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Evaluation *Alpinia galanga* Essential Oil for Anti Asthmatic Activity in Animal Models

Jyotsna Laksmhi Patil[1], Suma US[1]*, Mamatha MK[2] , Priya Shetti[2]

[1]KLEU'S college of pharmacy, Belgaum, Karnataka 590016
[2]Mallige college of pharmacy, Bangalore 560090

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Corresponding Author:
E-mail : Sumau316@gmail.com
Mob.: +919164657721

Abstract

The aim of the present study was to evaluate antiasthmatic activity of *Alpinia galanga* essential oil by using various animal models.Essential oil of *Alpinia galanga* was procured from GR herbals, Indore & Chemical analysis was carried out through GC-MS. In present study investigated the contraction inhibition activity on tracheal chain using histamine (10 µg/ml). For *in vivo* studies PCD time was observed in 0.1% histamine exposed guinea pigs. The present findings demonstrated significantly decrease the airway inflammation induced by ovalbumin. Hence the present study verified that *A.galanga* essential oil bearing antihistaminic, anti-inflammatory effect.

1 Introduction

Asthma or Chronic obstructive pulmonary disease also called as Emphysema. It affects 10 million people , resulting annually in 2 million emergency room visits, 500,000 hospitalize and 5000 death[1]. Medicinal plants have been used for various ailments by an alternative system of medicine and folklore treatments. However many ethnopharmacological uses are yet to be justified scientifically for the rational and safe use. Volatile oil containing plants used as an acute and chronic bronchitis and also shows anti-inflammatory effect on trachea and tracheids which reduces asthma[2].

Alpinia galanga (Fam: Zingiberaceae) is a well-known medicinal herb used for the remedy of various disorders. Traditionally rhizome of *Alpinia galanga* is used as anti-tubercular, hypothermia, bronchial catarrh, antimicrobial, expectorant, reduce sputum, dilates bronchioles and reduce asthma[3-7].

The present study focuses on the possible beneficial effects of *Alpnia galanga* essential oil on asthma.

2 Material and Methods

2.1 Chemicals

Sodium chloride, potassium chloride and Ovalbumin were purchased from Sigma chemicals USA, Disodium hydrogen phosphate, Histamine hydrochloride, Tween-80, Foetal bovine serum, Alumina, Ammonium chloride,chloroform, diethyl ether and sodium dihydrogen phosphate were purchased from Himedia Pvt Ltd Mumbai, Dexamethasone from centaur pharmaceuticals Goa, Trisodium citrate from Rankem, New Delhi. Disodium EDTA from Nice chemicals Pvt Ltd, Cochin.

2.2 Plant collection

Alpinia galangal was collected from Belagaum.

2.3 Needle selection

The appropriate needles were selected for dose administration (Table 1).

2.4 Procurement of essential oil

Essential oil of *Alpinia galanga* along with certificate of Analysis (COA) were procured from GR HERBALS, 11-B Kankariya Road, Industrial Area, Hatod, Dist-Indore-453111, and essential oil was stored at 2 to 8 ^{0}C.

Table 1: Oral feeding Needles and their description

Purpose	Needle size
Oral dosing needle for mice	24G, 1/2 ", curved ball ended
Oral dosing needle for guinea pigs	18G, 1 and 1/2 ", curved ball ended

2.4.1 Phytochemical investigation[8]

Essential oil was subjected to Phytochemical screening using GC-MS.

The GC instrument - Shimadzu Chromatogram

Initial oven temperature - 40°C

Final oven temperature - 280°C

The Gas Chromatography/Mass Spectrometer (GC/MS) of the essential oils was carried out using an Agilent Gas Chromatography. The oven temperature was set from 40°C – 280 °C. Helium was used as the carrier gas at a flow rate of 5 ml/min, with a split ratio of 1: 200. 0.5 µl of the essential oil solution was manually injected into the GC/MS.

2.5 Pharmacological investigation

2.5.1 Animal selection

Male Albino mice weighing 18-26gm were procured from Sri. Venkateshwara Enterprises, Bangalore and Guinea pigs weighing 300-600gm were procured from Central Animal House, JNMC College Belagavi. Albino mice were housed in solid bottom polypropylene cages with a stainless steel grill on top. Guinea pigs were housed in open wooden slab. Animals kept with bedding of clean paddy husk at an ambient temperature and humidity, with12–12 h light and dark cycle. The animals were kept on *ad libitum* feed and water, after fifteen days of acclimatization period; they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) Resolution NoKLECOP/IAEC/Res.17-31/08/2013

2.5.2 Animal dose selection[9,10]

According to literature reviews we confirmed that the LD_{50} of *Alpinia galanga* is 2000mg/kg, thus present study was to evaluate antiasthmatic activity of the dose 200mg/kg($1/10^{th}$) as a lower dose and 400mg/kg($1/5^{th}$) as a higher dose of LD_{50} was selected

2.6 Preparation of drug and reagents

2.6.1 Preparation of test drugs

For *in vitro* model- 100µg/ml of *A.galanga* essential oil was prepared in 1% tween 80.

For *in vivo* models- *A.galanga* essential oil was emulsified with water in proportion of 2:2:1 (Essential oil:Water:Tween 80).

2.6.2 Kreb's solution[8,10]

The compositions of kreb's solution are mentioned below:

Chemicals		Kreb's solutions (gm/liter)
NaCl	-	6.9
KCl	-	0.35
$CaCl_2$	-	0.28
$MgSO_4.7H_2O$	-	0.28
$NaHCO_3$	-	2.1
KH_2PO_4	-	0.16
Glucose	-	2.0

2.6.3 Phosphate Buffered Saline (pH 7.4)

- Measure a volume of 800 ml of Double distilledH2O with a graduated cylinder and transfer to an Erhlenmyer's flask.
- Add a magnetic stir bar to the Erhlenmyer's flask and place the flask on a magnetic stir plate. Adjust the speed of the magnetic stir bar so that oxygen is not introduced into the solution while it is rapidly mixed. Transfer to the flask: (a) 8 g of NaCl; (b) 0.2 g KCl; (c) 1.44 g of Na2HPO4; (d) 0.25 g of KH2PO4.
- Allow the solutes to dissolve for 3 to 5 min.
- Ensure that there are no remaining particles of undissolved salts in the solution before adjusting the pH. If particles are present, continue stirring vigorously.
- Reduce the speed of the magnetic stir bar so that the solution is gently mixing.
- Ensure that the pH meter has been properly calibrated (see and rinse the pH probe with ddH2O. Remove the excess water from the probe tip (without touching the probe tip) with a clean paper towel. Place the pH probe into the solution.
- Slowly add 1 M HCl dropwise with a transfer pipette and allow the HCl to fully dissolve into the solution. Stop stirring the solution.
- Measure the pH with the pH meter.
- Repeat Steps #8 through #10 until the pH of the solution is 7.4.
- Pour the solution into a fresh graduated cylinder and adjust the final volume to 1 litre double distilled water.

- Transfer the solution to a container that is suitable for autoclaving at 15 lb/in2.
- Autoclave the solution on liquid cycle at 15 lb/in2 for 20 min.

2.6.4. Alum precipitated OVA[9]

Dissolve 50µg of OVA in 0.2ml of PBS which contains 1mg of $Al(OH)_3$ for each animal.

2.6.5 10mM Na2-EDTA

Molecular weight of Na_2-EDTA= 372.24

Dissolve 3.72gm of Na2-EDTA in 100ml of distilled water and make up to 1 litre.

2.6.6. ACK Buffer

ACK lysing buffer is used to lyse red blood cells in a preparation which contains white blood cells.

Formula

NH_4Cl 8,024 mg/l (53,49g/mol)

$KHCO_3$ 1,001 mg/l (100,12g/mol)

$EDTA.Na_2{\cdot}2H_2O_3$.722 mg/l (372,24g/mol)

2.7 Methodology

2.7.1 Spasmolytic activity on guinea pig isolated tracheal chain[10,]

Many research studies reported that there is a preponderance of H1 excitatory and a scanty population of H_2 receptors on isolated guinea tracheal preparation. There is a contractile response of different agonists like Ach, histamine, 5-Hydroxytryptamine and bradykinin on guinea pig trachea.

Preparation of drug solution

Test drug was emulsified in 1% tween 80(100µg/ml).

Histamine (10 µg/ml) dissolved in distilled water

2.7.2 Histamine induced bronchospasm in guinea pigs

On day 1 and 7, guinea pigs were exposed to 0.1% w/v of histamine dihydrochloride aerosol in histamine chamber. On days 1 to 7, animals were received treatment drugs. The progressive dyspnoea was observed in animals when exposed to histamine aerosol. The end point, pre-convulsion dyspnoea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion.

Percentage increased in time of PCD = (1-T1/T2) × 100

Where: T1 = time for PCD onset on day 0

T2 = time for PCD onset on day 7

2.7.3 Ovalbumin induced sensitization in mice

Ovalbumine used as a exogenous antigen. On day 1 and 7, positive control group mice were systemically sensitized with a subcutaneous injection of a suspension containing 50µg of ovalbumin (OVA) and 1mg aluminum hydroxide [$Al_2(OH)_3$] in 200µl of phosphate buffer solution (PBS).

2.8 Statistical Analysis

Results were expressed as Mean ± S.D., where n= 6. Differences among data were determined using one way ANOVA followed by Dunnette's multiple comparison test (Graph Pad Prism software, version 5.01). $p<0.05$ was considered statistically significant.

3. Results

3.1 Phytochemical analysis

Essential Oil was subjected to phytochemical screening using GC-MS.

The GC instrument - Shimadzu Chromatogram

Initial oven temperature - 40^0C

Final oven temperature - 280^0C

The Gas Chromatography/Mass Spectrometer (GC/MS) of the essential oils was carried out using an Agilent Gas Chromatography. The oven temperature was set from 40°C – 280 °C. Helium was used as the carrier gas at a flow rate of 5 ml/min, with a split ratio of 1: 200. 0.5 µl of the essential oil solution was manually injected into the GC/MS.

As a result of chemical investigation, the essential oil obtained from *Alpinia galanga* L. identified the presence of α-pinene, β-pinene, Limonene, Isobornyl formate and Eudemia 4(14)11 diene (Fig 1)

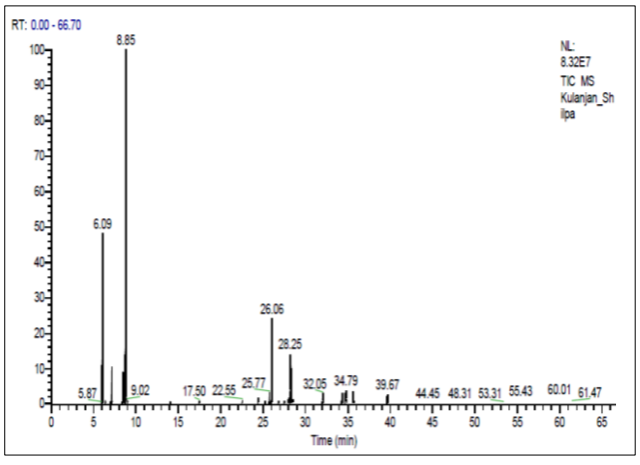

Fig 1: Qualitative analysis of *Alpinia galanga* essential oil

3.2 Effect of Alpinia galanga essential oil on contraction of tracheal smooth muscle

Guinea pig tracheal chain is much more sensitive for dose relative contraction of agonist like histamine. In isolated tracheal chain preparation, there was statistically significant(***$p<0.001$,

**p<0.01, *p<0.05, ns- non significant in 2µg ,1 & 4 µg,8 µg and 16 µg of histamine respectively) decrease in contraction of tracheal smooth muscle in presence of *Alpinia galanga* essential oil and it showed 57.6±14.61(%) inhibition of contraction on smooth muscles (Table 2).

Table 2: Effect of *Alpinia galanga* essential oil on contraction of tracheal smooth muscle

Histamine Dose (µg)	Height of response before treatment (cm)	Height of response after treatment (cm)	% inhibition of drug due to test drug
1	2.03±0.19	0.47±0.07**	76.50±4.36
2	2.5±0.06	0.77±0.18***	68.98±7.83
4	2.83±0.07	1.33±0.28**	53.17±9.35
8	3.06±0.09	1.63±0.39*	47.30±11.96
16	3.1±0.15	1.75 ± 0.75^{ns}	42.05±20.25

All the values were expressed as Mean± SEM using one way ANOVA followed by Dunnette's multiple comparision test, where n=5, a- when compared to asthma control. (***p<0.001, **p<0.01, *p<0.05, ns- non significant)

3.3 Effect of *Alpinia galanga* essential oil on histamine induced bronchospasm in guinea pig

Histamine causes very strong smooth muscle contraction. After introducing histamine aerosol to guinea pig (highly sensitive for histamine) it produces Pre-convulsion dyspnea (PCD).The time taken to initiates PCD was observed. There was no significant difference in PCD time in asthmatic control, there was significant (p<0.001) increase in PCD time after treatment with dexamethasone (2mg/kg) and significant (p<0.001, p<0.01) increases the time with the dose of *Alpinia galanga* 200mg/kg & 400mg/kg, respectively (Table 3).

Table 3: **Effect of *Alpinia galanga* essential oil on histamine induced bronchospasm in guinea pig**

Animals	PCD Time (sec.)							
	Control		Asthma+Dexamethasoe (2 mg/Kg)		Asthma+*A. galanga* essential oil (200 mg/Kg)		Asthma+*A. galanga* essential oil (400 mg/Kg)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	144	179	69	239	90	170	57	160
2	132	109	170	400	94	186	107	242
3	119	125	90	315	105	221	127	231
4	91	99	107	341	99	168	98	272
5	85	98	117	325	126	237	105	295
6	112	106	135	319	92	195	118	225
Mean	112.67 21.17	1193 28.13	114.67 32.23	318.17 47.28^{***}	101.00 12.22	196.172 5.26^{**}	102 22.20	237.5 42.27^{***}
% protection	-	11.43 6.24	-	64.97 6.81	-	48.29 4.37	-	57.14 9.16

Values are expressed as mean ± SEM for 6 animals in each group One Way ANOVA followed by Dunnett's Multiple Comparison Test., ***P<0.001 when compared with Diseased., **P<0.01 when compared with Diseased

Table 4 and 5 demonstrated Leukocyte count in BALF elevated in response to the inflammation due to the OVA exposure. Asthmatic showed significant (P<0.001) elevation in leukocyte count and there was elevation of leukocyte count was observed in aluminium hydroxide sensitised group whereas significant (P<0.001) decrease in dexamethasone group and high dose (400mg/kg) of Alpinia galanga group showed significant

decrease in leukocyte count as compared with asthmatic control.

There was significant (P<0.001) increases AEC level in asthmatic group (380.9±26.47) as compared to normal group (47.43±5.66) whereas after treatment with dexamethasone significant (P<0.001) decreases to 87.09±10.49 and high dose of *Alpinia galanga* treatment group showed (P<0.001) decreases to 159.8±16.88 of AEC as compared to asthmatic control.

Table 4: Effect of *Alpinia galanga* essential oil on Total Leukocyte Count in BALF in control and experiments

	Total Leucocyte count (cells/µl)					
Animal	**Normal**	**$Al_2(OH)_3$**	**Asthma**	**Asthma+Dexamethasone (2 mg/Kg)**	**Asthma+*A.galanga* essential oil (200 mg/Kg)**	**Asthma+*A.galanga* essential oil (400 mg/Kg)**
1	250	200	1350	500	950	700
2	200	300	1200	450	850	650
3	250	300	1300	450	1050	750
4	200	250	1400	400	950	600
5	150	200	1250	550	900	700
6	250	300	1350	400	1050	650
Mean±SEM		258.33 49.16 ###	1308.33 73.60***	458.33 58.45***	958.33	675

Table 5: Effect of *Alpinia galanga* essential oil on Absolute Eosinophil Count in blood in control and experimental mice

	Absolute Eosinophil Count in Blood (cells/µl)					
Animal	**Normal**	**$Al_2(OH)_3$**	**Asthma**	**Asthma+Dexamethasone (2 mg/Kg)**	**Asthma+*A.galanga* essential oil (200 mg/Kg)**	**Asthma+*A.galanga* essential oil (400 mg/Kg)**
1	45.55	53.22	353.51	74.32	175.50	141.53
2	47.00	57.41	374.50	89.00	197.50	179.83
3	54.74	53.59	401.96	101.36	218.67	174.32
4	40.67	49.39	416.50	79.33	229.49	138.32
5	43.00	45.00	388.49	81.83	240.00	161.51
6	53.59	47.83	350.18	96.70	188.73	163.51
Mean±SEM	47.43±5.66	51.07±4.5	380.9±26.47###	87.09±10.49***	208.3±255***	159.8±16.88***

3.4 Effect of *Alpinia galanga* essential oil on IgE antibody concentration in blood serum in control and experimental mice

Ovalbumin induced model of allergic airway inflammation (inflammatory mediators) switches B-lymphocytes to produce IgE antibodies. There was significant (P<0.001) increased serum IgE level in asthmatic group (14.03±0.32) as compared to normal group (1.843±0.18) whereas after treatment with dexamethasone it decreases significantly (P<0.001) to 2.465±0.21 and high dose of *Alpinia galanga* treatment group showed 2.74±0.20 serum IgE level which was highly significant as like dexamethasone (Table 6).

4 Discussions

The present study was aimed to evaluate the anti-asthmatic activity of *Alpinia galanga* wild. essential oil using various animal models like isolated guinea pig tracheal chain model, bronchial hyperactivity in guinea pigs and ovalbumin induced sensitization in mice. Phytochemical analysis of procured *Alpinia galanga* essential oil was assessed by using

GC-MS, showed presence of flavonoids such as α pinene, 1,8 cineol, humelene, geranyl acetate, isobornyl formate, eudemia 4(14)11 diene, cubenol, viridiflorol, isomenthome, piperinol, geranyl acetate,terpene-4-ol,1,8 cineol, linolool.

The present study was planned to evaluate the actions of test drug on various aspects of asthma like bronchoconstriction, eosinophilia, allergy associated with inflammation using various *in-vitro* and *in-vivo* animal models.

Table 6: Effect of *Alpinia galanga* essential oil on IgE antibody concentration in blood serum in control and experimental mice

	Immunoglobulin E Concentration in Serum (ng/ml)					
Animal	**Normal**	**$Al_2(OH)_3$**	**Asthma**	**Asthma+Dexamethasone (2 mg/Kg)**	**Asthma+*A.galanga* essential oil (200 mg/Kg)**	**Asthma+*A.galanga* essential oil (400 mg/Kg)**
1	1.84	1.96	13.57	2.21	3.17	2.46
2	2.02	1.88	14.26	2.53	3.27	2.94
3	2.07	2.09	13.8	2.25	3.91	2.74
4	1.66	1.98	14.49	2.76	3.73	3.01
5	1.61	2.21	14.03	2.6	4.41	2.67
6	1.86	2.35	14.03	2.44	4.23	2.65
Mean±SEM	1.843±0.18	2.078±0.18	14.03±0.32	2.465±0.21	3.787±0.50	2.745±0.20

In the present study ovalbumin was used as an allergen which acts as an antigen presenting cell carrier antigen to induce and precipitate the asthmatic reaction by switching T cell receptors especially Th 2 type lymphocytes.Ovalbumin induced model of allergic airway inflammation demonstrates that IgE levels in blood and eosinophilic infiltration in the lungs are markedly increased in asthmatic condition.

The purpose of this study was to evaluate antiasthmatic activity of *Alpinia galanga* essential oil using animal models.

The *A. galanga* essential oil showed smooth muscle relaxation activity which is primary treatment of asthmatic attack. By observing this effect, the present study suggests that essential oil was effective on smooth muscle (bronchoconstriction) in allergic condition.

In histamine induced bronchospasm model, *A. galanga* showed dose dependent % protection against PCD, which proved that histamine that antihistaminic activity. The present study also suggest that it significantly decrease the airway inflammation induced by ovalbumin hence the present study proved that essential oil bearing antihistaminic, anti-inflammatory effect which has essential to treat asthma. On the basis of the present study it is concluded that the essential oil of *A. galanga* possess antiasthmatic activity.

Further studies are needed to investigate the possible mechanism of action and the active constituents responsible for the activity are to be isolated and identified to validate the therapeutic potential.

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6 Conflict of Interest

No

7 Author's contributions

JLP conducted the research and sus wrote the paper; MMK and PS prepared the tables and alignment.

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