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Evaluation of Anti-asthmatic Activity of Aqueous Extract of *Achillea mellifolium* Linn Flowers

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Abstract

The present study was designed to evaluate the anti-asthmatic activity of aqueous extract of flowers of *Achillea mellifolium Linn.* belonging to the family Asteracae (Compositae). The anti-asthmatic activity of the extract 5, 10, 15, 20, 25, 30mg/ml was evaluated in guinea pig ileum, guinea pig trachea chain, rat ileum, rat fundus isolated preparations by using standard drugs Histamine, Acetyl Choline & 5- HT respectively. The extract significantly inhibited all the response produced by the inflammatory mediators used and maximum significant anti-asthmatic activity was observed at the doses of 25, 30 mg/ml.

Key words: *Achillea mellifolium*, anti-asthmatic activity, acetyl choline, histamine, 5-HT.

Introduction

Asthma is a chronic disease with spastic contraction of smooth muscle in the bronchioles characterized by difficult breathing with wheezing. Asthma, a common, chronic inflammatory disorder of the airways, associated with pronounced health and economic consequences, has been identified as one of the five pressing global lung problems [1]. It has many causes but more specifically due to inflammation of air passage, hypersensitivity of afferent glossopharyngeal and vagal ending in larynx and afferent trigeminal endings in the nose, pulmonary edema and congestion of lungs caused by left ventricular failure (cardiac asthma).

The prevalence of childhood asthma with wheeze and/or wheezy bronchitis, ranges from 9.9 to 33% [2]. There is considerable mortality and morbidity due to asthma [3,4], the majority of which is avoidable.
There are two types of asthma. They include (i) atopic (childhood onset, extrinsic, type – I) and (ii) non- atopic (adult onset, intrinsic, type - II). Atopic occurs in children and young adults who

have atopic (type-I) hypersensitivity to foreign proteins. Again when the same antigen comes into contact the antibody/antigen reoccurs resulting in release of histamine and other factors increase mucous secretion and muscular contraction that narrows the airways. Attacks become less frequent and less severe with age. Non-atopic occurs in adult life with no childhood history and is associated with chronic inflammation of upper respiratory tract. Eventually impaired lung ventilation leads to hypoxia, pulmonary hypertension and right sided heart failure.

Anti asthmatic drugs like corticosteroids, theophylline, salbutamol are widely used in the treatment of asthma but these drugs produce some adverse effects like immune suppression, cardiac problems [5]. Now a days the approach on herbal medicine to reduce the adverse effects has been increased and *Achillea mellifolium* is one of the traditional herbal medicine claim which has anti-asthmatic property. So, the aim of the study was to give a scientific evaluation of anti-asthmatic property of Acetyl choline

Achillea mellifolium contains essential oils, sesquiterpenes, flavonoids, alkaloids, tannins, achillin, leucodin, desacetylmatricarin, 8 alpha angeloxy-derivatives [6]. Its major component Chamazulene showed strongest antimicrobial activity [7]. The most frequently reported medicinal uses were for treating gastrointestinal ailments (50%), skin injuries and problems(25.6%), followed by respiratory, urinary-genital and cardiovascular problems (20.5%, 20.5%, 19.2% respectively). The flowers, rich in chemicals are converted by steam into anti-allergenic compounds. The flowers are used for various allergic mucus problems, including hay fever and harvested during summer and autumn.

Materials and Methods

Plant material- The fresh flowers of *Achillea mellifolium* was collected locally in the month of January and authentified by Prof. P.Jayaraman (Ph.D.), Director-Plant Anatomy Research Centre (PARC) Tambaram. After authentication the flowers were dried at room temperature to free from moisture. The flowers were subjected to size reduction to get coarse powder of desired particle size. The coarse powder was then stored in a clean dry air tight container.

Preparation of Achillea mellifolium Extract

The powdered material was subjected to maceration with solvent Chloroform for 7 days. The extract was then concentrated to $3/4^{th}$ of its original volume at 55^0 C. The concentrated extract was then taken in a china dish. The plant extract was subjected for preliminary phytochemical analysis.

Animals- Albino rats (150-200gm) and Guinea pig (400-500gm) of either sex supplied by the Animal House of SRM University, Chennai were used for the study. All the animals were housed in the cages with natural light-dark cycle and fed with standard pellet food and water *ad libitum.* The study was approved by the Institutional animal Ethical Committee, CPCSEA (IAEC) of SRM College of Pharmacy, Chennai. Ethical norms were strictly followed during all experimental procedure.

The different models used for the study are a) Guinea pig ileum preparation, b) Guinea pig tracheal chain preparation, c) Rat fundus preparation, d) Rat tracheal chain preparation.

a) Guinea pig tracheal chain preparation

Guinea pig of either sex (200-500 g), starved overnight but allowed free access to water were used. The animals were killed by a blow on the head and exsanguinated. The trachea was dissected out and cut along its length on the dorsal surface. Incomplete transverse cuts were made along the segments of the cartilage to produce a zigzag strip. The isolated trachea was mounted in a 30 ml organ bath containing Tyrode solution, maintained at 37±1 °c and gassed with air. The tissue was equilibrated for 45 min during which the bath solution was replaced every 10 min. At the end of the equilibration period, histamine (1000 µg/ml) induced contraction as well as effect of extract (up to 30 mg/ml) was recorded. At the end of the equilibration period, acetyl choline (1000µg/ml) induced contraction as well as effect of extract (up to 30 mg/ml) was also investigated. A drug tissue contact time of 1 min was maintained. Responses were recorded on smoked paper recorder [8, 9].

b) Guinea pig ileum preparation

A segment of the guinea pig ileum (approximately 2 cm long) removed from a freshly killed animal was suspended in a 30 ml organ bath containing tyrode solution maintained at 37^{o} C and gassed with air. After an equilibration period of 45 min, contractile responses were established for histamine (1000µg/ml) and acetyl choline (1000µg/ml). The effect of the extract on histamine and acetyl choline induced contractions was investigated [8-11].

c) Rat stomach strip preparation

Adult albino rats (200-250 mg) of either sex were killed by a blow on the head and exsanguinated. The abdomen was opened and the stomach removed. The fundus portion of the head and was cut into a sheet, from where strips of about 2-3 cm long were prepared. The strip was suspended in tyrode solution and after 45 min equilibration period, the effects of extract (up to 30 mg/ml) on 5-hydroxytryptamine (5-HT, serotonin) induced contractions were recorded [9]

d) Rat Ileum Preparation

Adult albino rats (200-250 mg) of either sex were killed by a blow on the head. The animal kept overnight fasting is sacrificed as per CPCSEA recommended guidelines. The abdominal cavity is quickly opened and a piece of ileum is isolated and is placed in a petry dish containing Kreb's solution maintained at 37^{0} C. The mesentry of ileum is removed and the interior content is washed by Kreb's solution with the help of pipette. The tissue is mounted in mammalian bath and connected to isotonic frontal writing lever. The tissue is allowed to stabilize for 30 min. The responses to Acetylcholine and plant extract were recorded [12].

Statistical analysis

The data are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of ANOVA, followed by Dunnetts t-test where $P<0.001$ was considered statistically different.

Results

The preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, tannins, flavonoids, and polyphenols.

Guinea pig Tracheal chain Preparation

The aqueous extract of *Achillea mellifolium* showed the inhibitory action on CRC of acetylcholine using guinea pig trachea preparation. The maximum inhibitory action was at the concentration of 30mg/ml.

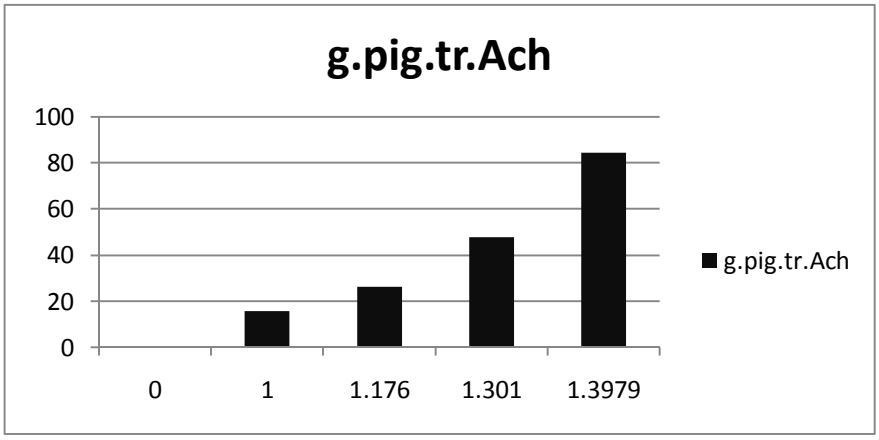

Fig.1. Effect of aqueous extract of *Achillea mellifolium* on CRC of acetyl choline using Guinea pig tracheal chain preparation

Guinea pig ileum Preparation

The aqueous extract of *Achillea mellifolium* showed inhibitory action on histamine on guinea pig ileum preparation. The maximum inhibition was shown at the concentration of 30mg/ml.

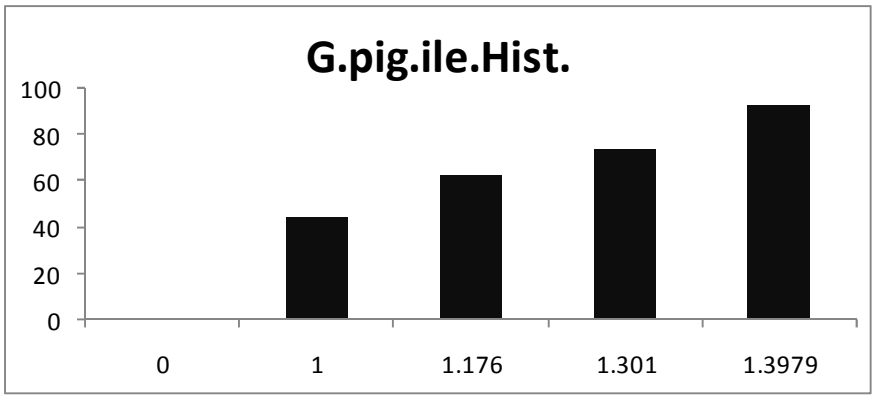

Fig.2. Effect of aqueous extract of *Achillea mellifolium* on guinea pig ileum preparation using histamine as agonist

The aqueous extract of *Achillea mellifolium* showed inhibitory action on acetylcholine using guinea pig ileum preparation. The maximum inhibition was shown at the concentration of 30mg/ml.

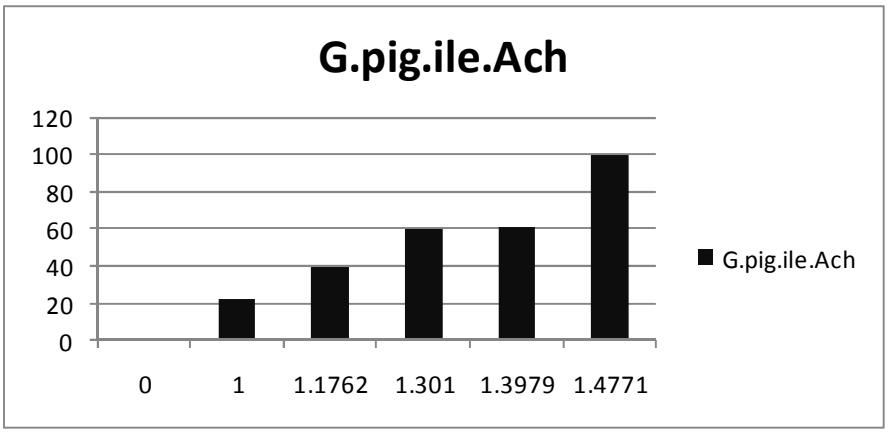

Fig.3. Effect of aqueous extract of *Achillea mellifolium* on CRC of acetyl choline using Guinea pig ileum

Rat Fundus Preparation

The aqueous extract of *Achillea mellifolium* showed inhibitory action on CRC of 5-HT using rat fundus preparation. The maximum inhibitory action was at the concentration of 30mg/ml.

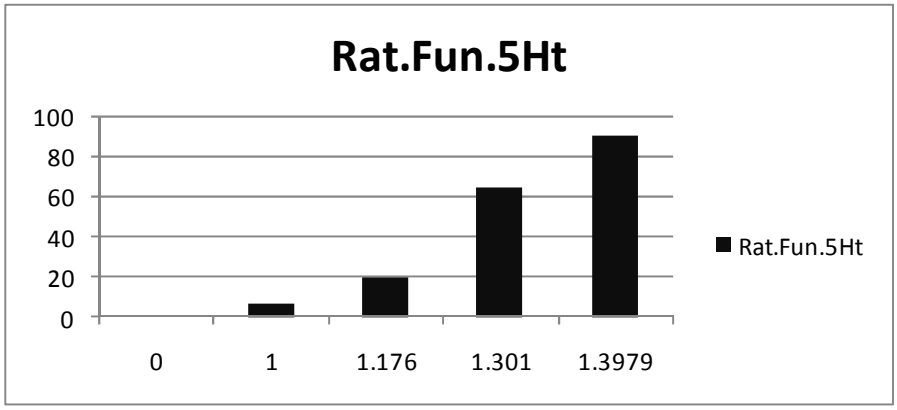

Fig.4. Effect of aqueous extract of A*chillea mellifolium* on CRC of 5HT using rat fundus

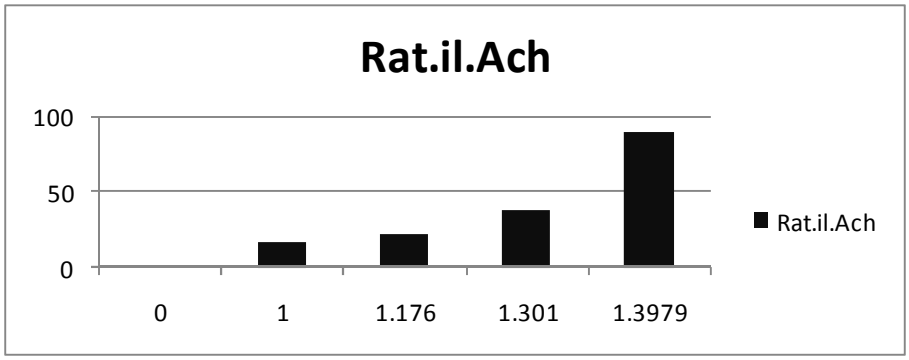

Fig.5. Effect of aqueous extract of *Achillea mellifolium* on CRC of Acetylcholine using rat ileum

Rat Ileum Preparation

The aqueous extract of *Achillea mellifolium* showed inhibitory action on CRC of acetylcholine using rat ileum preparation. The maximum inhibitory action was shown by 30mg/ml.

Discussion

The aqueous extract of *Achillea mellifolium Linn* inhibited trachea contractions induced by histamine, serotonin, and acetylcholine. These agents are implicated in various ways in the pathogenesis of asthma [13]. Histamine is the most implicated mediator in bronchconstriction that accompany asthma [14]. Although the role of 5-HT in asthma is uncertain; it is a potent bronchoconstrictor [15] and also increases acetylcholine release from airway nerves via 5-HT3 receptors [16]. Acetylcholine on its own can cause bronchconstriction by activating efferent cholinergic fibers secondary to the stimulation of the sub-epithelial afferent fibers by inflammatory mediators such as histamine [17]. The aqueous extract exhibited the least activity against histamine and 5HT; however, it was most potent at inhibiting acetyl choline induced contraction.

In animals, 5-HT brings about contraction of smooth muscles by stimulating the 5-HT_2 receptors. The pronounced inhibitory activity of aqueous extract against contraction induced by 5-HT in rat fundus strip indicates that the inhibitory activity may be helpful for anti-asthmatic action but its selectivity about the sub types of 5-HT2 receptor antagonism is not cleared. Therefore, it is likely that the chloroform extract interacted with 5-HT to bring about enhanced activity at this receptor, leading to physiological antagonism of the contractile effect at the 5-HT2 receptors.

Furthermore, the relaxation of histamine and Acetyl choline precontracted trachea by the extracts indicates their potency in ameliorating established asthma. Airway hyper responsiveness in asthma is attributed in part to changes in autonomic regulation particularly increased parasympathetic activity [18]. The extracts decreased acetylcholine-induced contraction of the guinea pig ileum, this inhibitory activity might be by the antagonism on the muscarinic receptors, especially at higher doses.

Airway obstruction/bronchconstriction or airway hyper responsiveness in asthma is believed to be a direct consequence of air way wall inflammation [19]. Mechanisms that possibly underlie this anti-inflammatory activity include inhibition of the actions of inflammatory mediators such as histamine, effect on adrenocorticoid hormone and immunosuppression.

So the possible mechanism of its anti asthmatic activity is might be due to its inhibitory action on histamine, acetyl choline and 5-HT release. Previous studies showed that it also possess anti-inflammatory action which could also be one of the possible mechanisms. Further studies are needed for exact mechanism of action and isolation of active constituent for its activity.

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