were divided into six group's containing five animals in each group.On 1st day, group II to VI were sensitized with 20µg Ovalbumin and 2mg of alum suspended in 0.1 ml saline solution by an i.p. route6.On 14th day, a booster injection of alum-Ovalbumin mixture was given to same group.From day Results obtained were studied statistically by calculating standard error of mean (SEM) and % 23rd to 27th respective drug treatment was given to respective group.After that day 28th to 30th animals were exposed to aerosolized OVA albumin (1%) for 20 min three times a day7.Then the rats were sacrificed and tracheal catheter was inserted in trachea for collecting the BAL fluid (Bronchoaleveolar lavage). BAL fluid was collected by lavaging the lung with aliquots of 1ml phosphate buffer saline total recovery volume per rat was approximately 1.5ml.Total polymorphonuclear cells i.e. Neutrophils, Eosinophils, lymphocytes, monocytes, alveolar macrophages, epithelial cells were counted in BAL fluid and histopathological evaluation of lung tissue was carried out[9].

Table 2: Treatment schedule for OVA induced airway inflammation in rats

Sn	Groups	Treatment	Parameter
1	Control	0.5% CMC (1mg/kg) p.o.	24hrs after the last dose of OVA, Total and differential cell count in BAL Fluid and lung histopath
2	Negative Control	0.5% CMC (1mg/kg) p.o.	
3	Standard	Montelukast (10mg/kg)	
4	TCLE 125	125mg/kg	
5	TCLE 250	250mg/kg	
6	TCLE 500	500mg/kg	

Histo-pathological study of lungs: Histological analysis of lungs from non-sensitized vehicle treated group showed normal lung histology. In contrast, histological sections of lung tissue in ovalbumin sensitized rats showed airway inflammation, infiltration of eosinophils, lymphocytes and sub mucosal edema of the lungs. Treatment with Tinosporacordifolialeaves extract (125, 250, 500mg/kg) decreased infiltration of inflammatory cell and airway lumen plugging thereby decreasing inflammation in dose dependent manner[7,9].

Experimental design and Biostatistics - All groups were run simultaneously in both methods of evaluation at identical experimental conditions in parallel experimental design. One way ANOVA were taken as statistical method for testing the hypothesis. All test groups were tested against negativecontrol. Significant difference i.e. error $p<0.05$ was taken as sufficient level to reject null hypothesis.

4. Results

latency with respect to control for each participating group[15].

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Table 3: Effect of Tinosporacordifolialeaves extract (125, 250 and 500mg/kg) and Montelukast (10mg/kg) on OVA induced Airway inflammation in rats.

Groups	Control	Negative Control	Standard	TCLE 125	TCLE 250	TCLE 500
Total Cell Count	2052 ± 101.14	10650 ± 451.13$^{\#}$	4448 ± 145.21**	8408 ± 319.60**	6578 ± 189.38**	5446 ± 188.30**
Neutrophils	21.8 ± 2.04	40.4 ± 2.73$^{\#}$	29.8 ± 2.60**	32.2 ± 1.07**	31.8 ± 1.99**	30.2 ± 1.16**
Lymphocyte	27.6 ± 1.33	39.6 ± 2.93$^{\#}$	29 ± 2.49**	35.6 ± 0.68^{ns}	32 ± 0.44*	30.8 ± 0.49**
Eosinophils	5.2 ± 0.20	9.4 ± 0.40$^{\#}$	5.6 ± 0.40**	7.2 ± 0.37*	6.8 ± 0.37**	6.4 ± 0.24**
Monocyte	6.8 ± 0.58	8.8 ± 0.37$^{\#}$	7 ± 0.31**	8 ± 0.32*	7.6 ± 0.40*	7.4 ± 0.24**
Macrophage	5.4 ± 0.24	8.4 ± 0.24$^{\#}$	6 ± 0.55**	9 ± 0.31^{ns}	7.6 ± 0.24*	6.4 ± 0.25**
Epithelial Cells	5 ± 0.54	8.6 ± 0.24$^{\#}$	5 ± 0.31**	6.8 ± 0.37*	6.2 ± 0.49*	5.6 ± 0.24**

Control = 0.5% CMC (1mg/kg); Negative control = Ovalbumin; Standard = Montelukast (10mg/kg);TCLE 125 = Tinosporacordifolia leaves extract (125mg/kg); TCLE 250 = Tinosporacordifolia leaves extract (250mg/kg); TCLE 500 = Tinosporacordifolia leaves extract (500mg/kg); (n=5) Values are expressed as Mean ± SEM, Data was analyzed by One-Way ANOVA followed by Dunnett's tests *P < 0.05, ** P < 0.01 as compared with Negative control group and #P<0.05 when negative control group was compared with control.

Table 4: Histopathological report of rat lungs

Sr. No.	Code No.	Congestion of blood vessels and hemorrhages	Emphysema	Bronchial hyperplasia, degenerative changes with MNC infiltration	Infiltration of MNC around Bronchi	Inflammatory changes with consolidation of lung with aggregated MNCs	Pneumonic foci	Overall pathological grade (lesion score)
1	Control	NAD	NAD	+	+	+	NAD	Minimal (+)
2	Negative Control	+++	+++	++++	+++	+++	++++	Severe (+++)
3	STD	NAD	NAD	NAD	+	+	NAD	Minimal(+)
4	TCLE 125	+	+	++	++	+	+	Mild (++)
5	TCLE 250	+ (focal)	+	++	+	+	+ (focal)	Mild (++)
6	TCLE 500	NAD	NAD	NAD	+ (focal)	+ (focal)	NAD	Minimal (+)

Note: Overall Grade score as-NAD =No Abnormality Detected, Minimal changes (+), Mild changes (++), Moderate changes (+++), Severe changes (++++). Focal and minimal changes may not be significant for alteration of functional capacity of the organ.

5. Discussion

Etiology of asthma points different causes of disease like genetics, immunological, allergic, hypersensitivity, chemical exposure, environmental and seasonal factors[23]. Asthma is a chronic respiratory disease characterized by airway hyper responsiveness, mucus hyper secretion, bronchial inflammation, and elevated IgE levels.In OVA induced airway inflammation there is typical repitative exposure of allergen with bronchial smooth muscle which induces condition that closely match to allergic asthma. Pathological scenario of asthma is complex and it is mixed effect of histamine, leukotrienes, acetylcholine, prostaglandins, interleukines secretions in-situ[24]. There are serveral ways of treatment applied and used by physicians to manage Asthma clinically. Intranasal ovalbumin challenge in rats resulted in activation of hyper responsiveness via Th2 cells, together with other inflammatory cells such as macrophages, lymphocytes,

Figure 2: Effect of Tinosporacordifolia and Montelukast on histopathology of lungs:

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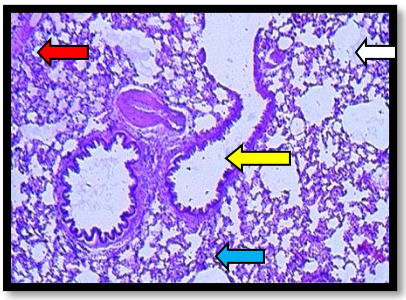
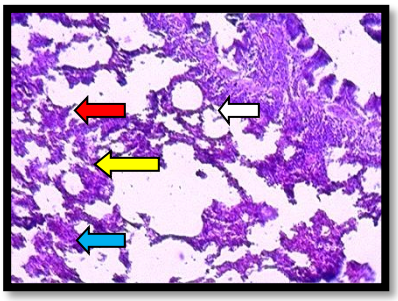
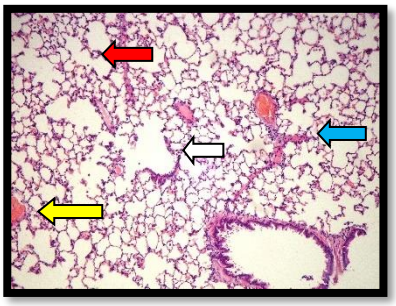
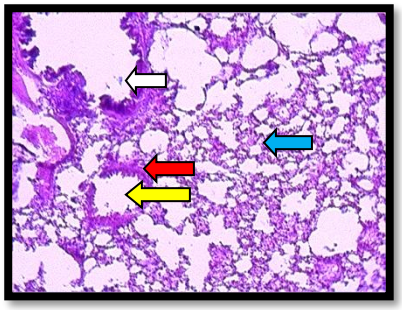
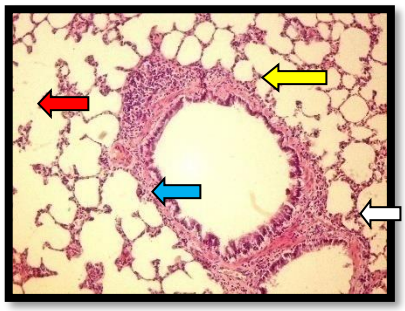
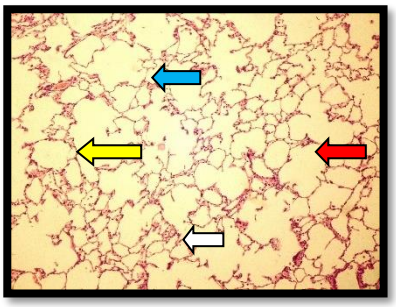
Control	Negative control	TCLE 250
		
Standard	**TCLE 125**	**TCLE 500**
		

photograph showing emphysema (white arrow), vascular changes (red arrow), cellular changes (blue arrow), bronchial debris (yellow arrow) H&E stain

neutrophils, Eosinophils as well as mast cells in the Bronchoalveolar lavage fluid and lung tissue which play critical role in the initiation, development and chronicity of this disease[5].Simultaniously there is infilteration of different leukocytes and mast cell in the affected areas as the disease progress.

Eosinophil is one of the primary effector cells in airway inflammation, and produces a wide range of inflammatory mediators[17], including granule-derived cationic proteins such as eosinophil major basic protein (MBP), lipid mediators such as prostaglandins and leukotriene C4 (LTC4), and cytokines[6].

The histo-pathological examination showed milder pathology, reduced inflammatory cell infiltrates and a lower density of Eosinophils in the lungs of treated rats, which further supported our results from the BALF analysis[7].

In OVA induced airway inflammation model, Tinosporacordifolia leaves extract significantly reduces eosinophil, lymphocyte, and neutrophil count[8] in BALF. It also shows significant decrease in Emphysema, Hemorrhage, Bronchial Hyperplasia and Mononuclear cells in histopathology report. These are the typical characteristics of antiasthmatics[9].

The present study was aimed to evaluate the anti-asthmatic activity[10] of Tinosporacordifolia leaves extract by using in-vitro model like isolated goat tracheal chain preparation and in-vivo models like Carrageenan induced pleurisy in rats and OVA induced airway inflammation in rats which we conducted successfully. Out of these different studies, only distinct and significant results are presented and described in this research article.

6. **Conclusion**:

In the present study, we did the pharmacological evaluation of a herbal drug, Tinosporacordifoliafor its anti-asthmatic effect. In summary, our data suggest that, In OVA induced airway inflammation model, Tinosporacordifolia leaves extract significantly reduces Eosinophils, Lymphocytes & Neutrophils and also shows significant decrease in Emphysema, Hemorraghes, Bronchial Hyperplasia, and Mononuclear cells in Histopath report.Thus, anti-asthmatic activity[10] of Tinosporacordifoliacan be attributed to broncho-dilating, anti-allergic, anti-histaminic and anti-inflammatory activity suggestive of its potential in treatment and prophylaxis of asthma.

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