

Bronchodilator Activity in Traditional Medicines: Gift of God Kingdom

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1. Introduction

Since ancient times humanity has depended on the diversity of plant resources for food, clothing, shelter, and traditional medicine to cure myriads of ailments. Early humans recognized their dependence on nature in both health and illness. Physical evidence of the use of herbal remedies has been found some 60,000 years ago in a burial site of a Neanderthal man uncovered in 1960 in a cave in northern Iraq. Here, scientists found great quantities of plant pollen, some of which came from medicinal plants still used today. The first written records detailing the use of herbs in the treatment of illness are in the form of Mesopotamian clay tablet writings and Egyptian papyrus. Led by instinct, taste and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or non-active, and also which combinations or processing methods had to be used to gain consistent and optimal results. Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopeias. Traditional medicine evolved over centuries, depending on local flora, culture, and religion.

Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, particularly plants and with many based on their use in traditional medicine. By using medicinal chemistry and combinatorial chemical and biosynthetic technology, novel natural product leads will be optimized on the basis of their biological activities to yield effective chemotherapeutic and other bioactive agents (Cragg et. al. 2005).

During the past decades, public interest in natural therapies, namely herbal medicine, has increased dramatically not only in developing countries but mainly in industrialized countries (Calixto, 2000). The market for ayurvedic medicines is estimated to be expanding at 20% annually. Sales of medicinal plants have grown by nearly 25% in India during 1987-96, the highest rate of growth in the world. The global trade in medicinal plants is of the

order of US\$ 800 million per year. Export statistics available between 1992 and 1995 indicate that India exported about 32,600 tonnes of crude drugs valued at \$US 46 million. China with exports of over 120,000 tons per annum (US\$ 264.5 million) and India with over 32,000 tons per annum dominate the international market. The annual export of medicinal plants from India is valued at Rs. 1200 million. Two of the largest users of medicinal plants are China and India. Traditional Chinese Medicine (TCM) uses over 5000 plant species, India uses about 7000. According to Export Import Bank, the international market for medicinal plant related trade is to the tune of US\$ 60 billion having a growth rate of 7% per annum. China's share in world herbal market is US\$ 6 billion while India's share is only US\$1 billion. The World Bank estimated global trade in medicinal and aromatic plants and their products at US \$ 5 trillion by 2050 AD. Global herbal market is around \$ 70.5 billion with an average annual growth of 10-12% per annum. In European union, it contributes to around 45% (\$ 32 billion), rest of the Europe 4% (2.8 billion), North America 10% (7.8 billion), Asia 19% (12.2 billion) and others 7% (4.6 billion) (Handa, 2007).

Complimentary alternative medicine therapies continue to gain popularity as modalities for the treatment of atopic disorders, such as asthma, allergic rhinitis, and atopic dermatitis. In Chinese, Japanese, Korean, Indian, and Western cultures, herbal therapies are commonly used for allergies. Although well controlled scientific studies have been performed for many of the Asian herbal therapies, and some basic studies have been performed for various herbal components (active ingredients), more needs to be done to assess the composite effects of many of these remedies (Zuckerman et al., 2002) Complementary and alternative medicines (CAMs) are used in more than 80% of the world's population and are becoming an increasing component of the US health care system, with more than 70% of the population using CAM at least once and annual spending reaching as much as \$34 billion.

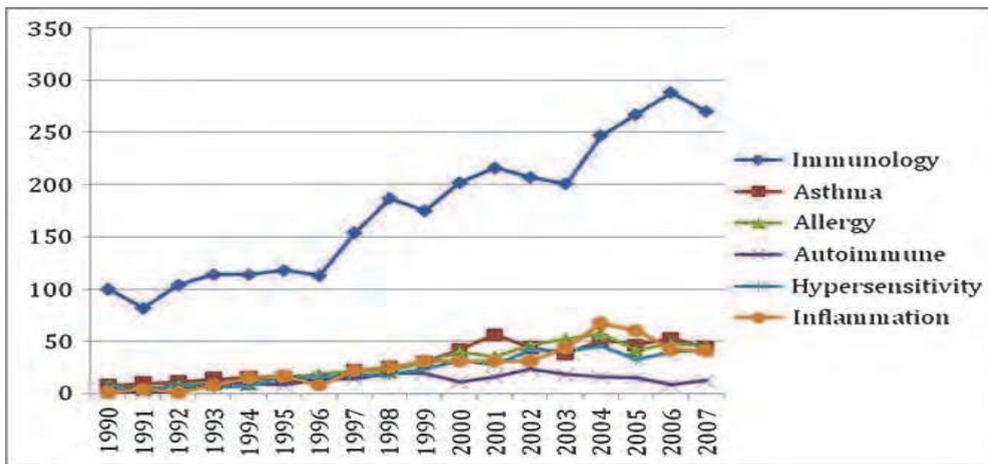


Fig. 1. Annual CAM publications related to allergy and immunology. The numbers of articles published and available for search through PubMed using the search terms complementary medicine and immunology, asthma, allergy, autoimmune, hypersensitivity, or inflammation are shown.

Since the inception of the National Centre for Complementary and Alternative Medicine, there has been an enormous increase in the number of basic science and therapy-based clinical trials exploring CAM. The subspecialty of allergy and immunology represents a particularly fertile area with a large number of CAM therapies that have been shown to affect the immune system. Research has shown that phytoconstituents such as resveratrol, quercetin, and magnolol may affect transcription factors such as nuclear factor- κ B and the signal transducer and activator of transcription/Janus kinase pathways with resultant changes in cytokines and inflammatory mediators. Clinically, there have been hundreds of trials looking at the effect of CAM on asthma, allergic rhinitis, and atopic dermatitis.

2. Bronchitis and related diseases

Bronchitis is described as the inflammation of the bronchial tubes (inflammation = itis). The inflammation causes swelling of the lining of these breathing tubes, narrowing the tubes and promoting secretion of inflammatory fluid.

Bronchiolitis is a term that describes inflammation of the smaller bronchi referred to as bronchioles. In infants, this is usually caused by respiratory syncytial viruses (RSV) and affects the small bronchi and bronchioles more than the large. In adults, other viruses as well as some bacteria can cause bronchiolitis and often manifest as persistent cough and at times production of small plugs of mucus.

Acute bronchitis describes the inflammation of the bronchi usually caused by a viral infection, although bacteria and chemicals may also cause acute bronchitis. Acute bronchitis is a cough that begins suddenly, usually due to a viral infection involving the larger airways. Colds (also known as viral upper airway infections) often involve the throat (pharyngitis) and nasal passages, and at times the larynx (resulting in a diminished hoarse voice, also known as laryngitis). Symptoms can include a runny nose, nasal stuffiness, and sore throat. Croup usually occurs in infants and young children and involves the voice box and upper large airways (the trachea and large bronchi).

Chronic bronchitis for research purposes is defined as a daily cough with sputum production for at least three months, two years in a row. Chronic bronchitis is a diagnosis usually made on the basis of clinical findings of a long term persistent cough usually associated with tobacco abuse. From a pathologic standpoint, characteristic microscopic findings involving inflammatory cells seen in airway tissue samples make the diagnosis. When referring to pulmonary function testing, a decrease in the ratio of the volume of airflow at 1 second when compared to total airflow is less than 70%. This confirms the presence of obstructive airways disease of which chronic bronchitis is one type. Certain findings can be seen on imaging studies (chest X-ray, and CT or MRI of the lungs) to suggest the presence of chronic bronchitis; usually this involves an appearance of thickened tubes.

Asthma: Asthma is a chronic inflammatory disease that affects about 300 million people worldwide, a total that is expected to rise to about 400 million over the next 15–20 years. Most asthmatic individuals respond well to the currently available treatments of inhaled corticosteroids and β -adrenergic agonists; however, 5–10% has severe disease that responds poorly. Asthma is a life threatening respiratory condition that causes:

1. The lining of the airways to become swollen
2. The body produces thick mucous

3. Tightness of the muscle around the airways.

This combination of problems interferes with the exchange of oxygen and carbon dioxide in the lungs **Figure 2**.

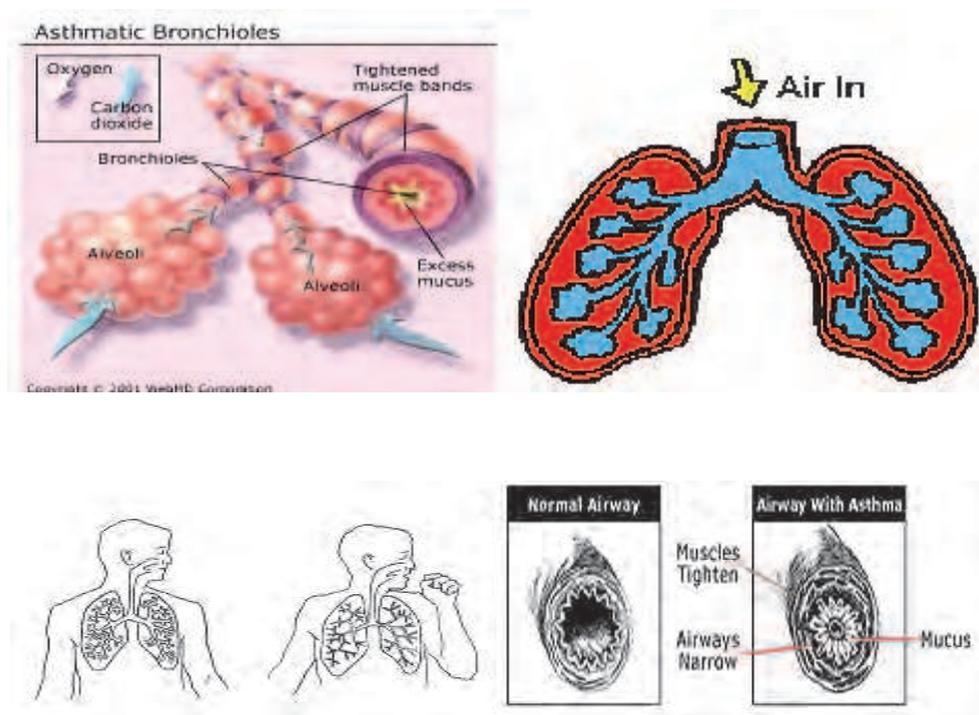


Fig. 2. Bronchitis and related diseases

3. Symptoms

- Breathlessness
- Wheezing
- Sputum Production
- Difficulty in talking
- Dyspnoea
- Tightness of Neck Muscle
- Coughing after physical activity
- Whistling Sound while breathing
- Frequent coughing
- Feeling Frightened, exhaustion
- Chest Tightness
- Greyish or bluish colouring of lips

4. Triggers or mediators responsible for bronchitis and related diseases

List of agents	Events triggering asthma
<i>Respiratory infection</i>	Respiratory syncytial virus (RSV), Rhinovirus, Influenza and Para-influenza virus, Mycoplasma pneumonia bacteria
Allergens	Airborne pollens (grass, trees, weeds), house-dust, mites, animal dander, cockroaches, fungal spores
Environment	Cold air, fog, ozone, sulfur dioxide, nitrogen, tobacco smoke, wood smoke
Emotions	Anxiety, stress, laughter
Exercise	particularly in cold, dry climate
Drugs/preservatives	Aspirin, NSAIDs, Sulfites, Benzalkonium chloride, β blockers,
Occupational stimuli	Bakers (flour dust), farmers (hay mold), spice and enzyme workers, printers (Arabic gum), chemical workers (azodyes, anthraquinone, ethylenediamine, toluene, diisocyanates, PVC), plastics, rubber and wood workers (formaldehyde, western cedar, dimethylethanolamine, anhydrides)

Table 1. List of agents responsible as triggers in **bronchitis and related diseases**

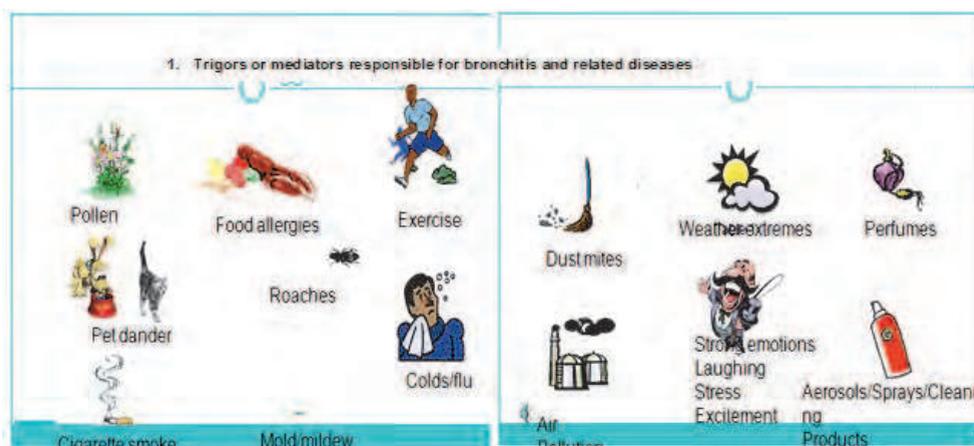


Fig. 3. Agents responsible as triggers in bronchitis and related diseases

5. Traditional medicines as bronchodilator and used in related diseases

Plants	Vernacular name	Traditional Uses
<i>Adhatoda zeylanica</i> Medic. F: Acanthaceae	Addasaramu	2-3 spoonfuls of leaf extract given for about a month.
<i>Ailanthus excelsa</i> Roxb. F: Simaroubaceae	Peddamanu	Bark decoction administered orally in 2 spoonfuls thrice a day for about one month
<i>Azima tetracantha</i> Lamk. F: Salvadoraceae	Uppi Teega	Leaf juice administered orally, 2 spoonfuls, twice a day for about 20 days.
<i>Bambusa arundinacea</i> (Retz.) Willd. F: Gramineae	Veduru	Leaf decoction administered orally, 3 spoonfuls, twice a day for about one month.
<i>Barleria cuspidata</i> Heyne.ex Nees F: Acanthaceae	Nelambram	Root decoction administered orally, 2 spoonfuls 3-4 times a day for 7days.
<i>Barleria prionitis</i> L. F: Acanthaceae	Mulla Gorinta	Stem ground with honey and ginger ,made into dry pillets and administered , 2 pillets, twice a day for amonth.
<i>Blumea mollis</i> (D.Don.) Merr. F: Compositae	Kukka Pogaku	Dried leaves smoked with wrapping leaves of <i>Diospyros mealanoxylon</i>
<i>Boerhavia diffusa</i> L. F: Nyctaginaceae	Atika Mamidi	Root extract is administered orally, one spoonful a day for 15days.
<i>Calotropis procera</i> (Ait.) R.Br. F: Asclepiadaceae	Jilledu	Flower powder mixed with honey and administered, 2 spoonfuls, twice a day for a month
<i>Cassia fistula</i> L. F: Leguminosae, Sf; Caesalpinoideae	Rela	Fruits ground with roots of <i>Hemidesmus</i> and the paste administered in 10g twice a day about 20 d.
<i>Curculigo orchioides</i> Gaertn. F: Hypoxidaceae	Nela Tadi	Rhizome extract administered, 2 spoonfuls, twice a day for about 2 months or till cure.
<i>Datura metal</i> L. F: Solanaceae	Erri Ummetta	Fruits ground and made into small pills with honey and 2 pills taken twice a day for about 3 months
<i>Desmodium triflorum</i> (L.) DC. F: Leguminosae, Sf; Papilionatae	Munta Manda	Root decoction given in 2 spoonfuls twice a day for about 10d.
<i>Lepidagathis cristata</i> Willd. F: Acanthaceae	Suryakanta	Powder of shade dried whole plant mixed with honey in 2 spoonfuls is administered twice a day for a bout 20d.

Plants	Vernacular name	Traditional Uses
<i>Nerium oleander</i> (L.) F: Apocynaceae	Ganneru.	Flowers ground with jaggery and the extract administered in 2 spoonfuls twice a day for about 2 months.
<i>Opuntia stricta</i> (Haw.) Haw. F: Cactaceae	Naga Phanni	Fruits are warmed and the juice given in 2 spoonfuls thrice a day for about 2 weeks.
<i>Passiflora foetida</i> L. F: Passifloraceae	Tella Jumiki	Leaf decoction administered in 2 spoonfuls with fruit juice of <i>Terminalia chebula</i> thrice a day for about one month.
<i>Pergularia daemia</i> (Forssk.) Chiov. F: Asclepiadaceae	Dustapa teega	Leaf decoction taken in 2 spoonfuls 2-3 times a day for about 15d.
<i>Phyllanthus emblica</i> L. F: Euphorbiaceae	Pedda Usiri	Fruits ground with tubers of <i>Cyperus rotundus</i> and leaves of <i>Tinospora cordifolia</i> and the paste administered with honey in 2 spoonfuls twice a day for about one month.
<i>Phyllanthus reticulatus</i> Poir. F: Euphorbiaceae,	Puli Chettu	Root decoction with honey administered in 2 spoonfuls twice a day for one month.
<i>Piper longum</i> L. F: Piperaceae	Pippallu	Whole plant ground with leaves of <i>Adhatoda zeylanica</i> and made into powder. A spoonful of powder is taken once in day for 20d.
<i>Portulaca quadrifida</i> L. F: Portulacaceae	Sanna pappu koora	Whole plant extract mixed with honey and administered in 2 spoonfuls thrice a day for about 20d.
<i>Solanum surattense</i> Burm.f. F: Solanaceae	Mulla	Root decoction administered in 2-3 times a day for about one month.
<i>Tragia involucrata</i> L. F: Euphorbiaceae	Durada gondi	Root powder cigared with leaves if <i>Diospyros melanoxylon</i> and smoked to reduce suffering
<i>Tylophora fasciculata</i> Ham. F:Asclepiadaceae	Veripala teega	Tender leaf juice administered in 2 spoonfuls twice a day for 20 – 30d.
<i>Vicoa indica</i> (L.) DC. F: Compositae	Adavi poddu tirugudu	Leaf juice administered in 2 spoonfuls twice a day for 15d
<i>Vitex negundo</i> L. F: Verbenaceae	Vavili	Leaf juice with dried powder of <i>Zingiber officinale</i> given in 2 spoonfuls twice a day for about 20d.
<i>Zaleya decandra</i> (L.) Burm.f.F: Aizoaceae	Tella garijelu	Root juice administered in 2 spoonfuls twice a day for about 20d.

Table 2. List of traditional medicinal plant drugs for the treatment of bronchial diseases (Madhu et al., 2010).

6. Reported bronchodilators from medicinal plant drugs

Plant	Part used	Extract/Active principle	Probable action in asthma
<i>Achyranthus aspera</i>	Roots	Oily preparation	Decreased ESR, Decreased total Eosinophil count. (Sharadini,1985)
<i>Adhathoda vasica</i>	Leaves Roots	Alkaloids	Bronchodilator, Anti- anaphylactic (Sharadini,1985)
<i>Albizzia lebeck</i>	Flower, Bark	Decoction	Anaphylaxis, Histamine Induced Bronchospasm (Tripathi & Das,1977)
<i>Belamcand chinensis</i>	Leaves, Rhizome	50% Ethanolic Extract	Histamine Induced Bronchospasm (Singh & Agrawal, 1990)
<i>Bupleurum falcatum</i>	Roots	saikosaponin-A (SSA), triterpenoid glycoside.	Inhibited the passive cutaneous anaphylaxis reaction in rats & antagonism of the histamine action and inhibition of allergic mediators (Park et al., 2002).
<i>Benincasa hispida</i>	Fruit Pulp	Metanolic Extract	Histamine & Ach Induced Bronchospasm(Anilkumar D., Ramu,2002)
<i>Boswellia serrata</i>	Root	Boswellin, boswellic acids	Inhibit LT biosynthesis and block synthesis of 5-HETE & LTB4 (Gupta,1998)
<i>Curcuma longa</i>	Rhizome	Tumerones, curcuminoids	Inhibits histamine release from rat peritoneal mast cells (Ammon & Wahl,1991)
<i>Eugenia caryophyllis</i>	Flower buds, leaves	Eugenol	Antianaphylaxis, inhibits C 48/80 induced anaphylaxis(Sharadini,1985)
<i>Picrorhiza kurroa</i>	Roots	Picrorrhizin	Inhibits release of histamine and SRS-A (Doshi & Shetge, 1983)
<i>Solanum xanthocarpum</i>	Herb	Salasodin	Bronchodilator (Govindan & Viswanathan, 1999)
<i>Tenospora. cardoifofia</i>	Stem	Aqueous extract	Mast cell stabilizing activity (Nayampalli & Desai,1986)
<i>Tamarandus indica</i>	Whole plant	Indolizidine alkaloid.	Bronchodilatory, membrane stabilizing (Sharadini,1985)
<i>Vitex. negundo</i>	Leaves	Alcoholic extract	Bronchodilatory, membrane stabilizing (Saraf & Nair,1995)
<i>Calotropis gigantea</i>	Flower	α & β calotropeol, β -amyryn, ,giganteol	Bronchodilator anti-inflammatory (Sangraula and Kumar 1999)
<i>Calotropis procera</i>	Flower	α & β calotropeol, β -amyryn, giganteol	Bronchodilator anti-inflammatory (Sangraula and Kumar 1999)

Plant	Part used	Extract/Active principle	Probable action in asthma
<i>Cedrus deodara</i>	Wood	Himacholol	Mast cell stabilizing activity (Shinde <i>et al.</i> , 1999)
<i>Centipeda minima</i>	Whole plant	Pseudoguaienolide, sesquiterpen, lactones, flavonoids	Inhibits passive cutaneous anaphylaxis in rats (Wu <i>et al.</i> , 1985)
<i>Clerodendrum serratum</i>	Leaves	Aqueous extract.	Bronchodilator (Gupta, S.S., 1994)
<i>Inula. racemosa</i>	Roots	Aqueous, alcoholic	Anti-histaminic, Anti-serotonergic (Srivastava <i>et al.</i> , 1999)
<i>Sarcostemma brevistigma</i>	Twigs	Alkaloid fraction	Inhibits passive cutaneous anaphylaxis in rats (Saraf and Patwardhan, 1998)
<i>Tephrosia purpurea</i>	Whole plant	Ethanollic extract	Bronchodilatory, antianaphylactic (Gokhale and Saraf, 2000)

Table 3.

7. Bronchodilator activity of two combined component of alkaloidal fraction of *Ailanthus excelsa* Roxb

Asthma is a chronic inflammatory disease that affects about 300 million people worldwide, a total that is expected to rise to about 400 million over the next 15–20 years (Kumar *et al.*, 2010_a). *Ailanthus* is a deciduous tree belonging to the family Simarubaceae, and widely distributed in Asia and North Australia. Commonly it is known as a Plant of Heaven. The bark of this plant is used as an anthelmintic, expectorant, antiasthmic, antispasmodic and antipyretic (Kumar *et al.*, 2010_b). *Ailanthus excelsa* is reported to be useful in a many ailments like asthma, allergy, bronchoconstriction etc. In the present study two combined Component of alkaloidal fraction of stem bark of *Ailanthus excelsa* Roxb.(AFAE) was for the first time evaluated for its bronchodilator activity.



Fig. 4.

7.1 Materials and methods

Plant material: Stem barks of *Ailanthus excelsa* Roxb were collected in Aug. 2008 from local area of Pimpri, pune-18 (INDIA) and identified by the RRI of Ayurveda Kothrude, Pune (INDIA). A voucher specimen - 899 was authenticated. Stem barks were dried, powdered, passed through 40 mesh sieve. The powdered material was extracted with methanol (95%) using soxhlet apparatus (10%). The brown extract (2gm) of stem bark of *Ailanthus excelsa* roxb. was used to prepare an alkaloidal rich fraction. The alkaloidal fraction was 500 mg and this Alkaloidal fraction was subjected to column chromatography for isolation of pure constituents using different polarity solvent and silica (60-120) as adsorbent. The component was isolated in chloroform: methanol (4:1) proportion (50 mg) and the same was checked for its purity by performing TLC in Beznene: methanol (4:1) solvent system. A single spot with Rf value of 0.56 was recorded. The isolated sample was then subjected to GC-MS which however showed the same to be a mixture of two components having close resemblance to each other and the fragmentation pattern was almost same and the retention time gap was very small. The molecular weights were 413 and 429. Alkaoidal tests were positive for the constituents.

Animal: Albino rats (Wistar strain) and mice (musmusculus strain) of either sex weighing 150-200gm rats and 20-25gm mice respectively were used. They were housed in microolon boxes with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining Institutional Animal Ethical Committee clearance (198/99/CPCSEA).

Acute toxicity studies: AFAE was safe upto 1000mg/kg and based on the results of preliminary toxicity testing the doses of 10, 20 and 40mg/kg p.o were chosen for further experiments.

7.2 Bronchodilator activity

Effect of test drug on isolated goat trachea chain preparation: Isolated adult Goat tracheal tissue was obtained immediately after slaughter of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution which was continuously aerated and maintained at 37 ± 0.5 °C. Tissue was allowed to equilibrate for 45 min. under a load of 400 mg. A dose response curve for histamine was taken in variant molar concentrations, by maintaining 15 min time cycle. After obtaining a dose response curve of histamine on trachea, the AFAE was added to the respective reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa were plotted to record dose response curve of histamine, in absence and in presence AFAE (Bhujbal et al., 2009).

Milk induced leukocytosis and eosinophilia: Mice were divided into five groups, six animals in each group. Animals belonging to group-I received distilled water (DW) 10 ml/kg, (p.o.). Animals belonging to group II, III, IV, V received boiled and cooled milk injection in dose of 4 ml/kg, (s.c.). Animals belonging to groups III, IV and V received AFAE in dose 10, 20 and 400 mg/kg, p. o. respectively, 1 hr before milk injection. Blood samples were collected from each mouse from the retro orbital plexus, under light ether anaesthesia. Total leukocyte count and total eosinophilia count was done in each group before drug administration and 24 hr after milk injection. Difference in Total leukocyte count and total eosinophilia count before and 24hr after drug administration was calculated.

Clonidine-induced Mast Cell Degranulation: Rats were divided into five groups, six animals in each group. Animals belonging to group-I received vehicles 5 ml/kg, (p.o.) Animals belonging to group-II received Sodium cromoglycate 50 mg/kg, (i.p.). Animals belonging to group-III, IV and V received AFAE in dose (10, 20 and 40mg/kg, p.o.) respectively. The treatment was continued for 7 days. On day 7th, 2 hour after the assigned treatment mast cells were collected from the peritoneal cavity. 10 ml of normal saline solution was injected into peritoneal cavity and abdomen was gently massaged for 90 second. The peritoneal cavity was carefully opened and the fluid containing mast cells was aspirated and collected in siliconised test tube containing 7 to 10 ml of RPMI-1640 Medium (pH 7.2- 7.4). The mast cells were then washed thrice by centrifugation at low speed (400-500 rpm) and the pallet of mast cells were taken in the medium. The mast cells suspension approximately (1×10^6 cells/ml) was challenged with 0.5 μ g/ml of clonidine solution and stained with 1 % toluidine blue and observed under high power microscope field (400 X). Total 100 cells were counted from different visual areas and the number of intact and degranulated cells was counted. The percent protection was calculated (Kumar et al., 2009).

Bronchoalveolar lavage and lung histology in rats: Animals were divided into five groups each group containing six animals. All the animals were sensitized by an intraperitoneal injection of 1ml alum precipitate antigen containing 20 μ g of ovaalbumin and 8mg of alum suspended in 0.9% sodium chloride solution. A booster injection of this alum-ovalbumin mixture was given 7 days later. Non sensitized animals were injected with alum only. Seven days after (15th day) second injection animals were exposed to aerosolized ovaalbumin(1%) for 30 min. Standard & test group was received Dexamethasone (1mg/kg, i.p.) as standard and AFAE 10, 20, 40mg/kg as test drug, 5 hr before antigen challenge. The rats were sacrificed at the end of study (24 hr after sensitization) and tracheal catheter was inserted in trachea. Bronchoalveolar lavage fluid was collected by lavaging the lung with 2 aquilots of 5 ml of 0.9% sodium chloride solution total recovery volume per rat was approximately 8ml. Total leukocytes and eosinophiles, neutrophiles were counted under microscope and Histopathological evaluation of lung tissue was carried out (Kumar et al, 2010c).

7.3 Statistical analysis

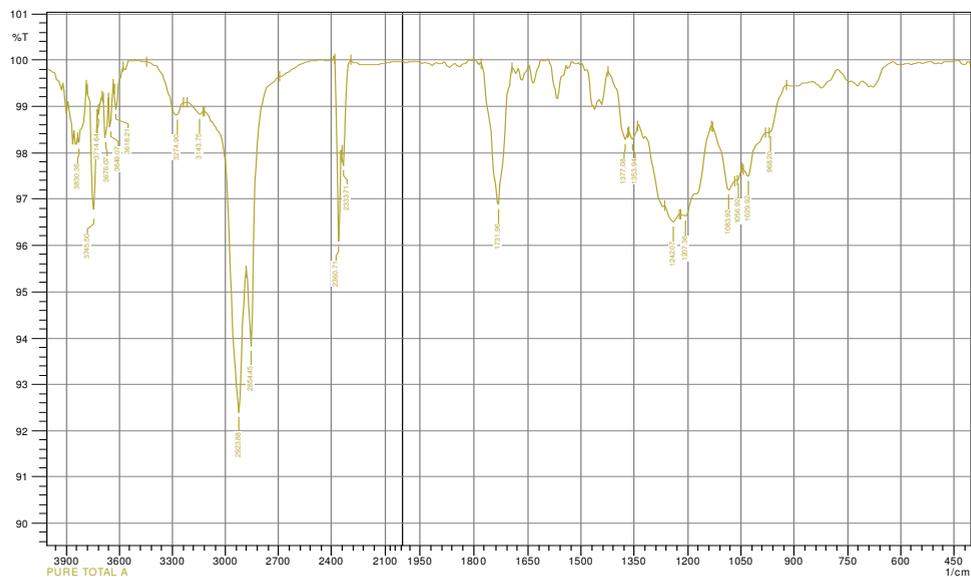
All values were expressed as mean \pm S.E.M. and data were analysed by ANOVAs followed by denest.

8. Results

Fraction quantity	Solvent proportion	Yield (mg)	colour	Identification test	TLC benzene: methanol (4:1)
500mg	Chloroform: methanol (80:20)	50 mg	brown	UV- 365 flurosent, positive for alkaloids	Single spot with Rf value - 0.56.

Table 4. Isolation and Characterization of AFAE

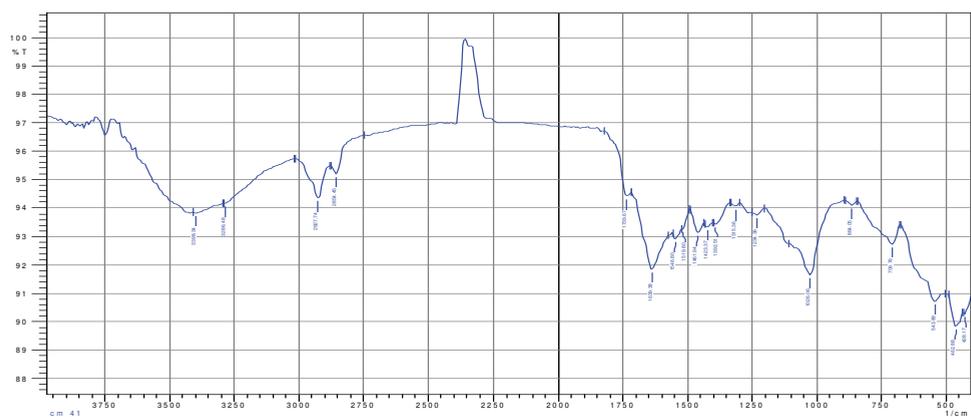
a. **Alkaloidal fraction:** U.V spectroscopy: UV λ max. -282, 242, 220, IR range:



968.20 1029.92 1056.92 1083.92 1207.36 1242.07 1353.94 1377.08 1731.96 2333.71 2360.71 2854.45
2923.88 3143.75 3274.90 3618.21 3649.07 3676.07 3714.64 3745.50 3830.36.

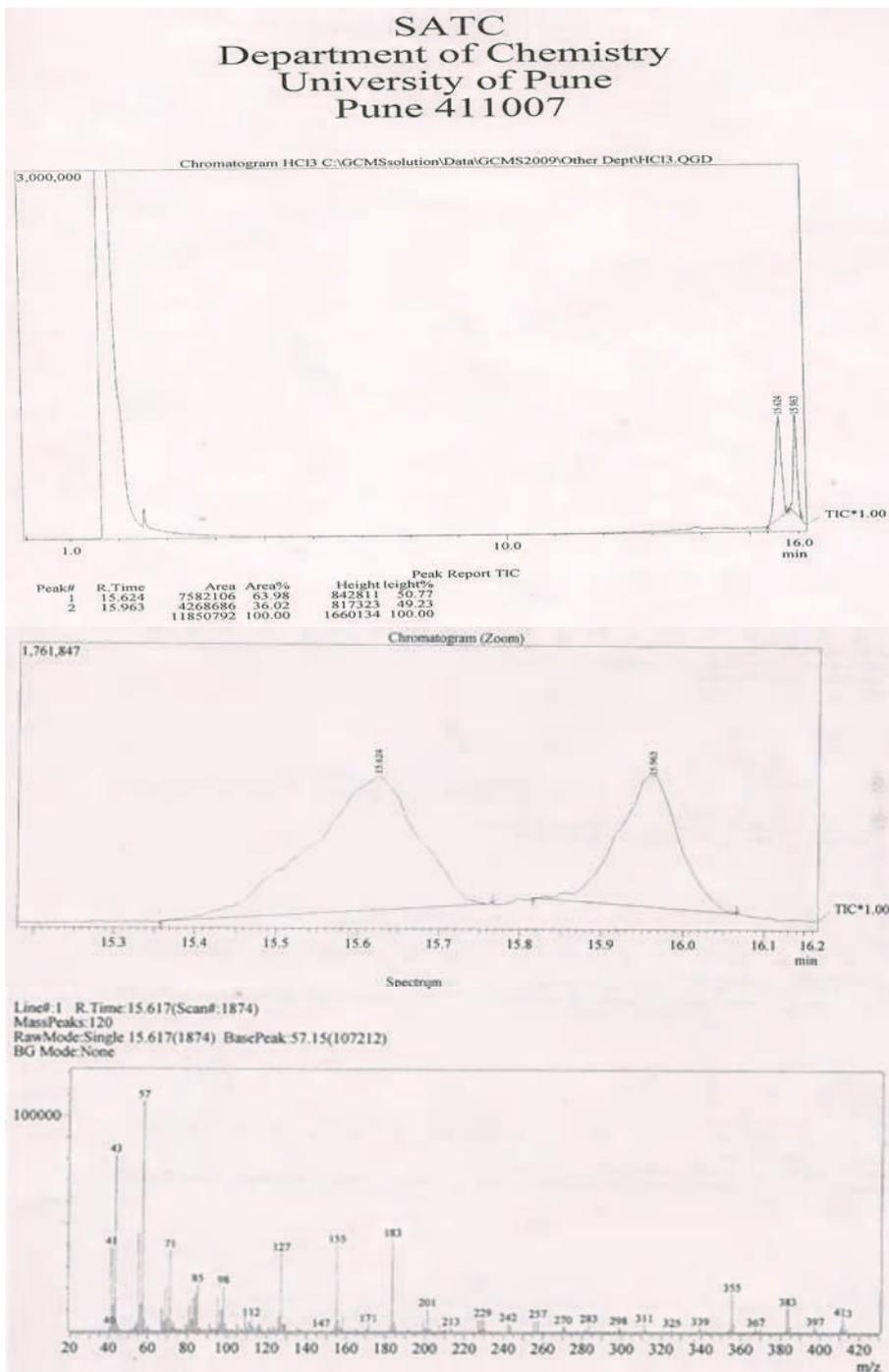
Fig. 5.

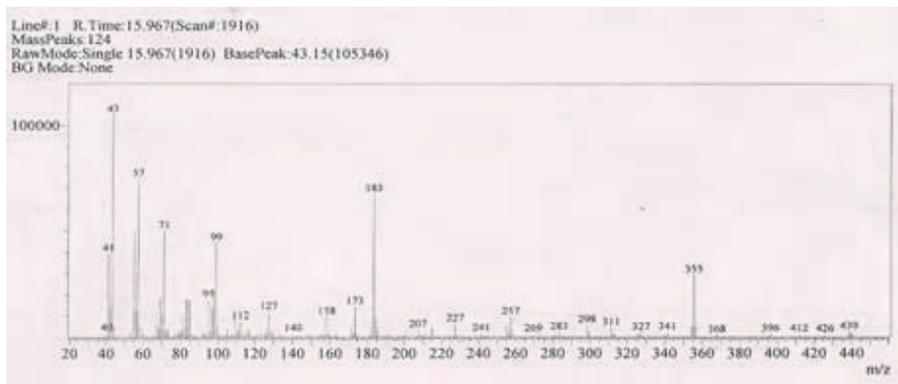
b. **AFAE:** U.V spectroscopy: UV λ max. - 209, 220, IR range:



428.17 462.88 543.89 709.76 864.05 1026.06 1234.36 1315.36 1392.51 1423.37 1461.94 1519.80 1546.80
1639.38 1739.67 2854.45 2927.74 3286.48 3398.34.

Fig. 6.



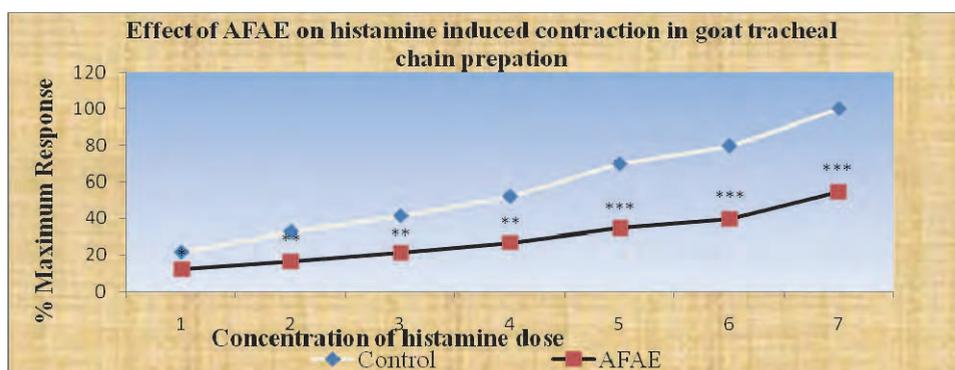


GC-MS

Fig. 7. The above compounds having very less retention time and they have similar ion peaks so in their structure there may be the possibilities of similarity.

8.1 Bronchodilator activity: In vitro

Effect of AFAE (30 μ g/ml) on Histamine induced contraction of isolated goat tracheal chain preparation: In the present study, it was observed that AFAE inhibits the contraction produced by histamine in these tissue preparations. Histamine (10 μ g/ml) was taken in different dose level and DRC was plotted in absence and in presence of *Ailanthus excelsa* extract. Study showed that AFAE inhibits significantly (* p <0.05, ** p <0.01, *** p <0.001) percentage contraction at concentration 30 μ g/ml in goat tracheal chain preparation. Dose dependent response relationship was seen (Figure 8).



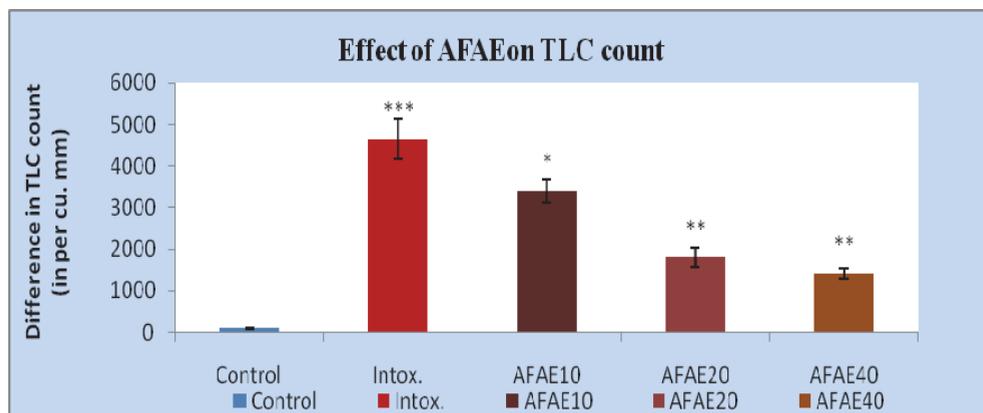
$n = 6$, Values are in Mean \pm SEM. Control = D.R.C. of Histamine in absence of AFAE. AFAE = D.R.C. of Histamine in presence of AFAE (30 μ g/ml). Statistical analysis done by using Student's t -test. (* p <0.05, ** p <0.01, *** p <0.001), significantly different from control.

Fig. 8.

8.2 In - vivo

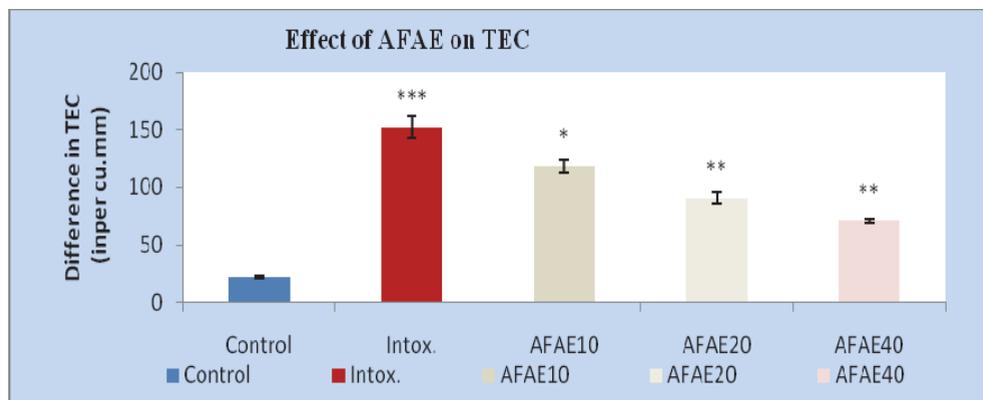
Milk induced leukocytosis and eosinophilia: Subcutaneous injection of milk at dose of 4 ml/kg produced a significant (***) p < 0.001) increase in the leucocytes and eosinophiles count

after 24 hr of its administration. In the groups of mice pretreated with AFAE at dose (10 mg/kg, 20 mg/kg and 40 mg/kg, p.o.), there was significant ($*p<0.05$, $**p<0.01$) inhibition of milk-induced Leucocytosis and eosinophilia (Figure 9 & 10).



n= 6, values are expressed in mean \pm SEM. Control = Vehicle (10 ml/kg, p.o.). Intox. = milk 4 ml/kg, $***p<0.001$, Intox. group compared with control group using student't, test and $*p< 0.05$, $**p<0.01$, AFAE compared to intox. Group using Statistical analysis done by ANOVA followed by Dunnet's test.

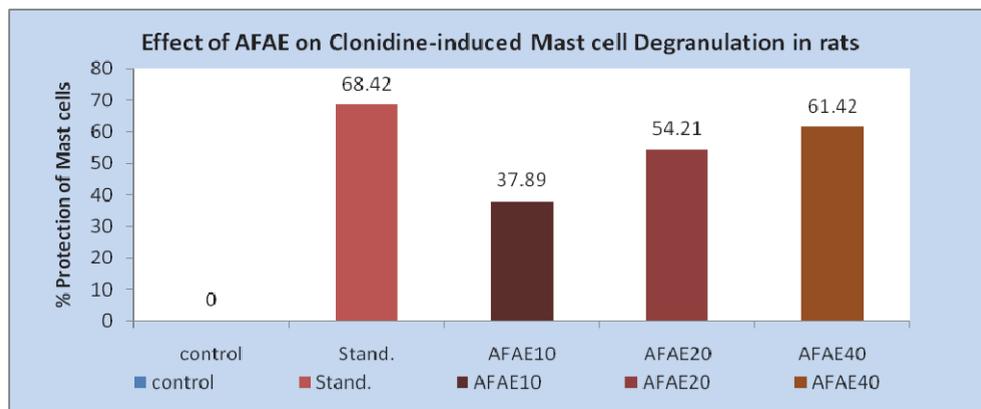
Fig. 9. Effect of AFAE on TLC count



n= 6, values are expressed in mean \pm SEM. Control = Vehicle (10 ml/kg, p.o.). Intox. = milk 4 ml/kg, $***p<0.001$, Intox. group compared with control group using student't, test and $*p< 0.05$, $**p<0.01$, AFAE compared to intox. Group using Statistical analysis done by ANOVA followed by Dunnet's test.

Fig. 10. Effect of AFAE on TEC

Effect of AFAE on clonidine-induced mast cell degranulation in rats: Clonidine induced mast cell degranulation was significantly ($**p<0.01$) inhibited by sodium cromoglycate (50 mg/kg, i.p.) and percent protection was found to be 68.42%. In the groups pre-treated AFAE (10, 20, 40 mg/kg, p.o) there was significant protection ($**p< 0.01$) of mast cells and the percent protection was 37.89, 54.21, and 61.42 % respectively (Figure 11).



n= 6, values are expressed in mean \pm SEM. Control = Distilled water (5 ml/kg, p.o.). Std. = Sodium cromoglycate (50 mg/kg, i.p.), Std., AFAE10, AFAE20, AFAE40 compared with Control (ANOVA followed by Dunnett's test), **p < 0.01.

Fig. 11. Effect of AFAE on Clonidine-induced Mast cell Degranulation in rats

Effect of AFAE on Bronchoalveolar lavage in rats: Persistent mucosal airway inflammation, associated with an increase in T helper type 2 (Th2) cytokine levels, eosinophil infiltration into the airways, and mucus and immunoglobulin (IgE) production, are the main features of allergic asthma. The infiltration of cells like eosinophils, neutrophils, monocytes, macrophages, lymphocytes, etc., increases the allergic asthmatic effect. Injection of ovalbumin 20 μ g + 8 mg alum in 1 ml (i.p.) on days 1 and 7 and 1% OVA aerosol on 15th day produced a significant (***) increase in the TLC. In the groups pretreated with standard drug dexamethasone (1 mg/kg i.p.), there was a significant (**) inhibition of ovalbumin-induced TLC and differential leukocyte count. The AFAE at doses of 10, 20, and 40mg/kg showed significant decrease in TLC and neutrophils, lymphocytes and monocytes, macrophages and eosinophils (*P < 0.05, **P < 0.01 [Table 5]. **AFAE40**

Sr. No.	Groups	Recoverable BAL Cells ($\times 10^3/\mu$ l)(Mean \pm SEM)					
		Total Cells	Neutrophile	Eosinophils	Lymphoctes	Monocytes	Alveolar Macrophages
1.	NS	186 \pm 8.12	19.6 \pm 3.69	13 \pm 1.55	6 \pm 1.34	4 \pm 0.71	4.8 \pm 0.58
2.	S	820 \pm 57.09***	122 \pm 10.08***	79.8 \pm 4.13***	39.2 \pm 3.8***	19.8 \pm .74***	16.6 \pm 1.08***
3.	Std	218 \pm 7.84**	52.6 \pm 1.17**	32.4 \pm 3.98**	10.6 \pm 0.66**	6.8 \pm 0.66**	6.2 \pm 1.36**
4.	AFAE10	384 \pm 24.97**	98.4 \pm 7.17*	66 \pm 4.44	18.4 \pm 2.06**	13 \pm 1.05**	12 \pm 0.71
5.	AFAE20	322 \pm 26.91**	76.8 \pm 3.61**	56.2 \pm 2.71**	18.6 \pm 1.03**	12 \pm 0.95**	13.2 \pm 0.97**
6.	AFAE40	246 \pm 12.39**	66.2 \pm 3.64**	45.2 \pm 4.83**	13.3 \pm 1.50**	9.4 \pm 0.86**	9.6 \pm 0.81**

n= 6, values are expressed in mean \pm SEM. NS = Non Sensitized group, Distilled water + 8mg alum in 1 ml (i.p.). S = Sensitized group, Ovaalbumin20 μ g+8mg alum in 1ml (i.p.) 1,7 day and 1% OVA aerosol on 15 day. Std. = Dexamethasone (1mg/kg, i.p.). NS compared with S by using student't, test***p < 0.001 and Std., AFAE10, AFAE20, AFAE40 compared with S (ANOVA followed by Dunnett's test), *p < 0.05, **p < 0.01.

Table 5. Effect of AFAE on Bronchoalveolar lavage in rats

Effect of AFAE on Histopathological evaluation of Lung Tissue: The histopathological evaluation of lung tissue showed the reduction of inflation and mediators of bronchoconstriction. The AFAE showed significant bronchodilator activity. Light micrograph of rat lungs collected from different treatment groups and the lungs were fixed in formalin and embedded in paraffin wax. Section of lung tissue were cut at 5 μ m thickness, mounted on glass slides, stained with hematoxylin and eosin (H \times E) and cells were identified as either eosinophils, neutrophils or mononuclear cells by standard morphology and 200 cells counted under 400X magnification (Figure 12).

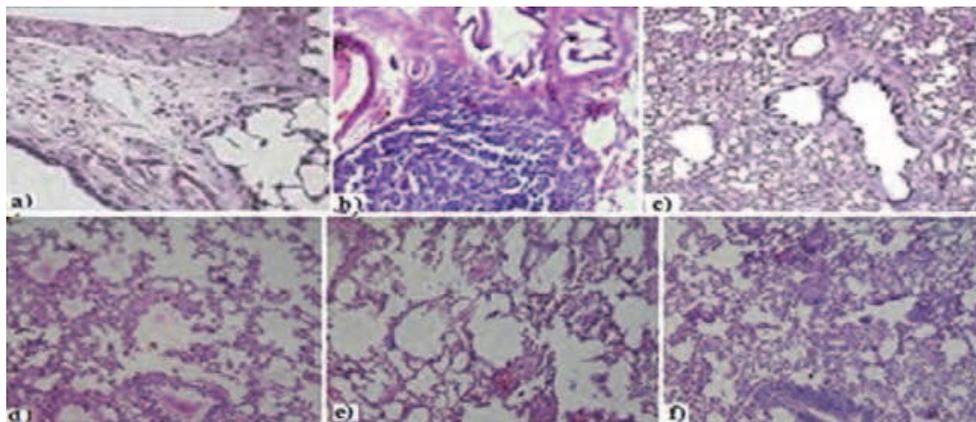


Fig. 12. Effect of AFAE on histopathological evaluation of lung tissue. Where a) = Absence of inflammatory cells, no edema in the lung tissue (NS- Non sensitized). b) = Abscess formation, fluid accumulation along with inflammatory cells, blood cells, edema (S- Sensitized). c) = a low magnification lung section from an antigen-challenged animals received (standard) dexamethasone (1mg/kg i.p.) showing absence of fluid accumulation around the blood vessel. d) = AFAE10 showing haemorrhages, emphysema, MNC and edema. e) = AFAE20 showing emphysema, MNC and haemorrhages. f) = AFAE40 showing emphysema.

9. Discussion

The role of plants serving as purifiers of air has been known to us, since times immemorial. To cope with the gradually increasing levels of toxic pollutants, tree plantation programs have been undertaken in different countries of the world to help in environmental cleanup. *Ailanthus excelsa* Roxb. (Simaroubaceae) is one such exotic avenue tree, the plantation of which has been encouraged under social forestry programs for large-scale tree plantation in different densely populated cities and towns of India [Mondal et al., 2007]. Histamine contracts the tracheobronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is easier to handle and to prepare; it is also much more sensitive than guinea pig tracheal chain. Therefore, the dose relative contractile responses of different agonists like ACh, histamine, 5hydroxytryptamine and bradykinin can be observed in isolated goat trachea. In the present study the isolated goat tracheal chain preparation; there is right side shift of Dose Response Curve (DRC) of histamine in the presence of AFAE indicating Bronchodilation [Bhujbal et al., 2009]. In the rat mast cell granules, the histamine

concentration has been calculated to be around 0.3 M. Both clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall. Clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80 [Lakdawala et al., 1980]. It is known that sodium cromoglycate; a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate. It has been known that all pharmacological agents that increase intracellular levels of cAMP relax airway smooth muscle and inhibit the release of autocoids from the tissue and basophils. The groups of animals pre-treated with AFAE resulted in a significant reduction in degranulation of mast cells and offered significant protection when challenged with clonidine indicating mast cell stabilizing activity which ultimately produces bronchodilation. The inflammatory response is characterized by an increase in the numbers of eosinophils and mast cells, mucus hypersecretion, and activation of T cells. Several studies have shown that T-helper type (Th2) cells play a major role in the initiation and maintenance of allergic airway inflammation and asthma through their increased production of Th2-type cytokines (IL-4, IL-5, and IL-13). These inflammatory cytokines also produced in the bronchial tissue by mast cells, alveolar macrophages, and epithelial cells, play a significant role in the pathogenesis of airway inflammation. The inflammatory mediators produce bronchoconstriction and the PAE helps to reduce the mediator which leads to bronchodilation. Ovalbumin increases the neutrophils, eosinophils, macrophages, monocytes, leukocytes, lymphocytes, epithelial cells, mucus etc., in BALF and AFAE helps to reduce all the allergic factors [Kumar et al., 2010].

Thus, it can be concluded from the results obtained in the present investigation that AFAE possesses significant bronchodilating activity. Hence, further detailed study needs to be conducted to separate the constituents and individually evaluate these phytoconstituent responsible to produce the above result and their clinical efficacy in the treatment of asthmatic patients.

10. Acknowledgement

I would like to thank Dr. R. K. Nanda, Santosh S. Bhujbal and other staff members of Dr. D. Y. Patil IPSR Pune for guiding me to conduct this valuable work.

I would like to thank NCL- Pune scientists and library staff members for their sport and help for literature review.

I would like to thank NTC- Pune for providing animals and Dr. Surayawanshi for providing histopathological evaluation (Omega labs. Pvt. Ltd.).

I would like to thank Dr. Rajesh Dabur for authentication of Plant in RRI-Pune, India.

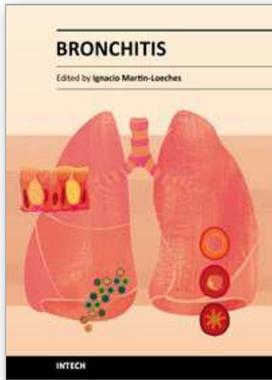
I would like to attribute special thank to Late Popinder Singh, Department of Pharmaceutical Sciences University of Kashmir-190006 for his content support and encouragement.

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Bronchitis

Edited by Dr. Ignacio Martín-Loeches

ISBN 978-953-307-889-2

Hard cover, 190 pages

Publisher InTech

Published online 23, August, 2011

Published in print edition August, 2011

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<http://www.intechopen.com/books/bronchitis/bronchodilator-activity-in-traditional-medicines-gift-of-god-kingdom>

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