Int J Pharma Res Health Sci. 2016; 4 (6): 1478-1482
DOI:10.21276/ijprhs.2016. 06.09
S Fathima *et al.*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Study of Antiasthmatic Activity of Ethanolic Extract of *Alternanthera Sessilis* Leaves

Syeda Nishat Fathima[1,*], Amreen Salwa[1], S. Anusha[2], Somaiya Fatima [3]

[1] Department of Pharmacology, Jayamukhi College of Pharmacy, Narsampet, TS, India
[2] Department of Pharmacognosy, Jayamukhi College of Pharmacy, Narsampet, TS, India
[3] Department of Pharmacology, Deccan School of Pharmacy, Hyderabad,TS, India

ARTICLE INFO

Received: 21 Nov 2016
Accepted: 18 Dec 2016

ABSTRACT

Background and Objective: Asthma is a common complex chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospasm. The present study was aimed to explore the activity of ethanolic extract of *Alternantherasessilis* leaves for antiasthmatic activity.
Methods: The asthmatic activity of *Alternantherasessilis*(500 mg/kg, p.o) was evaluated by Histamine aerosol induced bronchospasm in guinea pigs and bronchial hyperreactivity was studied on broncho-alveolar lavage fluid (BALF) in egg albumin sensitized guinea pigs and are compared with Mepyramine (8 mg/kg, p.o.) as standard.
Results: The occurrence of asphyctic convulsions were delayed by treatment with of ethanolic extract of *A.sessilis* leaveswhichsignificantly increased the percentage protection as compared to control following exposure to Histamine diphosphate aerosols. Significant decreased in the total leukocyte and differential leukocyte count in the BALF of the egg albumin sensitized guinea pigs was observed by administration of ethanolic extract of *A.sessilis* leaves (500 mg/kg, p.o., for 15 days,).
Conclusion: The data suggest that the Antiasthmatic activity of ethanolic extract of *Alternantherasessilis* leaves may be possibly due to the inhibition of antigen induced histamine release or reduction in leucocyte count.
Key words: Asthma, broncho-alveolar lavage, bronchospasm.

1. INTRODUCTION

Asthma is a common complex chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospasm. It is characterized by recurrent episodes of wheezing, coughing, shortness of breath and chest tightness. Common symptoms include wheezing, chest tightness, and shortness of breath [1]. The most current reviewed global estimate of

Corresponding author *
Syeda Nishat Fathima
Jayamukhi College of Pharmacy, Narsampet, TS, India
Email: syeda.nishu.fathima@gmail.com

asthma proposes that as many as 334 million people have asthma, and that the burden of disability is highest prevalence in industrialized countries [2]. Asthma pathophysiology includes a variable degree of airflow obstruction (related to bronchospasm, edema, and hypersecretion), bronchial hyper responsiveness, and airways inflammation apart from over 100 different inflammatory mediators and multiple inflammatory effects. Acute asthamatic symptoms arise due to activation of mast cells by IgE, whereas eosinophils, macrophages and T-helper 2 cells are involved in the chronic inflammation that underlies airway hyper responsiveness. Other inflammatory mediators that are involved in asthma are including lipid and peptide mediators, chemokines, cytokines and growth factors [3]. Presently used anti asthamatic drugs reduce the frequency of exacerbations and current medications that are used are anti-inflammatory drugs, in particular inhaled corticosteroids, bronchodilators such as short and long acting β_2 - adrenoceptor agonists, Leukotriene receptor antagonists, Mast cell stabilizers and Anti-IgE Therapy [4]. Inappropriate and long term use of these asthamatic medications causes development of cataracts and a mild regression in stature. In the past decade, research has been focused on scientific assessment of traditional drugs of plant origin for the treatment of various ailments. Since the time immemorial, various herbs are used as antiasthmatic with efficient therapeutic response. Examples of various plants and its extracts used in asthma are Adhatodavasica, Albizzialebbeck, *Allium sativum*, Artemiciacaerulenscens, Boswelliaserrata, Calotropisgigantea, *Coleus barbatus*,Calotropisprocera, Cedrusdeodara, Clerodendronserratum, Curcuma longa, Eugenia caryophylis, Eleocarpusspharicus, *Ginkgo biloba* ,Inularacemosa, *Lobelia inflata*Ocimum sanctum, Picrorrhizakurroa, Piper longum, Sarcostemmabrevistigma, *Scutellariabaicalensis*,Solanumxanthocarpum, *Tussilagofarfara*,Tephrosiapurpurea, Tinosporacordifolia,*Trifoliumpratense* Tylophoraasthmatica, Vitexnegundo, *Verbascumthapsus* etc. ones [5, 6]. Developing formulations using plants will not only be effective for management of diabetes but also will be economical and relatively safer than currently available treatments. In the present study ethanolic extract of *Alternantherasessilis* leaves are employed to evaluate the antiasthmatic activity as it is used as by folklore.

*Alternantherasessilis*linn (Amaranthaceae) is an annual or perennial prostate herb known by several common names, including sessile joyweed and dwarf copperleaf. The species occurs throughout tropical and subtropical regions of the world. It is a typical plant of the flood plain wetlands, margins of rivers, streams, canals, ponds, reservoirs and tanks in India used throughout South India; the Phytoconstituents present are hydrocarbons, ester, and sterols, such as stigmasterol, campesterol, ß-sitosterol, a- & ß-spinasterol, a-stigmasteanol and palmitates of sterol; it also contains 24-methylenecycloartanol, 5-a -stigmasta-7-enol and cycloeucalenol. Traditionally it is uses inophthalmia, gonorrhea, Galactagogue, Cholagogue, intellect promoting, strength, Abortifacient, Febrifuge, diuretic, leprosy, skin disease and dyspepsia, tonic and cooling. In-vitro and in-vivo studies has proven *Alternantherasessilis*to possessanti-diarrhoeal activity, anti-Inflammatory activity, anti-microbial activity, anti-oxidant activities, antipyretic activity, anti- ulcer activity, hepatoprotective activity, hematinic activity, hypoglycemic activity, nootropic activity and wound healing activity [7]. The present study has been carried out to explore the activity of ethanolic extract of *Alternantherasessilis* leaves for antiasthmatic activity

2. MATERIALS AND METHODS

Plant materials:

The fresh leaves of the young and matured plants *Alternantherasessilis*were collected in bulk from local area of Warangal, Telangana, India. The leaves were authenticated by authenticated by Dr. P. Veera Reddy, Professor, Government Ayurvedic College, Warangal, Telangana.

Drugs and Chemicals:

Histamine (Qualikems Fine Chem Pvt. Ltd., India), Mepyramine (Cadilla Healthcare Ltd, India) and Tween 80(S.D Fine Chemicals, India).Egg albumin, aluminium hydroxide, and other chemicals were purchased from Himedia Laboratories Pvt. Ltd., India. All other reagents used for the experiments were of high analytical grade.

Preparation of plant extract:

After collection, leaves were washed very carefully and clearly with water and dried under shade. The dried leaves were pulverized in an electrical processor and then the powder was separated.50 gram of dried powder material was extracted in a soxhlet apparatus with 200 ml. of absolute alcohol. The ethanolic leaf extract was then distilled, evaporated and dried in vacuum. All the extract were kept in desiccator and stored in a refrigerator for pharmacological experiment.

Animals:

Guinea pigs of either sex, weighing about 400-600 grams were used in experiments. Animals were housed in polypropylene cages maintained under standard condition (12 hours light / dark cycle; temperature 25± 3 ^{0} C; relative humidity 55 ± 5%) and had free access to standard pellet feed (Hindustan Lever Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. The experiments on animals were conducted in accordance with the IAEC and CPCSEA and our protocols were duly approved by the Institutional Ethical Committee.

Acute Toxicity Studies:

A preliminary pharmacological study was conducted to assess the gross behavioral effects and safety effects of the drug. The acute toxicity study was carried on mice weighing

about 20-25gm as per ICH guidelines [8].Overnight fasted mice received test extract at a dose of 100mg/kg intraperitoneally and mortality was observed for 14 days. If mortality was not observed for any animal then the procedure was repeated again with higher doses such as 300, 1000 and 2000 mg/kg.

The animals were observed continuously for 2 h for general behavioral, neurological, autonomic profiles and to find out percentage of mortality observations were tabulated according to Irwin's table [9]. For this the following check list was employed:

Stimulation: Hyperactivity, Piloerection, Twitching, Rigidity, Irritability, Jumping, Clonic convulsions, Tonic convulsions

Depression: Ptosis, Sedation, Loss of righting reflex (sleep), Loss of traction, Loss of Pinnal reflex, Catatonia, Ataxia, Loss of muscle rigidity, Analgesia.

Autonomic reflexes: Straub's tail, Laboured respiration, Cyanosis, Reddening, Abnormal secretions, balancing.

METHODS EMPLOYED IN SCREENING OF ANTIASTHAMATIC ACTIVIY:

Bronchial hyperreactivity by Histamine aerosol induced bronchospasm in guinea pigs [10]:

Guinea-pigs, which have been fasted for 24 h, were each exposed to an atomized spray of 0.2% histamine dihydrochloride aerosol 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 labored breathing leading to convulsions, asphyxia and death. The time of aerosol exposure until the time of onset of dyspnea leading to the appearance of convulsions, was recorded as the pre-convulsive time (PCT). This PCT was taken as the basal control value (T1). The animals were removed from the chamber and exposed to fresh air to recover, as soon as pre-convulsive dyspnea commenced. In the present experiments the criterion used was time for onset of dysponea and percent protection was calculated. 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 six animals each. Group 1 was served as the normal control group and treated with distilled water alone; Groups 2 served as the positive control and treated with Mepyramine (8 mg/kg, p.o.); Group 3-was treated with ethanolic extract of *Alternantherasessili s*(500 mg/kg, p.o) for 15 days last two hours after administration of last dose the animals were again subjected to histamine aerosol as on day 0 to determine PCT (T2). The protection offered by the treatment was calculated, using the formula

$$\%\text{protection}=\left\{1-\left(\frac{T1}{T2}\right)\right\}\times 100$$

Where, T1 = basal control value PCT on day 0 and T2 = PCT after Administration of test drugs on day 14.

Studies on broncho-alveolar lavage fluid (BALF) in egg albumin sensitized guinea pigs [11]:

Guinea pigs were selected and they were divided in groups of six animals each. Group 1 was served as the normal control group and treated with distilled water alone; Groups 2 served as the positive control and treated with Mepyramine (8 mg/kg, p.o.); Group 3-was treated with ethanolic extract of *Alternantherasessilis*(500 mg/kg, p.o); Group 4-sensitized group. Animals of group 2, 3 and 4 were sensitized with egg albumin (1 ml, 10% w/v, i.p.) on the 1st day. The animals of group 2 and 3 were dosed one daily with their respective treatments for fifteen days. On the 15th day two hours after last dose of treatment, all the animals of group 2, 3 and 4 were again challenged with egg albumin (0.5 ml, 2% w/v, i.v.) through saphenous vein. 3 hours after egg albumin challenge or just before the death of animals whichever occurs earlier, the animals were anesthesized and the trachea was cannulated. Into the trachea polyethylene tube was fixed and 1ml of phosphate buffered saline was injected by tuberculin syringe and 2ml of BALF was aspirated from it. The BALF suspension was centrifuged and stored at -70^{o} C. 1 in 10 dilutions was made in saline and differential leucocyte count and total white blood cells count were estimated by Automated Cell Counter.

Statistical Analysis [12]:

Values were expressed as Mean ± standard error of the mean. The Significance of differences among the group was assessed using one way analysis of variance (ANOVA). The test followed by Dunnett's multiple comparisons test of significance. p values less than 0.05 were considered as statistically significant.

3. RESULTS

The Histamine aerosol when given by nebulizer produces symptoms such as increased breathing frequency, forced inspiration, and finally a sphyctic convulsions. The occurrence of these symptoms can be delayed by *Alternantherasessilis* extract which significantly increased the percentage protection as compared to control following exposure to Histamine diphosphate aerosols. The above results are tabulated in table 1.

Table 1: Dose and PCT time

S.No	Treatment	Dose	PCT Time (sec) Before Treatment on Day 0	PCT Time (sec) After Treatment on Day 14	% Protection
1	Control(Saline)	1.0 ml/kg	104.75±8.93	112.23±14.81	0
2	Mepyramine	8 mg/kg, p.o.	97.53± 8.46	286.42±11.32**	65.94**
3	*A. sessilis*	500mg/kg, p.o	102.47±10.24	156.91±14.57*	42.10**

Values are mean ± S.E.M. (n=6) One way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control.

After fifteen days of treatment guinea pigs were again challenged with egg albumin and broncho-alveolar lavage fluid was studied. In the broncho alveolar lavage fluid significant increase in the total leukocyte count and differential leukocytescount were observed in sensitized group as compared to the control group. Sensitized guinea pigs when treated with *Alternantherasessilis* extract showed significant decrease in total leuocyte count and differential leukocytes count. The above results are tabulated in table 2.

Table 2: Total Cell count on treatment

S. No	Treatment	Total leukocyte Count (Cells/ml)	Neutrophil (Cells/ml)	Lymphocyte (Cells/ml)	Eosinophil (Cells/ml)	Monocytes(Cells/ml)
1	Saline, 1.0 ml/kg	5209.4±421.7	3264.6±364.6	1648.6±56.9	128.3±32.5	96.4±22.5
2	Sensitized group	9128.6±524.3	6984.4±351.4	1972.9±84.3	236.8±41.4	152.3±25.6
3	Mepyramine (8 mg/kg, p.o.)	5613.3±483.4**	3562.2±402.3**	1589.7±398.1**	124.4±31.7**	121.2±28.1**
4	*Alternantherasessilis*(500 mg/kg, p.o)	6806.5±692.1**	4203.9±381.2**	1663.4±42.7**	183.6±43.6**	103.5±21.3**

Values are mean ± S.E.M. (n=6) One way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to Sensitized group.

4. CONCLUSION

The present study was undertaken to evaluate the antiasthmatic activity of ethanolic extract of *Alternantherasessilis* leaves. Asthma is characterized as chronic airway inflammation and increased airway hyper-responsiveness. Histamine is a ubiquitous inflammatory mediator intimately associated with the pathology of airway inflammation seen in Asthma [13]. When Histamine was administered in the form of aerosol it produced dyspnoea leading to Convulsions by Stimulation of H1 receptors. Bronchial hypere activity induced by Histamine in guinea pigs was evaluated by measuring PCT Time. The ethanolic extract of *Alternantherasessilis* leaves significantly increased the PCT and percentage protection there by giving an impression that it has antihistaminic activity. Asthma is described by an inflammatory state resulting in activation of lung tissue cells and attraction and infiltration of leukocytes from the blood.The accumulation of leukocytes is a prominent feature of increase in bronchial hyper-responsiveness that occurs in asthma [14].Broncho-alveolar lavage fluid (BALF) in egg albumin sensitized guinea pigs showed increased in number of total leukocyte count and differential leukocytes count when ethanolic extract of *Alternantherasessilis* leaves was administered significant decrease in them was noted suggesting that the extract has anti-inflammatory effect as it reduces total and differential inflammatory cells.

5. CONCLUSION

The present observations provide evidence that ethanolic extract of leaves of *Alternantherasessilis* exhibited antiasthmatic activity. The extract may exert it effect by its anti-inflammatory and antihistaminic activity. However, further studies are required to establish molecular mechanism and to isolate or characterize the bioactive compounds, which are mainly responsible for the antiasthmatic action.

6. REFERENCES

1. O' Byrne PM,Postma DS. The many faces of airway inflammation– Asthma and Chronic obstructive pulmonary disease.Am J RespirCrit Care Med.1999; 159: S41-S66.
2. Asher I, Pearce N. Global burden of asthma among children. Int J Tuberc Lung Dis. 2014;18(11):1269-78.
3. Busse WW, Lemanske RF Jr. Asthma. N Engl J Med. 2001;344(5):350–62.
4. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. EurRespir J. 2014; 43(2):343-73.
5. Yaheya M,Ismail M. Antiasthmatic Herbal Drugs A Review. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 2(3):28-29.
6. Dnyaneshwar J T, Ravindra Y Patil. Some medicinal plants with antiasthmatic potential: a current status. Asian Pac J Trop Biomed. 2011; 1(5): 413–418.
7. Thomas M.W, Merish S, Tamizhamuthu M. Review of AlternantheraSessilis with Reference to Traditional SiddhaMedicine. International Journal of Pharmacognosy and PhytochemicalResearch2014; 6(2):249-254
8. ICH Topic S 7 A, Safety Pharmacology Studies for Human Pharmaceuticals. Note for guidance on safety pharmacology studies for human pharmaceuticals. [Internet].2015 [cited on 15 July 2015]. Available from: http://www.ema.europa.eu/pdfs/human/ich/053900en.pdf.
9. Irwin S. Comprehensive behavioral assessment: 1a A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia. 1968; 13(3):222-57.
10. Vogel HG. Drug Discovery and Evaluation-Pharmacological Assays. 2nd Edition. New York: Springer-Verlag Berlin Heidelberg. 2002. Chapter D Respiratory activity; 188-189.
11. Walls AF, Rhee YK, Gould DJ, Waiters C, Robinson C, Church MK, Holgate ST. Inflammatory mediators and cellular infiltration of the lungs in a guinea pig model of the late asthmatic reaction. Lung (1991) 169:227-240
12. Indranarayana A, Satyanarayana L. Biostatistics for Medical, Nursing and Pharmacy Students. New Delhi: Prientice Hall of India Private Limited. 2006; Chapter 16 General principles of Statistical test; 219-230, chapter 17: Statistical significance parametric and nonparametric test; 231-244.

13. White MV. The role of histamine in allergic diseases. J Allergy ClinImmunol. 1990;86(4):599-605.
14. Faoud TI. The Inflammatory Response in the Pathogenesis of Asthma.The Journal of the American Osteopathic Association. 2011; 111:S11-S17.

Conflict of Interest: None

Source of Funding: Nil