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EVALUATION OF THE ANTIASTHMATIC ACTIVITY OF AZIMA TETRACANTHA LAM LEAVES IN ANIMAL MODELS

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Abstract:

Asthma is a chronic disease that affects approximately 300 million people worldwide. Although wide range of drug is available, the relief is mainly symptomatic and short lived. Azima tetracantha leaves, is traditionally used to treat asthma. However, the scientific data on anti-asthmatic and anti- Cholinergic of this plant has got little attention. An attempt has been based on ethanolic extract of leaves of Azima tetracantha shown a tremendous effect on asthma when comparative study was done with normal and treated group. The anti-asthmatic activity of a 50% aqueous ethanol extract of dried and fresh leaves, and the volatile and fixed oils of Azima tetracantha was evaluated against histamine and acetylcholine-induced preconvulsive dyspnea (PCD) in guinea pigs fasted for 24 h were exposed to an atomized fine mist of 2% histamine dihydrochloride aerosol (dissolved in normal saline) 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 difficulty in breathing leading to convulsions, asphyxia and death. The time until signs of convulsion appeared is called Preconvulsion time (PCD). By observation experience was gained so that the Preconvulsion time can be judged accurately. As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. 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 Mepyramine (8 mg/kg) and polyherbal formulation (300 mg/kg) were administered intraperitonially 30 min prior to exposure. Animals, which did not develop typical asthma within 6 minutes, were taken as protected.

Keywords: *Antiasthamatic, Azima tetracantha, Histamine-induced model and Acetylcholine induced model.*

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INTRODUCTION:

Asthma is a disease of the human respiratory system in which the airways constrict and become narrow, often in response to a "trigger" such as exposure to an allergen, cold air, exercise, or emotional stress. Asthma affects 7% of the total population and approx 300 million worldwide. During attacks (exacerbations), the smooth muscle cells in the bronchi constrict, and the airways become inflamed and swollen with difficulty in breathing. Asthma causes 4,000 deaths a year in the US alone. Attacks can be prevented by avoiding triggering factors and by drug treatment[1].

There is limited information on field epidemiology of bronchial asthma in Indian adults. The results of the study indicated that from 73605 respondents (37682 men, 35923 women), one or more respiratory symptoms were present in 4.3-10.5% subjects. Asthma was diagnosed in 2.28%, 1.69%, 2.05 and 3.47% respondents respectively at Chandigarh, Delhi, Kanpur, and Bangalore, with overall prevalence of 2.38%. Female sex, advancing age, usual residence in urban area, lower socioeconomic status, history suggestive of atropy, history of asthma in a first degree relative, and all forms of tobacco smoking were shown as responsible ailments of asthma. Prevalence estimates of asthma in adults in this study pointed a high overall national burden of disease[2].

Asthma is one of the commonest chronic diseases of affluent societies. The striking increase in prevalence of asthma over recent decades in affluent societies and the rarity of this disease in less affluent populations confirms the importance of environmental and other factors in the cause of asthma, although which environmental and other factors are responsible is still not clear. The studies showed that genetic factors are also important in determining individual susceptibility to asthma. The results of genetic studies suggest that there are many genes with moderate effects rather than a few major genes. Asthmatic airways show inflammation with $CD4^+$ helper cells, mast cells, and eosinophils, characterizing the inflammatory response. Inhaled corticosteroids remain the cornerstone of treatment with the addition of long-acting -agonists as the next step if symptoms continue. Leukotriene antagonists, the only new drugs to reach the market in the past decade, have modest effects. A better understanding of the mechanisms underlying asthma and the genetic and environmental factors that predispose individuals to asthma will play a key role in understanding better preventative strategies and new therapeutic approaches [3].

It has been estimated that about 6000 articles are published every year about asthma in various indexed journals. The increasing prevalence of asthma has led to extensive research into its cause, pathophysiology, and management [3].

A few patients have asthma as part of a wider problem such as allergic broncho pulmonary aspergillosis[4], Churg-Strauss syndrome (Conron & Beynon, 2000) and aspirin-induced asthma[6]. Table 1.1 shows the main features of these disorders.

Historical background

The modern school of thought regarding asthma as a disease is so powerful that it is difficult to imagine asthma, as it was once conceptualized. During the seventeenth century, Once English physicians Thomas Willis and Sir John Floyer began arguing that asthma was different from other breathing disorders and is the same from person to person. They mentioned that asthma, as a specific form of disordered breathing, must be treated differently from other forms of breathlessness cases. By the late nineteenth century, physicians believed that asthma was a disease which had a specific set of causes, clinical consequences, and requirements for treatment, despite the diversity of individual experiences as shown in Table 1.1. Because of the spectrum of severity within asthma, some people with asthma only rarely experience symptoms, usually in response to triggers, where as other more severe cases may have marked airflow obstruction at all times [1].

Table 1.1. Classification of asthma [1]

Severity	Symptom Frequency	Symptoms at night	Peak expiratory flow rate or FEV_1 of predicted	Variability of peak expiratory flow rate or FEV_1
Intermittent	<once a week	≤ twice per month	≥80% predicted	<20%
Mild Persistant	>once per week but<once per day	>twice per month	≥80% predicted	20-30%
Moderate persistent	Daily	>once per week	60-80% predicted	>30%
Severe	Daily	Frequent	<60% predicted	>30%

Asthma exists in two states: the steady-state of chronic asthma, and the acute state of an asthma exacerbation. The symptoms are different depending on what state the patient is in. Common symptoms of asthma in a steady-state include: nighttime coughing, shortness of breath with exertion but no dyspnea at rest, a chronic 'throat-clearing' type cough, and complaints of a tight feeling in the chest. Severity often correlates to an increase in intensity of symptoms. Symptoms can worsen gradually and rather insidiously, up to the point of an acute exacerbation of asthma. It is a common misconception that wheezing is common in patients with asthma-some never wheeze, and their disease may be confused with another Chronic Obstructive Pulmonary Disease (COPD) such as emphysema or chronic bronchitis. An acute exacerbation of asthma is commonly referred to as an asthma attack. The cardinal symptoms of an attack are shortness of breath (dyspnea), wheezing, and chest tightness[7]. Some patients present primarily with coughing, and in the late stages of an attack, air motion may be so impaired that no wheezing may be heard. When present the cough may sometimes produce clear sputum. The onset may be sudden, with a sense of constriction in the chest, breathing becomes difficult, and wheezing occurs (primarily upon expiration, but can be in both respiratory phases). Asthma is classified according to the frequency of symptoms, Forced Expiratory Volume (FEV1) and peak expiratory flow rate (PEFR) [8]. Though symptoms may be very severe during an acute exacerbation, between attacks a patient may show few or even no signs of the disease[9].

Epidemiology

Asthma has no standard definition. Attempts to define asthma have generally resulted in descriptive statements invoking notions of variable airflow obstruction over short periods of time, sometimes in association with markers of airway hyper-responsiveness and cellular pathology of the airway[10].

Asthma is diagnosed clinically on the basis of symptoms of wheeze, dyspnea, and cough, and by objective evidence of variable airflow obstruction. Allergic asthma can present for the first time at any age, but incidence is highest in childhood[11]. Asthma in childhood frequently remits during adolescence but can recur in adult life[12]. Asthma in adults tends to persist for life and is probably associated with an accelerated decline in lung function[13]. Deaths from asthma are now uncommon, especially in children and young adults; in the past they have occurred in epidemics thought to have been caused by use of high dose formulations of relatively unselective β-agonist drugs.[14]

Asthma is an airway disease that can be classified physiologically as a variable and partially reversible obstruction to air flow, and pathologically with overdeveloped mucus glands, airway thickening due to scarring and inflammation, and bronchoconstriction, the narrowing of the airways in the lungs due to the tightening of surrounding smooth muscle. Bronchial inflammation also causes narrowing due to edema and swelling caused by an immune response to allergens[1].

1.3 Bronchoconstriction

During an asthma episode, inflamed airways react to environmental triggers such as smoke, dust, or pollen. The airways narrow and produce excess mucus, making it difficult to breath. In essence, asthma is the result of an immune response in the bronchial airways. The airways of asthma patients are "hypersensitive" to certain triggers, also known as ‘stimuli’ (see below). (It is usually classified as type I hypersensitivity). In response to exposure to these triggers, the bronchi (large airways) contract into spasm (an "asthma attack"). Inflammation soon follows, leading to a further narrowing of the airways and excessive mucus production, which leads to coughing and other breathing difficulties. Bronchospasm may resolve spontaneously in 1–2 hours, or in about 50% of subjects, may become part of a 'late' response, where this initial insult is followed 3–12 hours later with further bronchoconstriction and inflammation. The normal caliber of the bronchus is maintained by a balanced functioning of these systems, which both operate reflexively. The parasympathetic reflex loop consists of afferent nerve endings which originate under the inner lining of the bronchus. Whenever these afferent nerve endings are stimulated (for example, by dust, cold air or fumes) impulses travel to the brain-stem vagal center, then down the vagal efferent pathway to again reach the bronchial small airways. Acetylcholine is released from the efferent nerve endings. This acetylcholine results in the excessive formation of inositol 1, 4, 5-trisphosphate (IP3) in bronchial smooth muscle cells which leads to muscle shortening and this initiates bronchoconstriction[3].

1.4 Triggers of asthma (Stimulus that aggravate asthma response)

- Allergens from nature typically inhaled, which include waste from common household pests, the house dust mite and cockroach, as well as grass pollen, mold spores, and pet epithelial cells[15].

- Indoor air pollution from volatile organic compounds, including perfumes and perfumed products. Examples include soap, dishwashing liquid, laundry detergent, fabric softener, paper tissues, paper towels, toilet paper, shampoo, hairspray, hair gel, cosmetics, facial cream, sun cream, deodorant, cologne, shaving cream, aftershave lotion, air freshener and candles, and products such as oilbased paint [15]
- Association between some medications, including aspirin[16], ß-adrenergic antagonists (ß-blockers), and food allergies such as milk, peanuts, and eggs with asthma have been shown. However, not all people with food or other allergies have asthma [15]
- Use of fossil fuel related allergenic air pollution, such as ozone, nitrogen dioxide, and sulfur dioxide, which is thought to be one of the major reasons for the high prevalence of asthma in urban areas[7].
- Various industrial compounds and other chemicals, notably sulfites; chlorinated swimming pools generate chloramines-monochloramine, dichloramine and trichloramine in the air around them, which are known to induce asthma[17].
- Early childhood infections, especially viral upper respiratory tract infections. Children who suffer from frequent respiratory infections prior to the age of six are at higher risk of developing asthma, particularly if they have a parent with the condition. However, persons of any age can have asthma triggered by colds and other respiratory infections even though their normal stimuli might be from another category (e.g. pollen) and absent at the time of infection. In many cases, significant asthma may not even occur until the respiratory infection is in its waning stage, and the person is seemingly improving[7]. In children, the most common triggers are viral illnesses such as those that cause the common cold.
- Exercise or intense use of respiratory system in one or other way can also stimulate asthma attack. These are thought to be primarily in response to the exposure of the airway epithelium to cold, dry air.
- Hormonal changes in adolescent girls and adult women associated with their menstrual cycle can lead to a worsening of asthma. Some women also experience a worsening of their asthma during pregnancy, whereas others find no significant changes, and in other women their asthma diminished during their pregnancy[7].
- There is growing evidence that psychological stress is a trigger. It can modulate the immune system, causing an increased inflammatory response to allergens and pollutants. Cold weather can make it harder for patients to breathe. Whether high altitude helps or worsens asthma is debatable and may vary from person to person[18].

1.5 Bronchial inflammation

The mechanisms behind allergic asthma- i.e., asthma resulting from an immune response to inhaled allergens - are the best understood of the causal factors. In both people with asthma and people who are free of the disease, inhaled allergens that find their way to the inner airways are ingested by a type of cell known as antigen-presenting cells (APCs). APCs then "present" pieces of the allergen to other immune system cells. In most people, these other immune cells (T0 cells) "check" and usually ignore the allergen molecules. In asthma patients, however, these cells transform into a different type of cell (Th2), for reasons that are not well understood. The resultant Th2 cells activate an important arm of the immune system, known as the humoral immune system. The humoral immune system produces antibodies against the inhaled allergen. Later, when a patient inhales the same allergen, these antibodies "recognize" it and activate a humoral response. Inflammation results: chemicals are produced that cause the wall of the airway to thicken, cells which produce scarring to proliferate and contribute to further 'airway remodeling', causes mucus producing cells to grow larger and produce more and thicker mucus, and the cell-mediated arm of the immune system is activated. Inflamed airways are more hyper-reactive, and will be more prone to bronchospasm. The "hygiene hypothesis" postulates that an imbalance in the regulation of these Th2 cell types in early life leads to a long-term domination of the cells involved in allergic responses over those involved in fighting infection. The suggestion is that for a child being exposed to microbes early in life, taking fewer antibiotics, living in a large family, and growing up in the country stimulate the Th1 response and reduce the odds of developing asthma[19].

1.6 Factors that provoke asthma

The factors that commonly provoke asthma are allergens, upper respiratory tract infection, exercise, perfume and fumes, and changes in temperature and humidity. Some agents provoke asthma less frequently, but are important to restrict their use. These include drugs (aspirin and other nonsteroidal anti-inflammatory drugs, β-blockers), food additives (e.g. metabisulphite and tartrazine), and some food and drinks (e.g. peanuts, alcohol)[20] and Cola[21]. Some substances found in the workplace cause asthma

because of specific sensitisation, and occupational asthma accounts for up to 10% of asthma in young adults in Europe [22]. Occupational asthma is preventable and can lead to severe irreversible airway obstruction if the patient is not removed from the sensitizing agent. An occupational history should be taken from all patients with asthma and the history should be supplemented, when occupational asthma is suspected, with frequent peak flow measurements during days when the patient is and is not at work. Some of the common occupational substances that provoke asthma, although more than 200 sensitizing agents have been identified [23]. It is now well-established that environmental factors play a major part in the cause of asthma and allergy; family studies showed that a strong component of asthma risk is genetically determined. In the developed countries genetic factors are the major detectable determinants of individual disease risk whereas in the developing countries, the emergence of asthma probably reflects the strong effect of environmental exposures associated with economic development [7].

1.7 Pathophysiology of asthma

The fundamental problem in asthma appears to be immunological: young children in the early stages of asthma show signs of excessive inflammation in their airways. Epidemiological findings give clues as to the pathogenesis: the incidence of asthma seems to be increasing worldwide, and asthma is now very much more common in affluent countries.

In 1968 Andor Szentivanyi first described *The Beta Adrenergic Theory of Asthma*; in which blockage of the β2 receptors of pulmonary smooth muscle cells, causes asthma[24]. Szentivanyi's *Beta Adrenergic Theory* is a citation classic [25] using the Science Citation Index and has been cited more times than any other article in the history of the Journal of Allergy and Clinical Immunology, a renowned journal dedicated to allergy and immunology. In 1995, zentivanyi and colleagues demonstrated that Ig E blocks β2 receptors[24]. Since, overproduction of Ig E is central to all atopic diseases, this was a watershed moment in the world of allergy[26].

The pathology of asthma is characterized by various changes in the airways including mucus plugging, shedding of epithelial cells, thickening of the basement membrane, engorgement of the vessels, and angiogenesis, inflammatory cell infiltration, and smooth muscle hypertrophy and hyperplasia[27]. The pathogenesis of asthma can be broadly subdivided into inflammatory and remodeling components.

1.8 Diagnosis and assessment

Ascertainment of whether asthma is present is usually straightforward, and is based on a characteristic history and variability in lung function. In young children it can take some time to ascertain whether the child has persistent asthma rather than wheezy bronchitis [28]. In older patients, distinguishing severe chronic asthma from COPD can be difficult and the two disorders sometimes merge in those who have smoked cigarettes. Asthma can also be diagnosed incorrectly, especially in patients with inappropriate hyperventilation, dysfunction of the vocal cords, or obstruction of the upper airway. Diagnosis of asthma still relies on demonstration of variability (measurement of asthma severity) in lung function over time or improvement after a bronchodilator, prednisolone, or high-dose inhaled corticosteroid. Such variability can be measured with spirometry in the clinic or by regular peak flow measurements at home. Demonstration of bronchoconstriction after vigorous exercise can be useful in young people, whereas an increase in blood eosinophils could point to asthma in older patients. Other features associated with asthma, such as non-specific bronchial hyperresponsiveness to agents such as methacholine, are not specific enough to be a useful diagnostic test. The essential treatment for asthma includes oxygen, nebulised β2 agonists, and oral or intravenous corticosteroids to patients.

1.9 Management

A patient might have poorly controlled asthma due to poor management, severe asthma, or both. Poor compliance with treatment, especially inhaled corticosteroids, continues to be an important cause of poor asthma control and much effort is needed to ensure that patients understand why prophylactic treatment needs to be taken regularly and the dangers of poor compliance. Several developments have contributed to better asthma management including development of guidelines (The British Guidelines on Asthma Management, 1997), better use of peak-flow meters, and recognition of the need to improve patient education. Patients with asthma should be advised strongly not to smoke and to lose weight, if overweight, since these measures improve asthma control. Inactivated influenza vaccine is recommended and is safe in patients with asthma (The American Lung Assosiation Asthma Clinical Research Centers, 2001). Avoidance of allergens can be helpful for patients with specific allergen sensitivities. Complementary approaches to treatment, such as Yoga, help some patients, but overall, the effect seems to be small[29].

1.10 Pharmacotherapy

Drugs should be given by inhalation when possible so that the same beneficial effect can be achieved with a much smaller dose, thus causing lower systemic drug concentrations and fewer systemic adverse effects. Over the past two decades, many groups have tried to develop new types of drug for asthma, but only the leukotriene modifiers are on the market.

Most asthma guidelines include a stepwise approach to asthma treatment, which ranges from β-agonists alone for very mild intermittent asthma to oral corticosteroids for severe chronic asthma. A schematic hierarchy of the drugs considered most appropriate for different levels of asthma severity is shown in Figure 1.1. Treatment should be determined by symptoms, exacerbations, and lung function. There is some controversy about inhaled corticosteroids, which are very effective at suppressing inflammation in asthma. Symptoms and airway obstruction usually reappear when the drugs are discontinued.

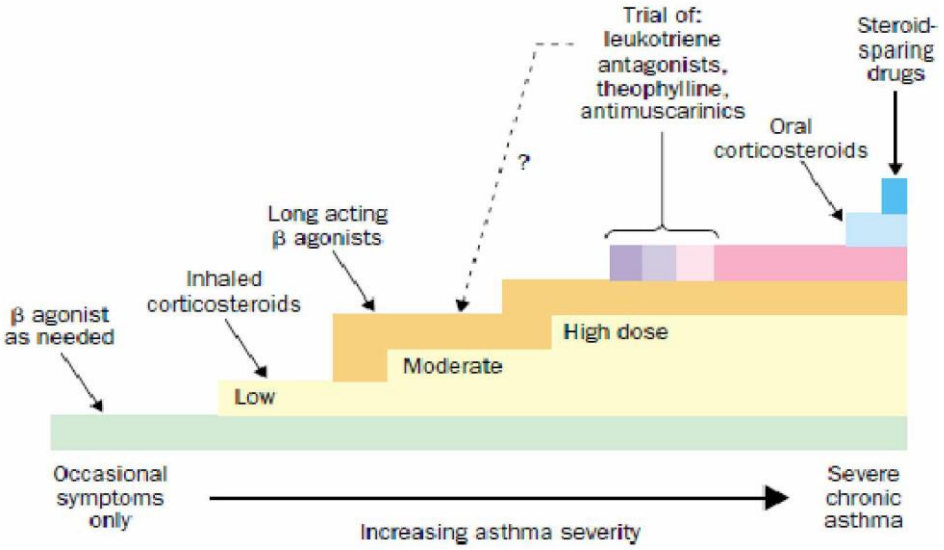

Figure 1.1. Treatment hierarchy used to add further drugs as asthma becomes increasingly severe

1.10.1 Inhaled corticosteroids

Inhaled corticosteroids are the cornerstone of treatment for asthma. They are especially helpful in patients with mild or moderate asthma, improving lung function, and reducing symptoms and exacerbations and they have been shown to reduce readmissions for asthma[31]and asthma deaths[30]. Inhaled corticosteroids are recommended for all patients requiring more than one puff a day from their β-agonist inhaler.

However, there are two limitations of inhaled corticosteroids. First, although small doses of an inhaled corticosteroid are very effective, the added benefit from higher doses is limited. Doubling [31] or quadrupling [32] the dose of an inhaled corticosteroid can improve asthma control, but it is usually better to add in a long-acting β-agonist. Second, long-term high doses of inhaled corticosteroids can cause systemic adverse effects, including reduced bone mineral density which is likely to predispose patients to osteoporotic fractures as they get older, and an increase in cataracts[34] and glaucoma.[35]

Since inhaled corticosteroids reduce the need for oral corticosteroids, which have many more adverse effects, the goal of treatment is to give the minimum dose of inhaled corticosteroid to maintain good control. Most patients with asthma can be managed on a low dose, which will produce maximum or near maximum benefit with minimum risk of long-term adverse effects (Figure 1.2). Fluticasone is twice as potent as beclometasone and budesonide. The dose of corticosteroids vary depending on the severity of asthma but will rarely be above 800 µg beclometasone or budesonide a day (equivalent to 400 µg of fluticasone a day) and usually preferred less than 400 µg a day (equivalent to 200 µg fluticasone a day) for most of patients.

1.10.2 β-agonists

Short-acting β-agonists such as salbutamol and terbutaline are very effective in prevention of

exercise-induced asthma and for relieving acute attacks of asthma. They do not provide benefit when given regularly[36] and are debatable, as some patients can even deteriorate. Patients should therefore take short-acting β-agonists only as required.

By contrast with short acting β-agonists, regular use of the long-acting β- agonists, salmeterol and formoterol, improves asthma control and reduces asthma exacerbations. Both drugs are effective for longer than 12h and should be taken twice daily. The main clinical difference between the two drugs is that formoterol has a rapid onset of action, similar to that of salbutamol, raising the possibility that formoterol could be used for symptom control in addition to regular administration. Asthma control was better with formoterol when compared with terbutaline for relief of asthma and it is now licensed for up to 72 μg a day. Thus, these drugs are now introduced at an earlier point in asthma management-i.e, for patients who remain symptomatic despite 400–800 μg of an inhaled corticosteroid. Both long acting β-agonists can be given in combination with an inhaled corticosteroid which improves patient compliance.

1.10.3 Theophylline, ipratropium, cromoglycate, and nedocromil

Theophylline is effective in asthma and has some anti-inflammatory(Fjellbirkeland et al., 1994) activity[37]. It is less effective than the long acting β-agonists [38] however, and its narrow therapeutic window and interactions with other drugs make it a less attractive option. More selective phosphodiesterase inhibitors have been developed but have not yet shown clear benefit over theophylline. Ipratropium and oxitropium are also effective bronchodilators but usually add little in patients with stable asthma who are already taking a β-agonist. Sodium cromoglicate and nedocromil have also been used in mild to moderate asthma therapy.

1.10.4 Prednisolone and steroid-sparing drugs

Oral corticosteroids cause much morbidity and patients on long-term oral steroids need careful assessment to be sure that such treatment is necessary. For those who require prednisolone, prophylaxis against osteoporosis needs to be considered, ideally with a measure of bone mineral density. Bisphosphonates and hormone replacement therapy decrease the loss of bone mineral density seen with oral corticosteroids[39].Some immunosuppressive drugs (methotrexate, ciclosporin, and gold) reduce oral steroid requirements in patients with severe asthma[40].

1.10.5 New drug therapy for asthma

Leukotriene modifiers consist of the lipoxygenase inhibitors such as zileuton, and the leukotriene antagonists such as montelukast and zafirlukast. The drugs are given orally and a single drug can therefore treat both rhinitis and asthma. Both types of drug are effective in patients with mild or moderate asthma[41]. Leukotriene antagonists cause some bronchodilatation within an hour of administration [42]and results of long term studies have shown a reduction in symptoms and exacerbations. Both drugs have shown efficacy when added to an inhaled corticosteroid. Montelukast was ineffective when added to other treatment in a pragmatic study of patients with more severe asthma[43]. Leukotriene antagonists should theoretically be useful in aspirin-induced asthma, but their effectiveness in these patients has been limited [44]. Churg-Strauss syndrome has occurred in association with use of the leukotriene antagonists, but whether it is due to a direct drug effect or unmasking of the syndrome as inhaled or oral corticosteroids are reduced is still uncertain [45].

1.11 Drugs that may be promising in near future

The suggestion that the anti-inflammatory effects of corticosteroids are due to gene silencing (transrepression), whereas the adverse effects are due to gene activation (transactivation) has led to interest in dissociated steroids that have only transrepression properties and should therefore have fewer adverse effects. Dissociated steroids produced unwanted effects on bone in animals, however, suggesting that some of their adverse effects are linked to transrepression[46]. The role of glucocorticoid receptor polymorphisms in determining the response to corticosteroids is also being investigated as is whether long-acting β-agonists enhance the anti-inflammatory effects of corticosteroids *in vivo* as shown *in vitro*. Increased understanding of the role of various cytokines and chemokines and the imbalance between Th1 and Th2 cells in asthma raises the possibility of interventions at different sites in the inflammatory cascade (Table 1.4). Some improvements in asthma control have been seen in early studies of soluble interleukin 4 receptors and selective Th2 cytokine inhibitors. Clinical studies of humanised monoclonal antibodies to Ig E, CD4+ cells, and interleukins 4 and 5 are under way. Antibodies to Ig E caused a large reduction in circulating free Ig E without major safety problems. The first large-scale clinical study [48] with an Ig E monoclonal antibody, omalizumab, in selected patients showed some clinical benefit and it enabled the dose of inhaled corticosteroid to be reduced.

The effects of antibodies to interleukin 5 and interleukin 12on clinical features of asthma have been disappointing. The need for regular injections to administer monoclonal antibodies is likely to restrict their role to patients with troublesome asthma. The completion of the human genome project is likely to fuel a new and important period of research into asthma therapy.

1.12 Drawbacks of modern medicines in asthma treatment

Despite the availability of a wide range of drugs for the treatment of asthma, the relief offered by them is mainly symptomatic. Moreover, the side effects of these drugs are also quite disturbing. Hence, a continuous search is on going to identify effective and safe remedies to treat bronchial asthma. Therefore, it is necessary to look for new solutions to manage this health problem. Although many drugs and interventions are available to manage asthma, in most instances these are expensive for a developing country like India and have several adverse effects. India is a country with a vast reserve of natural resources and a rich history of traditional medicine.

Although the contribution of modern synthetic medicine for elevating the human sufferings cannot be under-estimated, equally true is the fact that most of them leave unwanted harmful side/toxic effects on the human system disturbing the basic physiology. During the last three decades or so, there has been serious realization of these problems associated with synthetic drugs and as a result the world has started exploring the herbs as agents of therapy which, apart from being comparatively economical and easily available, are relatively free from the hazardous side effects, toxicity and development of resistance towards causative organisms. This does not mean that plants are hundred percent safe but in-depth review of literature and scientific work is still required in the field of medicinal plants regarding assessment of heavy metals and presence of toxins to call them safe Indian Medicinal Plants.

Significance of medicinal plants & traditional medicines in the management of asthma

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. It is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles. Due to lack of organized health care systems in developing countries, people with chronic diseases like asthma are among the worst suffers in their communities today. Hence, majority of the populations still have limited access or no access, especially those in remote areas, to modern medicines. Instead, they use traditional medicines for a range of disease complications. The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Different species of medicinal plants are used in the treatment of asthma. There are many natural herbs and herbal supplements that can be used for asthma treatment. Natural Asthma treatment incorporates vitamins, minerals and herbs to relieve symptoms and prevent further attacks. Among many disease or disorders, asthma is a serious disorder affecting large population of the world. Although there is a significant increase in the prevalence of number of patients suffering from asthma in every agegroup during the last decade, the largest increase of 73 percent was reported among children and young adults under the age group of 18 years. India has documented an estimated approx 15-20 million asthmatic patients. Some of the Medically advanced countries like United States of America have developed and maintained a national database of their citizens along with the natural allergy (asthma) causing plants detail, this detail includes the pollen releasing time, along with the main geographical distribution to assist its citizens in selection of a living as well as working place that may be free of trees responsible for causing asthma to particular individual. This reflects the seriousness of any country towards the healthcare needs of their citizens. The similar database or at least initiation of such type of database in India is need of the hour. Herbs are highly esteemed for millennia as a rich source of therapeutic agents for prevention and treatment of asthma and its ailments.

In the light of above background, the present study was aimed to screen Indian medicinal plants *Azima tetracantha* for the potential anti-asthmatic activity by Histamine induced and Acetylcholine induced bronchospasm in guinea pigs.

MATERIALS AND METHODS

1 Collection, identification and authentication of plant materials

The leaves of *Azima tetracantha* were collected. The collected plant samples were deposited in the Department of Pharmacology, Sural abs, Dilshuknagar, India for future reference.

7.2 Drugs and Chemicals

Drugs and Chemicals Compound disodium chromoglycate (DSCG), histamine dihydrochloride, acetylcholine chloride, atropine sulfate, mepyramine meleate, and toluidine blue were purchased from CAL Laboratories, Supplied by Kasliwal Brothers, Indore. Histamine solution was freshly prepared in

normal saline (NaCl, 8.5 g/l). All the other chemicals were of analytical grade.

Extraction

Extraction (11) Leaves of different plants were washed with distilled water to remove dirt and soil, and shade dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered materials (500 g) of each plant were defatted with petroleum ether (60-800) and then extracted separately with ethanol (95%, v/v) in a Soxhlet apparatus. The extracts were filtered and concentrated by distilling off the solvents and evaporated using water bath to get crude ethanol extract.

Figure 7.1 Soxhlet apparatus for the extraction of different plant Extracts

Experimental Animals

Antihistaminic and anticholinergic studies were conducted on guinea pigs (350-500 g) of either sex. For mast cell stabilization paradigm, adult Wistar albino rats weighing 140–160 g of either sex were used. All the Experimental animals were fed on commercial pellet diet (Poshak Ahar Pvt.ltd. India). They were group housed under standard conditions of temperature (22 ± 20C), relative humidity (60 ± 5%) and 12:12 light/dark cycle, where lights on at 0700 and off at 1900 h). They were divided in groups of ten animals each.

Group 1. The saline fed group served as control

Group 2. Treated with a standard drug. Before experimentation, the animals were kept on fast for 24 h but water was given ab de libitum.

During experiments, animals were also observed for any alteration in their general behavior.

All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).

7.6 Preparation of the test extracts

The polyherbal formulation was suspended in 1% SCMC in distilled water and administered orally or intraperitonially. The control animals were given an equivalent volume of SCMC vehicle. The standard group received various standard anti-asthmatic Drugs.

Preparation of plant extracts

7.5.1 Preparation of Extract:

Collection of plant materials.

The leaves of *Azima tetracantha* were collected from the outfield of Nimar Region, MP, India in February-March 2013. The plant materials were identified and authenticated by Dr. Rao, HOD, Botany and Plant Anatomy, Khargone, India.

7.12 Phytochemical screening of aqueous and ethanolic extracts of *Azima tetracantha*

Aqueous and Alcoholic extracts were chemically tested to know the presence of different primary, and secondary metabolites present in them as per the following scheme:

7.12.1 Tests for sterol/steroids

7.12.1.1 Salkowaski test

Few mg of the sample was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid and shaken. The development of red color in the chloroform layer indicates the presence of sterols/steroids.

7.12.2 Test for terpenoids

7.12.2.1 Liebermann–Burchard test

Few mg of the sample was dissolved in 1 ml of chloroform and few drops of acetic anhydride. Concentrated sulphuric acid was added by the side of the test tube. Production of purple color indicates the presence of triterpenoids and blue–green color indicates the presence of sterols.

7.12.3 Test for alkaloids

Few mg of the sample was taken in 5 ml of 1.5% v/v hydrochloric acid and filtered. The filtrate was then tested using following reagents:

7.12.3.1 Dragendorff's reagent

It is a solution of potassium bismuth iodide. It was prepared by dissolving bismuth nitrate (8 gm) in nitric acid (20 ml), and separately dissolving potassium iodide (27.2 gm) in water (50 ml), mixing the two solutions, and making up the volume to 100 ml. Above Dragendorff's reagent was sprayed on Whatman No. 1 filter paper then the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of a capillary tube. Development of an orange red color on the paper indicated the presence of alkaloids.

7.12.3.2 Mayer's Reagent

1.36 gm of mercuric chloride, and 3 gm of potassium iodide were dissolved in water to make 100 ml. To a little of each extract taken in dilute hydrochloric acid in a watch glass, few drops of the reagent was added, formation of cream-colored precipitate shows the presence of alkaloid.

7.12.3.3 Hager's reagent

It is a saturated solution of picric acid in water. When the test filtrate was treated with this reagent, yellow precipitate was obtained indicating the presence of alkaloids.

7.12.3.4 Wagner's Reagent

It is a solution of potassium triiodide in water which was prepared by dissolving 1.3 gm iodine in a solution of potassium iodide (2 gm) in water to make 100 ml. Formation of brown precipitate after addition of this reagent in extract indicates the presence of alkaloids.

7.12.4 Test for tannins

Sample was taken separately in water, warmed, and filtered. Tests were carried out with the filtrate using following reagents:

7.12.4.1 Ferric chloride test

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Dark green or deep blue color shows the presence of tannins.

7.12.4.2 Lead acetate test

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitate indicates the presence of tannins.

7.12.5 Test for flavonoids

7.12.5.1 Shinoda test

To the sample, 5 ml of 95% ethanol, few drops of HCl, and few magnesium turnings were added. The pink color shows the presence of flavonoids.

7.12.6 Test for carbohydrates

7.12.6.1 Molisch's test

About 0.1 gm of the sample was dissolved in 2 ml of water, and added 2-3 drops of 1% ethanolic solution of alpha naphthol, and then carefully poured 2 ml of concentrated sulphuric acid down the side of the test tube so that it forms a heavy layer at the bottom. A deep violet color is produced if carbohydrates are present.

7.12.6.2 Fehling's test

1 ml of Fehling's A and 1 ml of Fehling's B solutions were mixed and boiled for 1 minute. Equal volume of sample was then added, heated in boiling water bath for 5-10 minutes. First a yellow then brick red color shows the presence of carbohydrates.

7.12.6.3 Benedict's test

Equal volumes of the reagent, and sample were added. Mixture was then boiled for five minutes. Depending on the reducing sugar present a range of colors develops.

7.12.7 Test for proteins

7.12.7.1 Biuret test

To the sample 4% NaOH, and few drops of 1% $CuSO_4$ were added. Violet or pink color indicates the presence of proteins.

7.12.7.2 Xanthoproteic test

Sample was mixed with 1 ml of concentrated sulphuric acid; formation of precipitate shows positive test.

7.12.7.3 Millon's test

3 ml of sample was mixed with Millon's reagent, formation of precipitate indicates the presence of proteins.

7.12.8 Test for saponins

7.12.8.1 Foam test

A few mg of the sample was shaken vigorously with water. Honeycomb like foam indicates the presence of saponins.

7.12.8.2 Heamolytic test

Sample was added to one drop of blood placed on glass slide. Hemolytic zone indicated the presence of saponins.

7.12.9 Test for anthraquinone glycosides

7.12.9.1 Borntrager's test

To 3 ml of the sample, dilute sulphuric acid was added, boiled, and flittered. To the filtrate equal volume of chloroform was added, and shaken. After separating the organic layer, ammonia was added. Turning pink of ammonical layer indicates the presence of said glycosides.

7.12.10 Test for cardiac glycosides

7.12.10.1 Keller-Kiliani test

The sample was dissolved in acetic acid containing trace amounts of ferric chloride. It was then transferred to the surface of concentrated Sulphuric acid. A reddish-brown ring will be formed at the junction, and the color slowly changes to blue.

7.12.10.2 Legal's test

A few drops of pyridine, and sodium nitroprusside were added to the sample, and made alkaline. A pink or red color is obtained.

Pharmacological Screening (Antiasthamatic paradigms)

Histamine-induced Bronchospasm in guinea pigs

The guinea pigs fasted for 24 h were exposed to an atomized fine mist of 2% histamine dihydrochloride aerosol (dissolved in normal saline) 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 difficulty in breathing leading to convulsions, asphyxia and death. The time until signs of convulsion appeared is called pre-convulsion time (PCD). By observation experience was gained so that the Preconvulsion time can be judged accurately. As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. 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 ten animals each. Mepyramine (8 mg/kg) and polyherbal formulation (300 mg/kg) were administered intraperitonially 30 min prior to exposure. Animals, which did not develop typical asthma within 6 minutes were taken as protected.

Acetylcholine-induced bronchospasm in guinea pigs

Procedure was similar to the histamine aerosol study except that animals were exposed to aerosol of 0.5% acetylcholine in another set of animals (n = 10). Atropine sulphate (2 mg/kg) was used as a standard drug. Mast cell degranulation by compound 48/80 this was carried out as per the method described by Kaley and Weiner (1971) with little modification. Male albino rats were sacrificed by cervical dislocation. The animals were immediately injected with 15 ml of prewarmed (37°C) buffered salt solution (NaCl 137 mM; KCl 2.7 mM; MgCl 2; 1 mM; CaCl20.5 mM; NaH_2PO40.4 mM; Glucose 5.6 mM; HEPES 10 mM) into the peritoneal cavity, and massaged gently in this region for 90 s, to facilitate cell recovery. A midline incision was made and the peritoneum was exposed. The pale fluid was aspirated using a blunted plastic Pasteur pipette, and collected in a plastic centrifuge tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant discarded to reveal a pale cell pellet. The cell pellets were re-suspended in fresh buffer and re-centrifuged. Aliquots of the cell suspension were incubated with the test compounds or disodium cromoglycate, before challenge with compound 48/80. The aliquots were carefully spread over glass slides and the mast cells were stained with 1% toluidine blue and counterstained with 0.1% light green. The slides were dried in air and the mast cells counted from randomly selected high power objective fields (X450). The effect of polyherbal formulation on mast cells was studied by incubating the mast cells for 10 min with the above formulation in a concentration of 1, 10 or 100 µg/ml. In another set of experiments the mast cells which were pre-incubated with polyherbal formulations were exposed to the mast cell degranulator, compound 48:80 (10 µg/ml) and the incubation continued for a further 10 min. Then, the mast cells were carefully spread over glass slides. The percent degranulation of the mast cells

was calculated. Disodium cromoglycate (DSCG) (20 µg/ml) was included in one of the study groups for comparison.

Acute Toxicity Test (Determination of LD50)

The acute toxicity test (LD50) was determined according to the procedure described by Kabore Adama, Albino mice (20–25 g) of either sex were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10, 100 and 1000 mg/kg were administered intraperitoneally (i.p.), one dose for each group. The treated animals were monitored for 24 h for mortality and general behavior. From the result of the above step, four different doses of 250, 500, 1000 mg/kg were chosen and administered i.p. respectively to three groups of one mouse per group. The treated animals were again monitored for 24 h. The LD50 was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death. Preparation of polyherbal formulations the polyherbal formulations was composed of ethanolic extracts of Solanum xanthocarpum (50 mg), Aegle marmelos (50 mg), Caesalpinia bonduc (100 mg), and Murraya koenigii(100 mg).

7.11 Data analysis

The data are presented as mean ± S.E.M. Statistical significance was determined using One-way Analysis of Variance (ANOVA) followed by Dunnett's *t*-test or Chi-square test with *Yate's* correction factor. Differences were considered significant at $P < 0.05$.

RESULTS:

8.1 Extraction

Dried leaf powder of *Azima tetracantha* (500 gm) were extracted with water and alcohol (95% w/v) to get concentrated aqueous and alcoholic extract. The yields of extracts were as follows: AQEAT (20.48% w/w), ALEAT (11.45% w/w).

8.2 Acute toxicity test (Determination of LD50)

The LD50 of the AQEAT was calculated to be 2262.7 and 1131.4mg/kg by oral route. During the acute toxicity study, animals were observed for gross behavioral and morphological changes (respiratory distress, immobility, convulsion, loss of righting reflex etc.). Based on the results of acute study, doses were then selected. The plant extracts did not produce any significant changes in the normal behavior of the animals, and no toxic symptoms were seen at the dose levels studied.

8.3 Phytochemical screening of different plant extracts

Table 8.1: depicts results of screening of different plant extracts for various phytochemical constituents. AQEAT showed the presence of alkaloids, proteins, anthraquinone glycosides, flavonoids, saponins, sterols, tannins, and carbohydrates. ALEAT was found to contain alkaloids, flavonoids, saponins, steroids, and tannins.

Compounds	Name Of the Test	Leaf Extracts	
		Aqueous	Alcoholic
Alkaloids	Dragendroff's test	+	-
	Mayer's test	-	+
	Wagner's test	-	-
	Borntrager's test	+	+
Anthraquinone	Modified Borntrager's test	-	-
	Molisch's test	-	+
Carbohydrates	Anthrone reagent test	+	+
	Benedict's reagent test	+	+
Cardiac Glycosides	Kellar Kilani test	-	-
	Potassium hydroxide test	-	-
Flavonoids	Shinoda test	+	-
	Ammonia test	-	+
Glycosides	Benodict's test	+	-
	Fehling's test	-	-
Phenols	Ferric chloride test	-	-
	Phosphoimolybdic acid test	-	-
Phlobatannins	Hydrochloric acid test	-	-

	Xanthoprotein teswt	-	-
	Biurete test	-	-
Proteins	Million's reagent test	+	-
Quinines	Sodium hydroxide test	-	-
Reducing sugar	Benedict's reagent test	-	-
Resins	Turbidity test	+	+
Saponins	Foam test	+	+
Steroids/Terpenoids	Salkowski test	+	+
	Liberman-Burchard test	-	-
Tannins	Bramer's test	+	+

'+' found to be present, '-'found to be absent

8.4: RESULTS AND DISCUSSION:

The results of the present study revealed mast cell stabilization, antihistaminic and anti-cholinergic actions of polyherbal formulation. In the mast cell stabilization paradigm, 33% of mast cells were degranulated in the control group. Addition of DSCG (20 µg/ml), and the polyherbal formulation (100 µg/ml) reduced the percentage of mast cell degranulation (P< 0.01) compared to the control group. Compound 48/80 (10 µg/ml) produced about 76% degranulation of mast cells. Pretreatment with DSCG (20 µg/ml) and the polyherbal formulation (1, 10, and 100 µg/ml) significantly reduced (P< 0.01) degranulation of mast cells as compared to compound 48/80-tretaed control group (Table 1). The protection given by them at higher concentrations was comparable to that of DSCG, a potent mast cell stabilizing agent. These results suggest hat polyherbal formulation protects mast cells from compound 48/80-evoked degranulation. In the histamine aerosol study, the control animals showed convulsion during the first 3 min of the experiment. Prior treatment of polyherbal formulation (300 mg/kg, i.p.) protected the animals (Table 2) to a significant extent (P< 0.01) from the development of asphyxia produced by histamine aerosol confirming that it has antihistaminic activity. The role of histamine in asthma is well established (Nelson, 2003). The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins.

Table 1: Effect of polyherbal formulation on histamine-aerosol in guinea pigs

Treatment	**Dose mg/kg,ip**	**Preconvulsion time**	**Protection**
Control	Saline	130.2±1.42	-
Mepyramine	8	462±1.24	-
Polyherbal formulation	300	380±2.13	52.01

Values are mean ± S.E.M. (n = 10); **P< 0.01 vs. control; Dunnett's t-test after way ANOVA

Table 2: Effect of polyherbal formulation on acetylcholine induced bronchospasm in guinea pigs

Treatment	**Dose mg/kg,ip**	**Preconvulsion time**	**Protection**
Control	Saline	125.1±1.41	-
Atropine Sulphate	2	390±2.15	-
Polyherbal formulation	300	262±1.52	59.12

Values are mean ± S.E.M. (n = 10); **P< 0.01 vs. control; Dunnett's t-test after way ANOVA.

Table 3: Effect of polyherbal formulation on mast cell

Treatment	**Dose mg/kg,ip**	Weight of granuloma (mg)	Inhibition %
Control	Saline	120.2±2.41	-
Prednosoline	10	69.1±1.47	40.14
Polyherbal formulation	300	62±1.22	48.38

Values are mean ± S.E.M. (n = 10); **P< 0.01 vs. control; Dunnett's t-test after way ANOVA.

Acetylcholine-aerosol provoked bronchoconstriction in all animals of control group. The polyherbal formulation (300 mg/kg, i.p.) significantly protected animals (P< 0.01) from acetylcholine-induced bronchoconstriction. Mepyramine (a standard antimuscarinic drug, 2 mg/kg, i.p.) significantly prolonged pre-convulsion time to 462 sec (P< 0.01) and protected animals from asphyxia (Table 3). The LD50 of the polyherbal formulation was calculated to be 850.7 mg/kg, i.p. In the present study, we have used histamine and acetylcholine as spasmogens in the form of aerosols to cause immediate bronco constriction in guinea pigs. Mepyramine (8 mg/kg) were used as reference standard against histamine and acetylcholine induced bronchospasm respectively (Kumar A, Ramu P). The bronchodilatory effect of polyherbal formulation was found comparable to the protection offered by both the reference standard drug Mepyramine.

CONCLUSION:

Result study on *Azima tetracantha* Suggest that, there was appreciable decrease in severity of symptoms of asthma and also simultaneously improvement in lung function parameters. In conclusion, the results of present investigation suggest that, polyherbal formulation have significant bronchodilator activity against histamine and acetylcholine. However, further studies are suggested to establish molecular mechanism and also to isolate and characterize the active principles responsible for the action. Further study is ongoing to characterize the active principles of the extract which are responsible for antiasthmatic activity1

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