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EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF HYDROALCOHOLIC LEAF EXTRACT OF *MENTHA LONGIFOLIA* IN GUINEA PIGS & Human Patients

KEYWORDS

Anti-Asthmatic activity, preclinical studies, Guinea Pigs, *Mentha longifolia,* H1 antagonist, anti-inflammatory, prophylaxis, eosinophilia

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ABSTRACT The aim of the present study is to evaluate Anti-Asthmatic activity of hydro alcoholic leaf extract derived from the leaves of *Mentha longifolia* in Guinea Pigs. The study has found astounding results in the effective treatement of Asthma with reference to the preclinical studies done in Guinea Pigs. The results obtained in the present investigation that *Mentha longifolia* Leaves possess significant Anti-Asthmatic activity. At 150 & 300 mg/kg the anti-asthmatic activity of *Mentha longifolia* Leaves can be attributed to its bronchodilating, antihistaminic (H1-antagonist) and anti-inflammatory property, suggestive of its potential in treatment and prophylaxis of asthma. Clinical efficacy also decreased the eosinophilic levels in the treatment in asthmatic patients.

INTRODUCTION:

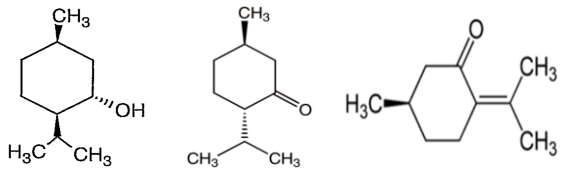
Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. Asthma is one of the most common chronic diseases of childhood, affecting more than 6 million children. Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world. The currently used drugs for the treatment of this disease in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. Asthma is characterized by a predisposition to chronic inflammation of the lungs in which the airways are reversibly narrowed. It occurs in 3 to 5% in all the people during their life span [1, 2]. Asthma affects 7% of the population of the United States, 6.5% of British people and a total of 300 million worldwide. India has an estimated 15-20 million asthmatics. Asthma is a respiratory disorder caused by allergic hypersensitivity reactions. It is a disease that does not respect the boundaries of age, race, gender and 5000 deaths occurring annually.

It is classified in to different types [3, 4]. They are Extrinsic Asthma occurs children and young adults who have atopic hyper sensitivity to foreign particles. An "Allergen" or an "antigen" is a foreign particle which enters in to the body. Extrinsic asthma is caused by this type of immune system response to inhaled allergens such as pollen, animal dander or dust mite particles. It stimulates the production of IgE antibodies that binds to the surface of mast cells and basophiles round the bronchial blood vessels. Intrinsic Asthma is not allergy-related, in fact it is caused by anything except an allergy, e.g.: Inhalation of chemicals such as cigarette smoke or cleaning agents, It is associated with chronic inflammation of Upper respiratory tract (URT), Aspirin trigger asthma, Stress, laughter, exercise, cold air, food preservatives or a myriad of other factors. Mixed Asthma name suggests, mixed asthma is a mixture of intrinsic and extrinsic asthma [5,6].

Inflammation is the response of a tissue and its microcirculation to pathogenic injury. It is characterized by generation of inflammatory mediators and movement of fluid and leucocytes from the blood to extravascular tissue [7]. A large number of drugs belonging to β2 agonists, corticosteroids, mast cell stabilizers, methylxanthines, leucotriene antagonists and others are in use for treating asthma. However none of them seems to be an ideal drug. The search for a new drug is still the need of the day. Herbal medicines are in great demand and are used by approximately 80% of the world's population. Their popularity is largely due to their presumed safety, efficacy, cultural acceptability, and lesser side effects compared with prescription medications [8]. There is high prevalence of usage of the alternative traditional system of medicines for the treatment of asthma. Ayurveda offers a unique insight into a comprehensive approach to asthma management through proper care of the respiratory tract. More than 400 medicinal plant species have been used ethnopharmacologically and traditionally to treat the symptoms of asthmatic and allergic disorders worldwide. Research on plants with medicinal properties and identification of the chemical components responsible for their activities have corroborated the traditional uses of ancient healing wisdom and lore and have proven the enduring healing potential of many plant medicines even in today's hi-tech community. The World Health Organization (WHO) has recognized herbal medicine as an essential building block for primary health care of vast countries like India and China. Herbal medicines are a treasure house of the information, from which we may derive leads to fill many blank spots in the modern medicine.

PLANT PROFILE:

Mentha longifolia is perennial herb 40-120 cm high with musty scent. Leaves are sessile or shortly petiolate usually oblong elliptical, hairs simple. Extremely variable in height, leaf size and shape and inflorescence. It is used as a folk remedy for treatment bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints due to their antiinflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue and anticatharral activities (Gulluce et al. 2007). It is represented by about 19 species and 13 natural hybrids, mainly perennial herbs, which grow wildly in damp or wet places throughout the temperate regions of Europe, Asia, Africa, Australia, and North America. Species of the genus Mentha have been reported to contain a range of components, including Cinnamic acids, Flavonoids, and Steroidal glycosides. However, the main active component of the genus Mentha is essential oil, which is reported to govern its various properties, which mainly consist of Menthol, Menthone, Pulegone, Piperitenone oxide, Cis-piperitone oxide, Thymol and Spathulenol [9, 10].



Menthol ($C_{10}H_{20}O$) **Menthone ($C_{10}H_{18}O$)** **Pulegone ($C_{10}H_{16}O$)**

MATERIALS AND METHODS:

Animals: AlbinoWistar Rats (150- 200g) and Dunkin-Hartley Guinea pigs (300- 600g) of either sex were used during whole period of

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experiments. The standard conditions of temperature (22 ± 2°C), with relative humidity (60 ±5%) and 12 h light/dark cycles were used. They were fed with standard pellet diet and water ad libitum [15].

Plant materials:
Fresh leaves of Mentha longifolia (3 kg) were collected in Chennai, authenticated by Prof. P. Jayaraman, a botanist, at the Department of Botany.

Extraction:
The plant materials were cleaned of adulterant and were coarsely ground. The powdered material (2 kg) was soaked in 70% methanol and 30% water for 3 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on a rotary evaporator at 37°C under reduced pressure (-760 mm Hg) to a thick, semi-solid mass of dark brown colour, i.e. the crude extract, yielding approximately 38% (w/w). Crude extract was solubilized in normal saline for use in the in vivo and in vitro experiments. Activity-directed fractionation of the crude extract was carried out by standard phytochemical procedures using different organic solvents. 80 g of extract was dissolved in distilled water. This was then introduced in a separating funnel and petroleum spirit (90–100 mL) was then added. This mixture was shaken vigorously, regularly allowing the air to escape out. It was kept for about 30 min to let the two layers separate. The upper layer of petroleum spirit was acquired and the same procedure was repeated twice and all the petroleum spirit layers were collected and concentrated in a rotary evaporator to obtain the petroleum spirit fraction (Ml.Pet). The remaining layer was evaporated and the resultant fraction was considered as the aqueous fraction (Ml.Aq). The yield of both fractions was 28.6% (w/w) and 40% (w/w), respectively [11].

PRECLINICAL STUDY:
Histamine Aerosol induced bronchoconstriction in Guinea pigs (in-vivo) [12-14]:
Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in *guinea pigs* by exposing histamine aerosol by an ultra-sound nebulizer in an aerosol chamber (30 x 15 x 15cm) made of Perspex glass. The required time for appearance of preconvulsive dyspnoea produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. *Guinea pigs* were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to n=4

Group 1 - Control and receive distilled water.
Group 2 and 3 - by oral intubation, 150 and 300 mg/kg of the plant extract, respectively,
Group 4 - Chlorpheniramine maleate, i.p 1mg/kg

After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hrs, to determine pre convulsive time (PCT).

The protection untaken by the treatment was calculated using the formula

$$\text{Percentage protection} = \frac{\text{Eta} - \text{Etb}}{\text{Etb}} \times 100 \text{ (\% Protection offered by the extract)}$$

Where, Eta is the mean of PCT before administration of test drugs.

Etb is the mean of PCT after administration of test drugs at 1 hr, 4 hr and 24 hrs [12-14].

RESULTS AND DISCUSSIONS:

Table No:-1 Effect of HLML against Histamine induced bronchoconstriction in guinea pigs:

GROUPS	Latent period of Convulsion (in sec.) (Mean ± SEM)			
	Before	1 hr	4 hr	24 hr
Chlorphenaramine maleate (1mg/kg), i.p.	15.3±0.87	53.4±1.96**	61.7±1.90**	27.4±1.07**
HLML 150 mg/kg, p.o.	13.9±1.02	25.6±2.01**	35.5±1.97**	24.7±0.97**
HLML 300 mg/kg, p.o.	15.8±0.73	41.7±1.67**	54.2±1.53**	26.8±1.57**

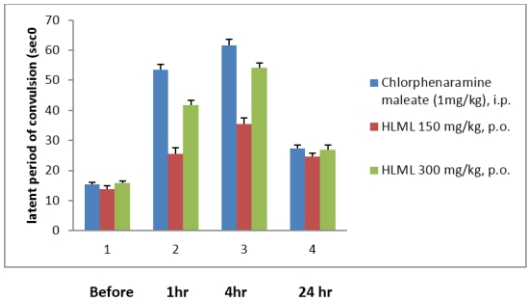
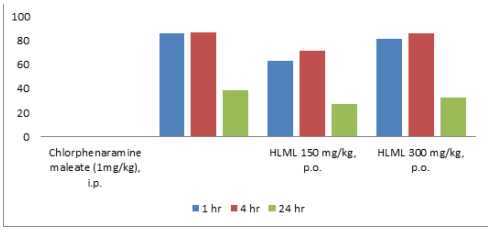


Figure No: - 1

Table No: - 2 % protection of HLML against Histamine induced bronchoconstriction in guinea pigs:

GROUPS	% Protection		
	1 hr	4 hr	24 hr
Chlorphenaramine maleate (1mg/kg), i.p.	86.3	87.2	38.7
HLML 150 mg/kg, p.o.	62.9	71.3	27.5
HLML 300 mg/kg, p.o.	81.5	85.9	32.6



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Statistical analysis:
All the values of *in vitro* and *in vivo* anti asthmatic activity were expressed as mean ± Standard error of mean (S.E.M) and was examined for significance by ANOVA (analysis of variance) and groups were compared by Dunnett's test for individual comparison of groups with control. P Value were measured moderate significant at P<0.01, <0.001 level.

Histopathology studies
Lung sections from HLML *i.p.* treated Guinea pigs were fixed in Formulin solution, embedded in paraffin, cut in 0.5-µm sections, and stained with haematoxylin-eosin. Inflammatory parameters in lung tissue (peribronchial, perivascular and parenchymal infiltration of inflammatory cells) were evaluated blindly by a senior lung pathologist. Total histology score was calculated [15] and graded from 0–4, where 0 = normal lung and 4 = diffuse maximal inflammation as shown in the table and figures 1 & 2.

CLINICAL STUDY:
Asthma induction and assessment of lung inflammation
Healthy human volunteers were sensitized with 0.0175 gm chicken ovalbumin (OVA) conjugated to 2% aluminium hydroxide in 0.035 ml saline were given intraperitoneally (*i.p.*). Protocol 1: Control humans were injected with saline.

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Protocol 2: HLML 150 mg/kg + OVA challenge.
Protocol 3: HLML 300 mg.kg + OVA challenge.

Note: Humans are treated with 10 mg tab montelukast on days 21, 22, and 23 only. Appropriate positive and negative controls were carried out in parallel with the treated groups. The induction with Ovalbumin was done on 1,7,14 and 21 days. On day 24, the blood samples are collected. [11-14].

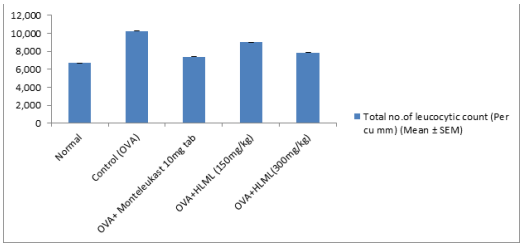
Analysis of bronchoalveolar lavage fluid (BALF) and serum:
The blood is collected using heparin into capillary tubes. Blood samples were centrifuged (10 minutes, 4°C, 1000 × *g*), and plasma was stored at −70°C until use. The levels of IL-4 (SRM medical College, Chennai) and total serum IgE were determined by ELISA according to the manufacturer's protocol [18-20].

OVA - specific Immunoglobulin E titre by ELISA
The blood is collected using heparin into capillary tubes. Serum OVA- specific immunoglobulin (Ig) E were measured as previously described , but with minor modifications; plaques were coated with anti-human IgE (SRM Medical college,Chennai) and levels of OVA specific IgE were expressed in arbitrary units of optical density.[21-22].

RESULTS
Effect of HLML on Ovalbumin induced eosinophilic airway inflammation:
HLML at 150 and 300 mg·kg significantly reduced the total number of cells ($p < 0.001$; n = 6), eosinophils (*** $p < 0.001$) in BAL compared with the untreated group of OVA sensitised Humans. HLML treatment during the challenges (days 21–23), sensitisation (days 1–20) significantly reduced the number of total cells in the BAL. It was as effective as 10mg tab of Montelukast. Similar results were obtained for peripheral blood count and eosinophil number. These results were found out by using Leishman's stain.

Table No:- 3
Analysis of HLML on Ovalbumin induced serum and alveolar fluid in humans:

Group treatment	Total no.of leucocytic count (Per cu mm) (Mean ± SEM)	Diff. in eosinophil count (Per cu mm) (Mean±SED)
Normal	6,700±12.67***	345±2.56***
Control (OVA)	10,235±31.56***	543±7.45***
OVA+ Monteleukast 10mg tab	7380±20.54	355±3.21
OVA+HLML (150mg/kg)	8990±26.89	390±3.67
OVA+HLML(300mg/kg)	7850±21.98***	360±3.35***



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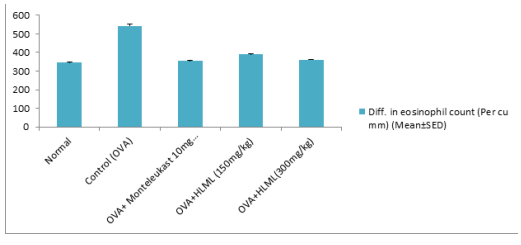

Figure No: - 4

Statistics: Statistical analyses were made using an ANOVA table followed by a Fisher's post-hoc

Test to determine statistical significance between groups (significant result $p < 0.0001$) as shown in the table and figures 3.

CONCLUSION:
Thus, it can be concluded from the results obtained in the present investigation that *Mentha longifolia* Leaves possess significant antiasthmatic activity. At 150 & 300 mg/kg the anti-asthmatic activity of *Mentha longifolia* Leaves can be attributed to its bronchodilating, antihistaminic (H1-antagonist) and anti-inflammatory property, suggestive of its potential in treatment and prophylaxis of asthma. Clinical efficacy also decreased the eosinophilic levels in the treatment in asthmatic patients.

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