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3.1 Introduction to Bronchial Asthma

Bronchial asthma is a major public health problem worldwide and the morbidity and mortality of asthma have increased in last few decades. The past decade has witnessed phenomenal increases in the incidences of asthma, asthma-related deaths and hospitalization. About 150 million people around the globe suffer from asthma and this number is rising. Worldwide, deaths from this condition have reached over 180,000 annually. Asthma affects over 5% of the population in the United States. Asthma accounts for 1.6% of all ambulatory care visits according to the national Ambulatory Medical care Survey and results in more than 470,000 hospitalizations per year (CDC 1998). The economic costs associated with asthma are estimated to exceed those of TB and HIV/AIDS combined (WHO 1998). Children have highest prevalence of asthma with a total of over five million children affected. India has an estimated 15-20 million asthmatics.

Bronchial asthma is a syndrome characterized by increased responsiveness of trachea and bronchi to various stimuli and manifested by acute, recurrent or chronic attacks of widespread bronchial- bronchiolar narrowing, variable in severity and usually of brief duration. According to the definition utilized by many physicians, asthma is not a disease but rather a syndrome, which unlike a disease cannot be attributed to one specific cause, but rather to several causes. The other way of defining asthma is where there is a recurrent 'reversible' obstruction of the airways in response to stimuli which are not in themselves noxious and which do not affect non-asthmatic subjects. Typically, all asthma patients with active disease have hyper responsive (hyper reactive) airways, manifest as an exaggerated bronchoconstrictor response to many different stimuli which can be allergenic, environmental, occupational, infections, pharmacological, exercise related and emotional (Fig. 1).
Asthma is a complex syndrome with many clinical phenotypes in both adults and children. Its major characteristics include a variable degree of airflow obstruction, bronchial hyperresponsiveness, and airway inflammation. For many patients, the disease has its roots in infancy, and both genetic factors (atopy) (Cookson 1999; Prescott et al 1998) and environmental factors (viruses, allergens and occupational exposures) (Stein et al 1999; Halonen et al 1999; Venables and Chan-Yeung 1997) contribute to its inception and evolution.

Airway obstruction in asthma is due to a combination of factors that include spasm of airway smooth muscle, edema of a of airway mucosa, increased mucus secretion, cellular (especially eosinophilic and lymphocytic) infiltration of the airway walls and injury and desquamation of the airway epithelium.
3.1.1 Classification of Asthma

There are two types of asthma, based upon the stimuli initiating. Extrinsic (allergic/ atopic) and intrinsic (idiosyncratic/ nonatopic) (Harsh Mohan 1998). Characteristics of extrinsic and intrinsic asthma (Table: 1) tend to occur so commonly in the same individual, however that this distinction has limited usefulness. It is useful however to identify a number of factors that are commonly implicated in causing an asthma attack.

**Table: 1**

**Types of asthma**

<table>
<thead>
<tr>
<th>Extrinsic asthma (allergic / atopic)</th>
<th>Intrinsic asthma (idiosyncratic/ nonatopic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset usually in childhood or early adult life.</td>
<td>Onset usually (but not invariable) in older adults. Begins after the age of 30 and tends to perennial and more severe. Status asthmaticus is more common in this group.</td>
</tr>
<tr>
<td>Family history of multiple allergies (asthma, hayfever, eczema) common (50%) well defined allergic history to a variety of inhaled allergens (atopy)</td>
<td>Family history of multiple allergies less common (20%).</td>
</tr>
<tr>
<td>Known external allergens.</td>
<td>No known external allergens.</td>
</tr>
<tr>
<td>IgE raised in 50-60% of subjects.</td>
<td>IgE normal or low.</td>
</tr>
<tr>
<td>Other allergies (hayfever and eczema) often present (54%).</td>
<td>Other allergies uncommon</td>
</tr>
<tr>
<td>Positive immediate skin tests.</td>
<td>Negative skin tests</td>
</tr>
<tr>
<td>Intermittent asthma.</td>
<td>More continuous asthma.</td>
</tr>
</tbody>
</table>

The asthmatic subjects have intermittent attacks of dyspnea, wheezing, and cough. In many subjects the asthmatic attack consists of two main phases as can be demonstrated by tests of FEV.
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- The immediate phase
- The late phase

However, in some subjects, only one of the phases may be obvious.

The immediate phase

The activation of cells bearing allergen-specific IgE initiates the early phase reaction. It is characterized primarily by the rapid activation of airway mast cells and macrophages. The activated cells rapidly release pro-inflammatory mediators such as histamine, eicosanoids, and reactive oxygen species that induce contraction of airway smooth muscle, mucus secretion, and vasodilatation. Inflammatory mediators induce microvascular leakage with exudation of plasma in the airways (Bousquet et al 2000). Plasma proteins also may promote the formation of exudative plugs mixed with mucus and inflammatory and epithelial cells. Together these effects contribute to airflow obstruction. Exercise induced asthma appears to involve only the phenomena of this first phase.

The late Phase

The second, late-phase response, i.e. the delayed response, occurs 6 to 9 hours after allergen provocation and involves the recruitment and activation of eosinophils, CD4 T cells, basophils, neutrophils, and macrophages (Bousquet et al 2000). The activation of T cells after allergen challenge may lead to the release of T-helper cell type 2 (Th2) like cytokines that may be a key mechanism of the late phase response (Kay 1992).

This phase is in essence an acute inflammatory reaction. The inflammation has special characteristics because asthma is not consistently associated with the inflammation seen, for example in bronchitis. There is infiltration not only by the usual inflammatory cells but also, and more specifically, by eosinophils and platelets. There is usually a blood eosinophilia and also some degree of loss of bronchial epithelium.
3.1.2 Pathophysiology

Asthma is characterized by a complex inflammatory response involving resident (e.g. mast cells, macrophages, nerves), recruited (e.g. lymphocytes, eosinophils, monocytes) and structural cells (e.g. epithelium, airway smooth muscle, fibroblast). These cells can synthesize and secrete a vast numbers of mediators, which may contribute to the bronchoconstriction, submucosal gland secretion, vasodilation, bronchial wall oedema, recruitment of inflammatory cells, airway remodeling and bronchial hyperresponsiveness observed in asthma.

Inhaled allergen challenge models contribute most to our understanding of acute inflammation in asthma. More recent studies have found substantial inflammation in bronchial-biopsy specimens from patients with asthma, even those with mild disease. These inflammatory changes can occur throughout the central (Haley et al 1998) and peripheral (Haley et al 1998; Kraft et al 1996) airways and often vary with the severity of the disease (Vignola et al 1998; Hamid and Minshall 2000). Further evidence of an inflammatory response in asthma is the presence of cytokines that mediate inflammation and chemotactic chemokines in bronchoalveolar-lavage fluid or pulmonary secretions (Barnes and Chung 1999).

The hallmark of asthma is of course airway inflammation. At the cellular level, events begin when macrophages present antigen to T lymphocytes that elaborate type 2 T-helper (Th2) cytokines such as interleukins 4, 5, and 13. In turn, IL-4 and IL-13 regulate IgE synthesis. (They allow B cells to switch to IgE). IgE binding to allergen can cross link the high affinity IgE receptor on mast cells, resulting in release of mediators that include histamine, prostaglandins, leukotrienes, and inflammatory cytokines. IL-5 mediates eosinophils recruitment and infiltration, acting in tandem with members of beta-chemokine family, especially eotaxin, macrophage inflammatory protein-1-alpha and monocyte chemoattractant protein-1. Eosinophils are considered pivotal cellular mediators of asthma. Recruitment of eosinophils requires the binding of adhesion
molecules on the cells to their counter receptors on vascular endothelial cells. Tumor necrosis factor alpha (TNF-α) of mast cells or macrophage origin upregulates endothelial expression of intercellular adhesion molecules 1 and 2, while IL-4 and IL-13 up regulates endothelial expression of vascular cell adhesion molecule-1. These interact with their ligands, lymphocyte function associated antigen-1 and very late acting antigen-4, on T cells and eosinophils, allowing cell migration from the vasculature. Subsequent infiltration of these cells into the airways associated with many of the pathologic changes seen in asthma.

Eosinophils produce immunoregulatory cytokines (IL-3, 4, 5, 6 and 8, granulocyte macrophage colony simulating factor, TNF alpha) growth factors (Transforming growth factors alpha), and proteins toxic to cells (Major basic protein eosinophils paraoxidase and eosinophils derived neurotoxin), thereby contributing to airway inflammation and over the long term remodeling (Fig. 2).

**Fig. 2 The role of eosinophils in allergic inflammation.**
Obtained from Busse WW, Lemanske RF.
3.1.2.1 Inflammatory cells

Epithelial cells
Bronchial epithelial cells traditionally have been considered as a barrier, participating in mucociliary clearance and removal of noxious agents. However, epithelial cells also participate in inflammation by the release of eicosanoids, peptidases, matrix proteins, cytokines, and nitric oxide (NO). IgE dependent mechanisms, viruses, pollutants, or histamines can activate epithelial cells. In fatal asthma, extensive epithelial shedding occurs. The integrity of airway epithelium may influence the sensitivity of the airways to various provocative stimuli (Bousquet et al 2000).

Eosinophils
Eosinophils play an effector role in asthma by release of pro-inflammatory mediators, cytotoxic mediators, and cytokines. Eosinophils migrate from their origin in the bone marrow to the airways by cell rolling, through interaction with selectin, and eventually adhere to the endothelium through the binding of integrins to adhesion proteins [vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1)]. Eosinophils survival is prolonged by IL-5 & GM-CSF. On activation, eosinophils release inflammatory mediators such as leukotrienes, particularly the cysteinyl leukotriene C4, which contracts airway smooth muscle, increases vascular permeability, and may recruit more eosinophils to the airways (Rothenberg 1998). It also releases granule proteins to injure airway tissue and intensify bronchial responsiveness (Bousquet et al 2000, Busse and Lemanske 2001).

Lymphocytes
Mucosal biopsy specimens from patients with asthma contain lymphocytes, many of which express surface markers of inflammations. There are two types of T-helpers CD4+ cells. Type 1 T-helper (Th1) cells produce IL-2 and interferon γ, both essential for cellular defense mechanism. Th2 cells produce cytokines (IL-4,-

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5,-6,-9 and -13) that mediate allergic inflammation. It is known that Th1 cytokines inhibit the production of Th2 cytokines and vice-a versa. The extent of imbalance between Th1 and Th2 cells (as indicated by diminished interferon γ production) during the neo-natal phase may predict the subsequent development of allergic disease, asthma, or both. Factors have been identified that enhance Th1-mediated responses and that are associated with a reduced incidence of allergy, asthma or both. These factors include infection with mycobacterium tuberculosis, measles virus, and hepatitis A virus; increased exposure to infections through contact with older siblings; attendance at day care during the first 6 months of life; and a reduction in the production of interferon γ (Busse and Lemanske 2001).

Alveolar Macrophages
The primary function of alveolar macrophages in the normal airway is to serve as “scavengers”, engulfing and digesting bacteria and other foreign materials. They are found in large and small airways, ideally located for affecting the asthmatic response. Mediators like PAF, LTB4, LTC4, LTD4 are produced by these cells and they produce inflammation (Bousquet et al 2000).

Neutrophils
Neutrophils may play a pivotal role in the disease process, at least in the sudden onset fatal cases as high numbers of neutrophils have been reported to be present in the airways of patients who died from sudden onset fatal asthma (Sur et al 1993).

Adhesion molecules
Adhesion molecules help adhesion of the various cells to each other and the tissue matrix to facilitate infiltration and migration of these cells to the site of inflammation. The adhesion molecules are divided onto families on the basis of their chemical structure. Some of which thought to be important in inflammation includes the integrins, immunoglobulin supergene family, selectins and
carbohydrate lignans including ICAM-1 and VCAM-1. Adhesion molecules are found on a variety of cells, such as neutrophils, monocytes, lymphocytes, basophils, eosinophils, granulocytes, platelets, endothelial cells, and epithelial cells, and can be activated by the many inflammatory mediators present in asthma. A major role of adhesion molecules is in the recruitment of leukocytes from the vascular lumen to tissues. Activation of the leukocytes or endothelial cells follows in response to a mediator or the initial adhesion event. Finally, firm adhesion of the leukocytes to endothelial cells surfaces allows diapedesis between endothelial cells and migration of leukocytes into the extracellular matrix. ICAM-1 and VCAM-1 are involved in the migration of lymphocytes and eosinophils (Busse and Lemanske 2001).

**Mast cells**
Mast cells are pivotal in the allergic response type I or the anaphylactic type—a rapidly progressing chain reaction that causes sudden attack of asthma. Mast cells are ubiquitous and are found around blood vessels in the connective tissue, in the lining of the gut and importantly in the lining of the upper and lower respiratory tract. These are large mononuclear cells heavily granulated, with granules containing a host of pharmacologically active substances. The allergen (antigen) enters into the human body through the respiratory tract, skin and/or Gastro Intestinal Tract (GiT). After the exposure to antigens, antibodies directed against specific antigens (i.e. IgE) are formed and are fixed to their respective receptors on the surface of the mast cells. This process is called sensitization of mast cells. During the second exposure to antigens, the antigens react with these antibodies at the cell surface. This event leads to a series of biochemical reactions. These migrate to the periphery in the secretory expulsion of the mast cell granules containing active substances (vasoactive amines and chemolytic amines) causing asthma attacks. This process is called “mast cell degranulation”. Mast cell degranulation releases interleukins, proteases, and other enzymes that activate the production of other mediators of inflammation.
3.1.2.2 Inflammatory mediators

Histamine
Histamine is stored in granules within mast cells and basophils and can be released under immunological conditions following the cross linking of antigen to high affinity IgE receptors present on the surface of mast cells and basophils or by non immunological stimuli (e.g. compound 48/80, calcium ionophore, substance P and hypo-osmolar solutions) (Liu et al 1991). Acute release of histamine following an allergic or non-allergic insult may lead to bronchoconstriction, which can be attenuated by selective H_1-receptor antagonists (Holgate and Finnerty 1989). There is evidence that histamine may also stimulate sensitized afferent nerves (Empey et al 1976). With regard to inflammatory cells, histamine has been shown to activate eosinophils (Raible et al 1994).

Prostanoids
Immediately following acute antigen challenge of asthmatic subjects, increased levels of prostaglandin (PG) F_2, PGD_2 and thromboxane (TX) B_2 are detected in bronchoalveolar lavage fluid (Liu et al 1990), which are derived from macrophages, airway epithelium and activation of mast cells. When inhaled, these prostanoids cause bronchoconstriction (Saroea et al 1995) and increase airway responsiveness to spasmogens unrelated to alterations in airway caliber (Fuller et al 1986), which suggests that prostanoids may play a greater role in modulating airway responsiveness.

Prostanoids are synthesized by cyclooxygenase (COX). Various pro-inflammatory cytokines stimulate the induction of COX_2 in human airway epithelium (Mitchel et al 1994) and smooth muscle (Johnson & Knox 1997) in culture suggesting that during inflammation, COX_2 expression may be augmented.
Some of the studies have shown that inhaled PGE$_{2}$ inhibits the development of the late asthmatic response unrelated to functional antagonism of airway smooth muscle contraction and attenuates the attendant increase in sputum eosinophilia (Gauvreau et al 1999). While other studies have shown that PGE$_{2}$ may have pro-inflammatory properties, which might play a role in the development of the allergic response by down regulating interferon (IFN)$\gamma$ and interleukin (IL)-12 productions from T-lymphocytes and monocytes, respectively, there by promoting T helper 2 lymphocyte development (Van der Pouw Kraan et al 1995). The potential anti and pro-inflammatory properties of PGE$_{2}$ may reflect activation of different prostanoid receptor subtype on different cells.

**Leukotrienes**
Cysteinyl leukotrienes, including LTC$_{4}$, LTD$_{4}$ and LTE$_{4}$ are endogenous bioactive lipid mediators derived from the 5-lipoxygenase pathways in mast cells, eosinophils and macrophages. They posses potent pro-inflammatory actions resulting in increased vascular permeability, mucus secretion and bronchial hyperresponsiveness, they are responsible for activation and recruitment of inflammatory cells and are very potent spasmogens of human airway smooth muscle (Drazen et al 1999). Cysteinyl leukotrienes are known to activate two receptors, CysLT$_{1}$ and CysLT$_{2}$, and the biological activity of the cysteinyl leukotrienes is mediated via activation of CysLT$_{1}$ (Lynch et al 1999). LTC$_{4}$, LTD$_{4}$ and LTE$_{4}$ increased the sensitivity of the airways to inhaled histamine which may be due to leukotrienes may alter the excitability of afferent nerves (McAlexander et al 1998) thereby increasing the sensitivity of the airways to indirect acting stimuli. Alternatively LTD$_{4}$ has been shown to promote the pulmonary recruitment of eosinophils in an animal model, by an IL-5 dependent manner (Underwood et al 1996), which could lead to an exacerbation of airway responsiveness.

**Bradykinin**
The release of klinis within the airway could lead to the activation of bradykinin receptors including $\beta_{1}$ receptors whose expression is regulated by inflammatory
cytokines and $\beta_2$ receptors present on various cells within the airway wall including vascular endothelium, airway smooth muscle, submucosal glands, nerves and airway epithelium (Mak & Barnes 1991). Clinical study suggests that bradykinin induces bronchoconstriction via activation of afferent nerves. Animal studies reveal that bradykinin simulates sub-population of afferent nerves, C-fibres (Fox et al 1993) and mediates the release of sensory neuropeptides from these (Saria et al 1988). The increase in airway responsiveness to bradykinin correlates with the number of eosinophils in bronchoalveolar lavage fluid, bronchial biopsies and sputum (Polosa et al 1998).

**Sensory neuropeptides**

The preprotackykinin-I (PPT-I) gene encodes substance P and neurokinin A while PPT-II encodes neurokinin B (Krause et al 1987). Alternate splicing of the PPT-I gene results in the formation of three mRNAs. Post translational processing of these mRNAs yields substance P. Studies have revealed that neutral endopeptidase which is localized in the epithelium of the lung of guinea-pigs (Kummer & Fischer 1991) and man (Johnson et al 1985) is responsible for the degradation of tachykinins (Nadel 1991). Three different types of tachykinin receptors i.e. NK$_1$, NK$_2$ and NK$_3$ exist in the ileum of various animal species. The effects of the tachykinins induced by activation of NK$_1$ receptors in the lung include mucus secretion, microvascular permeability and inflammatory cell recruitment and activation (Maggi 1995). Tachykinins contract human isolated bronchi via NK$_2$ receptors (Advenier et al 1992). In human studies, neuropeptides including substance P, calcitonin gene related peptide (CGRP), neurokinin A, neuropeptide Y and vasointesinal pepide (VIP) have been detected in the lung (Komatsu et al 1991). A common pathway by which various stimuli induce bronchial hyperresponsiveness might be the sensory nerves as it has been claimed that substance P containing nerves are more abundant in lungs obtained a autopsy from asthmatic as compared with healthy individuals (Ollerenshaw et al 1991). An elevated level of substance P like immunoreactivity has also been
detected in the sputum of patients with asthma or chronic bronchitis following hypertonic saline inhalation (Tomaki et al 1995).

**Endothelin**

Endothelins were originally discovered as potent vasoconstricor peptides, which are encoded by three distinct genes (Inoue et al 1989). They are formed via the action of endothelin converting enzyme (ECE). The expression of mRNA for the endothelins and ECE has been documented in human bronchial epithelial cells (Saleh et al 1997) and can be upregulated by a variety of pro-inflammatory cytokines (Shima et al 1995). The biological effects of endothelins are mediated via two receptors, designated as ET\textsubscript{A} and ET\textsubscript{B}, which are characteristic of G-protein coupled receptors. Endothelin is a potent contractile agonist of human airway smooth muscle (Knott et al 1995) and augments cholinergic nerve mediated responses in human airways in-vitro (Fernandes et al 1996), both effects mediated via the activation of ET\textsubscript{B} receptors. Few studies have examined the pro-inflammatory action of endothelins in the airways (Hay 1989). ET\textsubscript{A} – but not ET\textsubscript{B} receptor antagonists attenuated allergen induced recruitment of eosinophils in a murine model of inflammation (Fujitani et al 1997) in part by increased production of IFN\textgamma{} from pulmonary lymphocytes. Endothelins cannot be stored and requires de-novo synthesis, which may occur several hours after acute allergen challenge.

**Cytokines**

Cytokines are extracellular signaling proteins produced by different cell types including immune cells like T-lymphocytes. They act on target cells to cause a wide array of cellular functions like activation, proliferation, chemotaxis, immunomodulation, release of inflammatory mediators, growth and cell differentiation and apoptosis. CD\textsuperscript{4+} lymphocytes are classified as T-helper (Th\textsubscript{1}) cells, which provide immunity to pathogens and as T-helper (Th\textsubscript{2}) cells, that gives rise to allergic inflammation. Th\textsubscript{1} lymphocytes secrete IFN\textgamma{}, IL-2 and tumor necrosis factor (TNF)-\textbeta{}, while Th\textsubscript{2} lymphocytes secrete cytokines such as IL-3,
4, -5, -10 -13 and GM-CSF (Broide & Firestein 1991; Robinson et al 1996). IL-4 and IL-13 switch on B cells to produce IgE (Punnonen et al 1993). IL-5 promotes eosinophilic inflammation. IFNγ and IL-4 inhibit the growth and functions of other subset. Allergic reactions in the airways are caused by IgE sensitized mast cells and CD4+ Th2, whose activation leads to the infiltration of inflammatory cells, notably eosinophils, leading to tissue damage (Erb 1999). The differentiation, migration and pathobiological effects of eosinophils occur through the effect of GM-CSF, IL-3, IL-5 and certain chemokines such as eotaxin. A recent study has demonstrated that the adoptive transfer of Th0 and Th2 clones into severe combined Immunodeficiency (SCID) mice induces both and pulmonary eosinophilia and bronchial hyperresponsiveness following antigen challenge (Hansen et al 1999). Airway macrophages are important source of IL-1, TNF-α and IL-6. These cytokines then act on epithelial cells to release GM-CSF, IL-8 and RANTES (regulate on activation, normal T cells expressed and secreted) which amplify the inflammatory response and lead to influx of secondary cells such as eosinophils which themselves may release multiple cytokines. Cytokines also exert regulatory effect on the expression of iCAM-1 and VCAM-1, on epithelial cells of bronchial circulation and airway epithelial cells (Schleimer et al 1992). Some cytokines like IL-10 and IFNγ have anti-inflammatory effect on allergic inflammation (Barnes & Lim 1998).

Chemokines

Chemokines are chemotactic cytokines, which are potent chemoattractant of eosinophils, basophils, monocytes and T-lymphocytes. They are classified into two major groups. a) CXC chemokines, in which the first two-cysteine residues are separated by an amino acid and b) CC chemokines, in which the cysteine residues are adjacent to each other. Exacerbation of asthma leads to the synthesis and release of various chemokines, which can contribute to the recruitment of inflammatory cells to the airways. Chemokines are known to exert their effect through chemokine receptors CCR3, which belong to rhodopsin like G-protein coupled receptors (Barnes & Chung 1999). CCR3 mediates biological
effects of eotaxin and other eosinophil chemokines, such as RANTES, monocyte chemotactic protein (MCP)-3, MCP-4 and is expressed predominantly on eosinophils.

**Growth factors**

A variety of growth factors are thought to play a role in altering the structure of the airways. A number of growth factors, including platelet derived growth factor (PDGF), transforming growth factor (TGF) and epidermal growth factor (EGF) has been investigated in bronchial biopsies from asthmatic subjects (Chanez et al 1995). A number of in-vitro studies have shown that PDGF is a potent mitogen of human airway smooth muscle (Hirst et al 1996). TGFβ is a potent stimulant for fibroblast mitogenesis and is important in wound healing and fibrosis (Border and Noble 1994) plays a pleiotrophic role in the immune system (Torre-Amione et al 1990) and inhibits proliferation of airway smooth muscle (Cohen et al 1997). Eosinophils, fibroblasts and epithelial cells are the major sources of TGFβ (Vignola et al 1997). EGF induces airway smooth muscle proliferation (Cerutis et al 1997) and ET-1 potentiates EGF induced airway smooth muscle proliferation (Panettieri et al 1996).

**Proteases**

Tryptase, which is a mast cell serine protease affect fibroblast proliferation, degrade fibrinogen, generate C3a (Harvima & Schwartz 1993), simulate mucus secretion (Sommerhoff et al 1989) and degrade sensory neuropeptides (Tam & Caughey 1990). Thus, mast cell tryptase could play a role in regulation of haemostasis, mucus secretion and vascular permeability. Other proteases like thrombin induce proliferation of human airway smooth muscle (Panettieri et al 1995).
3.1.3 Airway Remodeling in Asthma

The rate of decline in lung function with age is greater in adults with asthma than in those without asthma (Lange et al 1998; Redington and Howarth 1997), and the ability to reverse the impairment in pulmonary function in many patients with asthma depends on the early recognition and treatment of the condition (Agertoft L, Pedersen, 1994; Haahtela et al 1991; Selroos et al 1995). Remodeling entails thickening of the airway walls, with increases in submucosal tissue, the adventitia, and smooth muscle (Kuwano et al 1993; Dunnill et al 1969; Hossain 1973). These features differ in asthma and chronic obstructive pulmonary diseases (Kuwano et al 1993), in allergic and nonallergic asthma (Paganin et al 1996), and with the severity of asthma (Kuwano et al 1993). The precise mechanisms underlying the remodeling process are under intense study. Recent observations in children with asthma (age, 5 to 12 years) (Ball et al 2000) suggest that preventing the progressive loss of lung function in childhood may require recognition and treatment of the disease during the first five years of life (Martinez et al 1995). Whether there is a mechanistic link between this loss of airway function and structural remodeling of the airway in early life is not yet known.

3.1.4 Genetic consideration

It has long been known that asthma and atopy run in families. Asthma, which begins in childhood generally, occurs in atopic individuals who produce significant amounts of IgE on exposure to small amounts of common antigens. This contrasts with those patients who develop asthma in adult life and who are non atopic, so called intrinsic or late onset asthma. First degree relatives of asthmatic patients have a higher prevalence of asthma when compared to relatives of nonasthmatic subjects (Haslett et al 2002). Screening families for candidate genes has identified multiple chromosomal regions that relate to atopy, elevated IgE levels and airway hyperresponsiveness. Evidence for genetic linkage of high total serum IgE levels and atopy has been observed on
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chromosomes 5q, 11q and 12q in a number of populations scattered throughout the world. Regions of the genome are demonstrating evidence for linkage to elevated total serum IgE levels. Excellent candidate genes exist for specific abnormalities in asthma within the regions that were identified in the linkage studies. For example, chromosome 5q contains cytokine clusters including IL-4, -5, -9 and -13. Other regions on chromosome 5q also contain the β-adrenergic receptors and the glucocorticoid receptors. Chromosome 6q contains regions that are important in antigen presentation and mediation of the inflammatory response. Chromosome 6q contains two genes that could influence atopy and airway hyperresponsiveness, including nitric oxide synthase (Mc Fadden 2003)
3.2 Pharmacotherapy of Bronchial asthma

The available agents for treating asthma can be divided into two general categories:

I. Bronchodilators

<table>
<thead>
<tr>
<th>β-adrenergic agonists</th>
<th>Metaproterenal, terbutaline, albuterol, formoterol, bitolterol, salmeterol, pirbuterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticholinergics</td>
<td>Ipratropium bromide, Tiotropium bromide</td>
</tr>
<tr>
<td>Methylxanthines</td>
<td>Theophylline, amimophylline, acepiphylline, diprophylline, proxophylline</td>
</tr>
</tbody>
</table>

II. Anti-inflammatory drugs

<table>
<thead>
<tr>
<th>Corticosteroids</th>
<th>Prednisolone, fluticasone, dexamethasone, beclomethasone dipropionate, budesonide, betamethasone.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-leukotrienes</td>
<td>Probilukast, Iralukast, Zieluton, Montelukast, zafirlukast, pranlukast.</td>
</tr>
<tr>
<td>Mediator release inhibitors</td>
<td>Cromoly Sodium, Nedocromil sodium.</td>
</tr>
</tbody>
</table>

I. Bronchodilators

Bronchodilator drugs have an anti-bronchoconstrictor effect that may be demonstrated directly in vitro by drug-induced relaxation of precontracted airways (Barnes 1988). Bronchodilators promptly reverse airway obstruction in asthmatics. This action believed to be mediated by a direct affect on airway smooth muscle. However, additional pharmacologic effects on the other airway cells (such as capillary endothelium to reduce microvascular leakage and mast cells to reduce release of bronchoconstrictor mediators) may contribute to the overall reduction in airway narrowing. Only three types of bronchodilators are in current clinical use: β-adrenergic agonist, methylxanthines, and anticholinergics.
1. β-adrenergic agonists

Epinephrine has been used to treat asthma since the beginning of the 20th century. β Adrenergic agonists are most widely used and effective bronchodilators for the treatment of asthma. Bronchodilation is mediated by β2 receptors; β2 selective drugs (Salmeterol and Furmoterol) have been developed that have very long duration of effect. β Adrenergic agonists leads to relaxation of bronchial smooth muscle that promote bronchodilation. Activation of adenylate cyclase increases the concentration of intracellular cyclic adenosine 3', 5'-monophosphate (cAMP), leading to activation of specific cAMP-dependent protein kinases that cause relaxation. Relaxation may also be due to inhibition of myosin phosphorylation. β-adrenergic agonists reverse bronchoconsriction irrespective of the contratile agent. β-adrenergic agonists prevent release of mediators from a number of inflammatory cells in vitro (Church and Hiroi 1987). In addition, β adrenergic agonists increase mucus secretion from submucosal glands and ion transport across airway epithelium. These effects enhance mucociliary clearance caused by asthma (Pavia et al 1980).

The inhaled route of administration is preferable to the oral route because adverse effects caused by systemic action of the drug are less and also because this route may be more effective. The inhaled drug reaches surface cells (e.g., mast cells or epithelial cells), which are less accessible to the orally administered drug.

Metaproterenal, terbutaline, albuterol, formopterol, bitolterol, salmeterol, and pirbuterol are the classic examples of selective β2-adrenergic agonists.

β-agonists improve respiratory symptoms and exercise tolerance despite the small improvement in spirometric measurements. The long acting β-agonists decrease infection exacerbations as an additional potential benefit. Salmeterol has been shown to reduce adherence of bacteria such as H. influenza to airway epithelial cells.
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β₂ selective agents cause tachycardia and palpitation by reflex cardiac stimulation secondary to peripheral vasodilation. Muscle tremor is caused by stimulation of β₂ adrenergic receptors in skeletal muscle and is the primary adverse effect of albuterol and bitolterol. Transient hypokalemia may be induced by high dose of these agents.

2. Anticholinergics

Datura plants contain the muscarinic antagonist stramonium and were smoked for relief of asthma 2 centuries ago. Now a days, atropine and ipratropium bromide are the most commonly available anticholinergics.

Anticholinergics specifically antagonize muscarinic receptors. They inhibit reflex cholinergic bronchoconstriction and do not significantly block the direct effects of inflammatory mediators such as histamine and leukotrienes on bronchial smooth muscle and vessels. When given by inhalation, anticholinergics produce bronchodilation by competitively inhibiting cholinergic receptors in bronchial smooth muscle. This activity blocks acetylcholine with the net effect being a reduction in cyclic guanosine monophosphate (cGMP) that normally acts to constrict bronchial smooth muscle. Anticholinergic drugs usually are less effective as bronchodilators in asthmatic subjects than β adrenergic agonists (Barnes 1987). Nevertheless, they may have an additive effect with β adrenergic agonists.

Atropine reduces mucociliary clearance in normal subjects and in patients with asthma and chronic bronchitis, but the quaternary derivative, ipratropium bromide, even when given in high does, has no such detectable effect either on normal subjects or in patients with airway disease (Pavia et al 1980).

Ipratropium bromide has been shown to decrease the effectiveness of voluntary cough on clearing mucus from the airways, which may affect its role in the treatment of patients who have excessive mucus production. Ipratropium has a
slower onset of action and a more prolonged bronchodilator effect compared with standard β₂-agonists and has been considered to be less suitable for use on an "as needed" basis for immediate relief of bronchospasm. The lack of systemic absorption of ipratropium greatly diminishes the anticholinergic side effects such as blurred vision, urinary retention, nausea, and tachycardia associated with atropine. A significant unwanted effect of inhaled ipratropium bromide is dryness of mouth and throat, bitter taste, cough and nausea. Nebulized ipratropium bromide may precipitate glaucoma in elderly patients because of its direct mydriatic effect on the eye. During sleep, ipratropium also has been shown to improve arterial oxygen saturation and sleep quality.

Tiotropium bromide is a long acting quarternary anticholinergic agent. Tiotropium in human lungs shows approximately 10 fold more potency than ipratropium and protects against cholinergic bronchoconstriction for greater than 24 hours.

3. Methylxanthines

Hyde Salter described the bronchodilator effect of strong coffee during the last century. Methylxanthines such as theophylline are related to caffeine and have been used to treat asthma since 1930. The methylxanthines may produce bronchodilation through numerous mechanisms, including

1. Inhibition of phosphodiesterase, thereby increasing cAMP levels,
2. Inhibition of calcium ion influx into smooth muscle,
3. Prostaglandin antagonism,
4. Stimulation of endogenous catecholamines,
5. Adenosine receptor antagonism, and
6. Inhibition of release of mediators from mast cells and leukocytes.

Theophylline inhibits release of mediators from mast cells, increases mucociliary clearance, and prevents the development of micro vascular leakiness, as would
an “anti-inflammatory” drug (Persson 1988). Theophylline also inhibits some functions of T lymphocytes, which may be relevant to control of chronic inflammation of the airway.

For nocturnal asthma, a single does of slow release theophylline at bedtime often is effective. This has been demonstrated to reduce overnight declines in FEV₁ and morning respiratory symptoms. Taken alone it increases exercise tolerance (Taylor et al 1985, Murciano et al 1989) without improving spirometry tests. Other theophylline salts, such as choline theophyllinate, offer no advantages over theophylline. The ethylenediamine component of aminophylline has been implicated in allergic reactions. Some derivates such as acepiphylline, diprophylline, and proxophylline, are less effective than theophylline (Weinberger 1984). The most common adverse effects are headache, nausea and vomiting, abdominal discomfort, and restlessness.

II. Anti-inflammatory drugs

Although the type of inflammatory responses may differ among diseases, inflammation is a common denominator of several lung diseases. Anti-inflammatory drugs suppress the inflammatory response by inhibiting

(1) Infiltration and activation of inflammatory cells as well as their synthesis
(2) Release of mediators
(3) Effects of inflammatory mediators themselves.

1. Corticosteroids

Since asthma is viewed as a chronic inflammatory disease and inhaled corticosteroids are known to have low toxicity, they may be considered as first line therapy (Barnes 1989). Prednisolone and dexamethasone were effective when they were given systematically to treat asthma but they had no anti-asthmatic activity when they were given by inhalation. Other corticosteroids e.g. beclomethasone dipropionate (BDP), betamethasone and budesonide, were effective in treating asthma when given by inhalation. The antiasthmatic potency
of an inhaled steroid is approximately proportional to its potency as an anti-inflammatory agent.

Corticosteroids inhibit the release of arachidonic acid metabolites and platelet activating factor (PAF) from lungs and macrophages by enhancing the production of proteins called lipocortin. Thereby they inhibit the formation of prostaglandins and leukotrienes. These effects occur because of ability of steroid – receptor complex to be transported to the nucleus, where it initiates DNA transcription of specific mRNAs. Corticosteroids potentially inhibit the accumulation of neutrophils, inhibit secretion of human pulmonary macrophages of leukotrienes and prostaglandins, inhibit formation of interleukins (ILs) such as as IL-1, IL-2, IL-3 and IL-5, inhibit degranulation and adherence of eosinophils, reduce number of circulating T lymphocytes and formation of an IgE binding suppressive factor. Steroids prevent and reverse the increase in vascular permeability due to inflammatory mediators and may therefore lead to resolution of airway edema (Erjefalt and Persson 1986). Corticosteroids remain the most effective therapy available for asthma but the legitimate fear of their adverse effects makes using them difficult. Steroids potentiate the effects of β adrenergic agonists on bronchial smooth muscle (Barnes 1989). Methylprednisolone is given i.v to patients with acute severe asthma. Inhaled steroids have no proven value in the management of acute asthma. Patients with chronic bronchitis occasionally respond to steroids, possibly because some have an element of undiagnosed asthma.

Corticosteroids inhibit release of ACTH and secretion of cortisol by a negative feed back effect on the pituitary gland. Adverse effects of corticosteroids include fluid retention, increased cell mass, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataract, and phychosis (Dajani et al 1981).
2. Anti-leukotrienes

Leukotrienes possess potent pro-inflammatory actions resulting in increased vascular permeability, mucus secretion and bronchial hyperresponsiveness. They are derived from the 5-lipoxygenase pathways in mast cells, eosinophils and macrophages. Anti-leukotrienes improve lung function and diminish symptoms, exacerbation rate and the need for rescue bronchodilator. These are drugs of choice in case of aspirin induced asthma, in which patients have high LTE\textsubscript{4} levels in urine and nasal secretions and even higher after taking aspirin (Christie et al 1992).

Leukotriene modifiers are drugs that modify the response of these mediators of inflammation by one of the four ways (Drazen et al 1996; O’Bryne et al 1997; Hay 1997).

Cysteinyl LT receptor Inhibitors
C-LTs promote eosinophil influx, bronchospasm and mucus hypersecretion, all are considered hallmark of asthma. C-LT receptor inhibitors antagonize or inhibit leukotriene predominantly LTD\textsubscript{4}. These agents inhibit phospholipases, prostaglandins, leukotrienes, and IL-1 synthesis. Probilukast and Iralukast belong to this class (Storms et al 1995; Drazen 1997; Lazarus 1998; Floreni and Rennard 1997).

5-lipoxygenase inhibitors
They prevent the formation of leukotrienes by blocking a 5-lipoxygenase pathway in their synthesis. Zileuton, ZD-2138, ABt-761 belongs to this class (Floreni and Rennard 1997).

5-lipoxygenase activating protein (FLAP) inhibitors
MK-0591 (Diamant et al 1995) and MK-886 (Friedman et al 1993) attenuated the early and late asthmatic response following antigen challenge but not the attendant increase in airway responsiveness to spasmogen.
Leukotrienes receptor antagonists
Montelukast, zafirlukast, pranlukast are selective and high affinity LT\textsubscript{1} antagonists (Adcock and Mathews 1998).

Zileuton has shown efficacy in exercise-induced asthma, aspirin induced bronchospasm and following chronic administration, an improvement in pulmonary function (FEV\textsubscript{1}) and a reduction in oral and inhaled corticosteroid use (Israel et al 1995, Tamaki et al 1997). Furthermore, in a small study, zileuton attenuated both airway and blood eosinophilia in nocturnal asthmatics (Wenzel et al 1995).

Zafirlukast has been demonstrated to attenuate the acute airway obstructive response to allergen and exercise challenge (Taylor et al 1991, Finnerty et al 1992) and to improve chronic asthma control both objectively (FEV\textsubscript{1}, nocturnal awakenings, β-agonist use) and subjectively (Spector et al 1995).

Montelukast has been shown to block the early and late response to allergen challenge following single dosing, to improve FEV\textsubscript{1} in both children (6-14 years) and adults (Altman 1998) and to protect against the development of exercise induced bronchoconstriction in both children and adults (Kemp et al 1998, Leff et al 1998). Tolerance to the bronchoprotective effects of montelukast in attenuating exercise-induced bronchospasm does not develop following at least 12 weeks of therapy (Leff et al 1998).

Pranlukast increases FEV\textsubscript{1} within 1 hour of dosing, improves patient summary symptom and nighttime asthma scores and reduces the use of rescue bronchodilators. In patients with moderate persistent asthma, it prevents exacerbations of asthma during reduction of high dose inhaled corticosteroids therapy (Tamaki et al 1997).
3. Mediator release Inhibitors

Cromolyn Sodium
Cromolyn Sodium (Sodium cromoglycate) is a derivative of khellin, an Egyptian herbal remedy. Cromolyn inhibited the release of mediators by allergen in passively sensitized animal and human lung preparations (Cox 1967). Cromolyn was classified as mast cell stabilizer. Cromolyn has variable inhibitory actions on other inflammatory cells including macrophages and eosinophils that may participate in allergic inflammation. In vivo chromolyn can block both the early response that may be mediated by mast cells to allergens and the late response and bronchial hyper responsiveness (Cockcroft and Murdock 1987). Cromolyn Sodium is used for prophylactic treatment and consequently needs to be taken regularly. It is the first choice anti-inflammatory drug for children because it has few adverse effects (Bernstein 1985). Cromolyn sodium is classified as an antiallergic drug because it appears to have a specific effect on allergy based inflammation. Several other drugs also may be included in this category.

Nedocromil sodium is a new drug used for prophylaxis. It has a similar pharmacologic profile of activity to cromolyn, is more potent in various tests, and may have a longer duration of action (Gonzales and Brogden 1987).

Ketotifen also is described as a drug to be used for prophylaxis against asthma (Martin and Bagglioni 1981).

Newer Targets In Asthma Therapy
The current pharmacotherapeutic approaches to asthma have several limitations. First, there is no known asthma cure and little evidence that prevention is possible in susceptible persons. Hence, patients continue to be at risk of symptoms and exacerbations. Mortality remains a severe problem. Finally, the medications have adverse effects. There is even some evidence, albeit conflicting, that cataract formation, osteoporosis and growth impairment, as
associated with systemic glucocorticoids, may arise from topical steroids, depending on dosages used. New inhalation devices and new generation beta-agonists are available. At the same time, new understanding of the molecular pathology of asthma has identified several novel therapeutic targets. Agents being tested in early phase clinical trials include antagonists of IgE, cytokines, adhesion molecules and transcription factors.

**TXA\(_2\) inhibitors**

TXA\(_2\) is potent bronchoconstrictor, mucus producer and blood and vessel permeability inducer and causes airway hyper responsiveness. Serabenast, domitroban and ozagrel are the examples of these (Kurosawa 1995). TXA\(_2\) synthetase inhibitor ozagrel reduced cough sensitivity to capsaicin (Fujimura et al 1995) and bronchoconstriction to acetaldehyde (Myou et al 1994). TXA\(_2\) antagonists BAYu3405 produced a modest decrease in airways responsiveness to methacholine following 2 weeks’ treatment in asthmatics (Aizawa et al 1996).

**Tachykinin receptor antagonists**

The first nonpeptide tachykinin receptor antagonist was CP-96345, which is a potent NK\(_1\) receptor antagonist (Snider et al 1991). SR 48968, GR 159897 and SR 144190 are selective nonpeptide NK\(_2\) receptor antagonists (Emonds-Alt et al 1997). SR 142801 (Sarahu et al 1997) and SB 223412 (Singh and Sanderson 1997) are selective NK-3 receptor antagonists.

**Tryptase inhibitors**

They inhibit both early and late reactions. APC-366 inhibited antigen induced late phase response and bronchial hyperresponsiveness to carbachol in sheep (Clark et al 1995). Lactoferrin disrupts the quaternary structure of tryptase, also attenuates antigen induced late response and bronchial hyperresponsiveness in allergic sheep (Elrod et al 1997).
Cytokine inhibitors
One of the novel approaches for the treatment of asthma is to target cytokines and develop cytokine modulators as drug. Two humanized anti-IL-5 monoclonal antibodies, Sch-55700 and SB-240563 reduces blood eosinophil count for several weeks and prevents eosinophils recruitment into the airways after allergen challenge in asthmatic patients (Leckie et al 2000). IL-5 signaling inhibitor GCC-AP0341 inhibited IL-5 mediated survival of eosinophils (Naito et al 1996). IL-4 receptor antibodies inhibited allergen induced airway hyperresponsiveness, goblet cell metaplasia and pulmonary eosinophilia in a murine model (Gavett et al 1997).

Chemokine inhibitors
A variety of chemokines, one of which is the chemoattractant eotaxin, are secreted by inflamed lung tissue thereby attracting eosinophils. Eotaxin receptor blockers are being investigated, as eosinophils are believed to be major contributors to the pulmonary damage seen in asthma. Monoclonal antibody (7B11) for human CCR3 has shown to completely block the binding and signaling of the known CCR3 ligands, thus blocking the chemotactic response of human eosinophils to all chemokines (Heath et al 1997).

Adhesion molecule antagonist
Interactions of eosinophils with intra cellular adhesion molecule-1 (ICAM-1) are thought to be necessary for eosinophils recruitment into airways. Antibodies to ICAM-1 blocked both eosinophils recruitment into the airways in the monkey model of asthma and importantly the increase in airway reactivity associated with allergen challenge (Gundel et al 1991, 1992; Wegner et al 1990).

Phosphodiesterase inhibitors
Considerable interest has been generated in the potential utility of isoenzyme-selective inhibitors of cyclic nucleotide Phosphodiesterase (PDE) in the treatment of asthma and other inflammatory disorders. The scientific foundation for this interest is based upon two fundamental principles. First, inhibition of PDE activity
increases the cellular content of two key second messengers, cAMP and cGMP, thereby activating specific protein phosphorylation cascades that elicit a variety of functional responses. Increases in cAMP content suppress a broad array of functions in inflammatory and immune cells. Both cAMP and cGMP mediate bronchodilation. PDE3 inhibitor enoxamine was shown to decrease lung resistance and increase compliance in patients with decompensated chronic pulmonary disease. Benzafentrine administered to normal volunteers by inhalation produced bronchodilation. Zaprinast is PDE5 inhibitor; it reduced exercise-induced bronchoconstriction but not histamine-induced bronchoconstriction. Most of the work now is focused on selectively targeting PDE4, primarily because inhibitors of this isoenzyme family have a notably appealing therapeutic profile; broad-spectrum anti-inflammatory activity coupled with additional bronchodilatory and neuromodulatory action. Rolipram, LAS-31025, RP-73401 and denbufylline are selective PDE4 inhibitors. SB 207499, V11294A, CP-220 and roflumilast are PDE4 inhibitors with less gastrointestinal side effects (Leeman et al 1987; Duplantier et al 1998).

**Endothelin modulators**

There are two approaches for ET-1 directed therapeutics- (1) Inhibitors of endothelin-converting enzyme (ECE), which mediates the synthesis of ET-1 from its precursor (Michael and Markewitz 1996) (2) Receptor antagonists of the effects of ET-1 at the end organ level. These agents reverse and/or prevent the increase in pulmonary artery pressure and vascular remodeling elicited by acute or chronic hypoxia. Examples are BQ-123, SB-217242 and bosentan (Winn et al 1996).

**Immunotherapy**

As demonstrated by Durham and Till (1998), conventional immunotherapy is associated with significant increase in mRNA for IL-12 and interferons, which are an important immunomodulator in converting Th2 responses to Th1. New immunotherapy using anti-IgE aimed at specific allergens that reduces serum
Review of literature

levels of IgE by more than 90% and inhibits the action of IgE by preventing binding of IgE to its receptors on mast cells and basophils. In this way it is able to inhibit the early and late reactions in asthma, reduce sputum eosinophils and reduce methacholine sensitivity. This recombinant human monoclonal antibody E25 is a humanized version of murine antibody and has been shown to selectively inhibit IgE production by human B cells in vitro. It decreases serum IgE levels in allergic patients and maximum bronchoconstriction was reduced upto 60% (Fahy et al 1997; Boulet et al 1997). A new form of immunotherapy is the use of gene vaccination with plasmid DNA. This modality induced a Th1 response that dominated over a Th2 response (Kline et al 1998).

Overall, it is abundantly clear that asthma pharmacotherapy must target airway inflammation and remodeling. While advances have already being achieved both in management guidelines and in new medications and inhalation devices, the disease’s morbidity and mortality remain significant and the disease itself is still incurable. From our growing understanding of asthma, new therapeutic agents can be expected to emerge, promising alternative approaches to this chronic and dreaded disease.
Diagnosis in Asthma

Pulmonary Function Testing
Pulmonary function tests provide objective, quantifiable measures of lung function. The measurements are very important in diagnosing and monitoring lung disorders, just as important blood sugar measurements are in diabetes, or blood pressure measurements are in hypertension. The recommendations of the National Asthma Education Program indicate that such testing is essential in the diagnosis and management of asthma because of evidence that both patients and physicians have inaccurate perceptions of the severity of asthma that contribute to delays in treatment (Bethesda, 1991). Indeed, underestimation of the extent of airflow (airway) obstruction is associated with increased mortality in asthma (Sly, 1989).

Potential uses of pulmonary function testing include the evaluation of patients with known or suspected lung disease; the evaluation of symptoms such as chronic cough, dyspnea, or chest tightness; monitoring the effects of exposure to dust, chemicals, or pulmonary toxic drugs; risk stratification prior to surgery; monitoring the effectiveness of therapeutic interventions and objective assessment of impairment or disability (American college of Physicians Consensus Statement 1990).

Pulmonary-function tests can identify abnormalities of lung function that might otherwise be overlooked and can exclude the possibility of some respiratory disorders such as chronic obstructive pulmonary disease. Physicians cannot identify obstructive or restrictive patterns reliably from history taking and physical examination alone (Hepper et al, 1969).

Spirometer is the most useful instrument, invented in 1846 by John Hutchinson. A person inhales as deeply as possible and then exhales as forcefully and completely as possible into a mouthpiece attached to the spirometer. A computerized sensor calculates and graphs the results. The results demonstrate
the movement of air into and out of the lungs during various breathing maneuvers. To determine the validity of spirometric results, at least three acceptable spirogram must be obtained. In each test, patient should exhale for at least six seconds and stop when there is no volume change for one second. The test session is finished when the difference between the two largest FVC measurements and between the two largest FEV₁ measurements is within 0.2 L (Crapo and Morris, 1989). Test quality remains the most important concern in lung-function testing. Variability (noise) is greater in pulmonary-function tests than in most other clinical laboratory tests because of the inconsistency of efforts by patients (Becklake, 1986) The elements that lead to high-quality test results are accurate equipment, good test procedures, an ongoing program of quality control, appropriate reference values, and good algorithms for the interpretation of results.

Role of Spirometry in Asthma

Spirometry is carried out

- At the time of initial diagnosis of asthma.
- After treatment is initiated and symptoms have stabilized to document attainment of near "normal" airway function,
- At least every 1 to 2 years to assess the maintenance of airway function.

Spirometric tracings should be examined to make sure they represent adequate effort by the patient, are reproducible, and contain no artifacts that would alter the test results. With computerized equipment, more than 20 different spirometric variables can be reported. It is important to resist the temptation to use more than a few such variables in the basic interpretation. Increasing the number of variables used in the test increases the number of false positive results (American Thoracic Society, 1991).

Each patient has an expected 'normal' value that is called a 'predicted' value. That means a person of particular ethnic origin, sex, age and height should have
Review of literature

a particular vital capacity and FEV1. This is read off from tables compiled after studying hundreds of normal persons. The measurements made on a patient are called observed values. The interpretation of lung-function tests usually involves comparing values measured in patients with reference values from studies of local populations of healthy nonsmokers.

In spirometry, there are several basic variables as follows:
The air within the lung at the end of a forced inspiration can be divided into four compartments or lung volumes. The volume of air exhaled during normal quiet breathing is termed “tidal volume” \( V_T \). The maximal volume of air inhaled above tidal volume is called the inspiratory reserve volume (IRV) and the maximal air exhaled below tidal volume is called expiratory reserve volume (ERV). The residual volume (RV) is the amount of air remaining in the lungs after a maximum exhalation (Fig. 3).

**Fig. 3 Lung volumes and capacities**

VC (VITAL CAPACITY) is the maximal volume of air that can be exhaled after a maximal inhalation or the maximal volume of air that can be inhaled after a maximal exhalation. It is equal to the sum of the IRV, ERV and \( V_T \). It can be measured as two variables: forced vital capacity (FVC) and slow vital capacity (SVC). The VC is approximately 75% of the total lung capacity (TLC). The TLC is the volume of air in the lung after the maximal inspiration and it is the sum of the four primary lung volumes (IRV, ERV, \( V_T \) and RV).

FVC (FORCED VITAL CAPACITY): The total volume of air that can be forcibly expired following a maximum inhalation. It is a non-specific indicator of pulmonary disease. Serial measurements are useful to measure deterioration or improvement in some patients. To measure FVC, the patient inhales maximally, then exhales as rapidly and as completely as possible. Normal lungs generally can empty more than 80% of their volume in six seconds or less.

FEV\textsubscript{1} (FORCED EXPIRATORY VOLUME in one second): The maximum amount of air that can be exhaled in the first second of expiration (measures how fast one can breathe out). Normally FEV\textsubscript{1} is 70 to 80 per cent of FVC. Reduced volumes suggest obstruction. Improvement after bronchodilation demonstrates reversibility. It is the most common screening method for detecting significant obstructive disease.

FEV\textsubscript{1}/FVC %: It is the proportion of total volume of air that can be expired in the first second of expiration. It is a specific measure of airway obstruction with or without restriction. Normally, this ratio is 75% or greater and any value below 70% suggests obstruction.

FEF\textsubscript{25-75} (forced mid expiratory flow rate): The average rate of airflow measured during the mid-portion of the forced vital capacity and regarded as a more sensitive measure of small airways narrowing than FEV\textsubscript{1}.
PEFR (peak expiratory flow rate): The maximum expiratory flow rate achieved that occurs very early in the forced expiratory maneuvers. This measurement is often used in the outpatient management of asthma because it can be measured with inexpensive peak flow meters.

MVV (maximum voluntary ventilation): It is the largest total volume of air that can be moved during a 10 to 15 second interval as a result of repeated voluntary effort. It is a simple test for overall breathing capacity. This test is non-specific since it integrates the results of respiratory muscle strength and control, elastic and resistive properties of the airways and patient motivation. The test is more sensitive to changes secondary to neuromuscular disease, particularly those affecting muscle endurance or coordination, than FVC alone. Thus MVV measurements are a useful tool for detecting trends (Fig. 4).

Fig. 4 Normal spirometric flow diagram.
(A) Flow volume curve. (B) Volume time curve.
Two basic types of lung dysfunction can be defined by spirometry: obstructive patterns and restrictive patterns. The primary criterion for airflow obstruction is a reduced FEV₁/FVC %. In obstructive lung conditions, the airways are narrowed, usually causing an increase in the time it takes to empty the lungs. Obstructive lung diseases can be caused by conditions such as bronchitis, infection and asthma. A restrictive pattern means that lung volumes are small. The primary criterion for this diagnosis is a reduction in total lung capacity (TLC), the volume of air in the lungs at the end of a maximal inhalation. In restrictive lung conditions there is a loss of lung tissue, a decrease in the lungs ability to expand or a decrease in the lung's ability to transfer oxygen to the blood. Restrictive lung disease can be caused by conditions such as pneumonia, lung cancer, scleroderma and multiple sclerosis. Other restrictive conditions may include chest injuries, obesity, pregnancy or loss of lung tissue due to surgery (American Thoracic Society, 1991).

<table>
<thead>
<tr>
<th></th>
<th>Obstructive Lung Disease</th>
<th>Restrictive Lung Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>Normal or Increased</td>
<td>Decreased</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>FEV₁ / FVC</td>
<td>Less than 75%</td>
<td>Greater or equal to 75 %</td>
</tr>
</tbody>
</table>
3.3 ASTHMA AND HERBS

A large number of plants which can cure asthma or at least alleviate the symptoms have been mentioned in various texts such as Charaka Samhita, Sushruta Samhita, Bhavprakash Nighanu etc. Some herbal alternatives employed in these conditions are proven to provide symptomatic relief and assist in the inhibition of disease development as well. These herbs therefore have multifaceted roles to play in the management of asthma. The early allopathic medicines for asthma were derived from plants.

Epinephrine has been used to treat asthma since the beginning of the 20th century. Datura plants contain the muscarinic receptor antagonists' stramonium and were smoked for relief of asthma two centuries ago. Strong coffee, which contain methylxanthines such as theophylline have been used to treat asthma since 1930. Ongoing research worldwide has provided valuable clues regarding the precise mechanism of action of these herbal alternatives. Medicinal plants contain a wide range of compounds that would serve as a 'leads' for the development of novel anti-asthmatic agents. Some such classic examples are of the plant Ammi visnaga and Boswellia serrata. Conventional anti-asthmatic compound, disodium cromoglycate was developed from the structural modification of furanochromone khellin isolated from the plant Ammi visnaga (Cox et al 1970). Antileukotriene drug has been recently been approved for the treatment of asthma. Bosewellic acid isolated from the plant Boswellia serrata is an effective antileukotriene compound (Safayhi et al 1997). Various plants used in the treatment of asthma are mentioned in Table 3.

Adhatoda vasica

The medicinal properties of Adhatoda vasica Nees (Natural Order: Acanthaceae), called Vasa or Vasaka has been recommended by Ayurvedic physicians for the management of various types of respiratory disorders. The leaves of the plant were found to contain an essential oil and the quinazoline
alkaloids vasicine, vasicinone and deoxyvasicine, which found to possess respiratory stimulant activity (Amin and Mehta 1959). Of the two alkaloids, vasicinone was found to be more potent than vasicine, with potential anti-asthmatic activity comparable to that of disodium cromoglycate (Atal 1980).

**Albizia lebbeck**

*Albizia lebbeck* has been used by Ayurvedic physicians for centuries in the management of asthma. The effect of decoction of the bark and flower were studied for its anti-asthmatic and anti-anaphylactic activity. The decoction protected the guinea pig against histamine and Ach induced bronchospasm (Tripathi and Das 1977). The decoction of the bark of *A. lebbeck* was also studied on degranulation rate of sensitized peritoneal mast cells of albino rats when challenged with antigen (horse serum) and triple vaccine was used as adjuvant. Disodium cromoglycate (DCG) and prednisolone were used for comparison. Studies revealed the significant cromoglycate like action on the mast cells, which has been attributed to the heat-sable and water-soluble saponins present in the plant (Tripathi et al 1979). Crude extract of seeds and a pure saponin fraction of Albizzzia have also been studied on the mast cells in the mesentery and peritoneal fluid of rats subject to anaphylaxis (Johri et al 1985).

**Boswellia serrata**

The gum resin of *Boswellia serrata*, known in Indian Ayurvedic system of medicine as Salai guggal, contains boswellic acid. It specifically inhibits leukotriene biosynthesis by inhibiting the activity of the enzymes, which leads to their formation. It also proved to be the most potent inhibitors of the classical component pathway of the inflammatory response. Boswellic acids also decrease the activity of human leukocyte elastase (HLE), which may be involved in the pathogenesis of emphysema. Boswellic acids are therefore effective in the prevention and or control of inflammatory processes, which are typically characterized by increased leukotriene formation (Safayhi et al 1997). Boswellia specifically blocks the synthesis of pro-inflammatory 5-lipoxygenase products,
including leukotrieneB₄ (Ammon et al 1991), which cause bronchoconstriction, chemotaxis, and increased vascular permeability. Therefore Boswellic acid might be used for their anti-allergic /anti-asthmatic activity.

**Tylophora asthmatica** (syn. Tylophora indica)

The medicinal properties of the plant *Tylophora asthmatica* have been known since ancient times. Powder from the dried leaves, root powder, and decoction of the leaves or infusion of the root bark have been used traditionally in the treatment of respiratory affections such as chronic bronchitis and asthma (Nadkarni 1976). Preparations containing dried, powdered plant material are available for the treatment of bronchial asthma and tropical eosinophilia. The anti-asthmatic activity of the plant is attributed to the presence of phenanthroindolizidine alkaloids, which has been isolated from the aerial parts of the plant (Ali and Bhutani 1989). A water extract of the plant showed anti-anaphylactic effect, leucopenia and inhibition of Schulz-Dale's reaction in experimental animals. The extract also showed brief nonspecific anti-spasmodic action in isolated tissues of g. pig ileum, rabbit duodenum, frog's rectus and rat stomach. The mode of action of the plant may be cell-mediated immunity (Haranath and Shyamalakumari 1975). The plant extracts were found to produce significant anti-inflammatory effects in rats (Manez 1990). Immunosuppressive and anti-inflammatory effects of *Tylophora asthmatica* are due to increased secretion of corticosteroids by adrenal cortex (Udupa 1991). *Tylophora asthmatica* also produced significant improvement in lung functions, when the effect of the plant was studied on the patients of bronchial asthma (Gore 1980).
### Table: 3 LIST OF PLANTS SHOWING ANTI-ASTHMATIC ACTIVITY

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Part of Plant used</th>
<th>Major chemical constituents</th>
<th>Activity studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achyranthes aspera</em></td>
<td>Roots</td>
<td>Achyranhine</td>
<td>Clinical (Suresh et al 1985)</td>
</tr>
<tr>
<td>(Amaranthaceae) Apamargah</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Adhatoda vasica</em></td>
<td>Methanolic extract of leaves</td>
<td>Vasicinol, vasicine</td>
<td>Reduced ovalbumin and PAF induced allergic reactions (Muller et al 1993)</td>
</tr>
<tr>
<td>(Acanthaceae) Vasa or Vasaka</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Albizia lebbeck</em></td>
<td>Stem bark</td>
<td>Saponins</td>
<td>Bronchodilator (Tripathi and Das 1977), Mast cell stabilizing (Tripathi et al 1979).</td>
</tr>
<tr>
<td>(Mimosaceae) Siris</td>
<td>Aqueous extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Allium cepa</em></td>
<td>Onion juice</td>
<td>The α and β unsaturated Thiosulphinates</td>
<td>Inhibit cyclooxygenase and 5-lipoxygenase (Bayer et al 1989)</td>
</tr>
<tr>
<td>(Liliaceae) Onion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aquillaria agallocha</em></td>
<td>Aqueous extract of stem</td>
<td>Triterpenoids</td>
<td>Inhibit compound 48/80 induced PCA, Mast cell stabilizing (Kim et al 1997)</td>
</tr>
<tr>
<td>(Thymelaeaceae) Agar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Artemisia caerulescens</em></td>
<td>Butanolic extract</td>
<td>Quercetin, isorhamnetin</td>
<td>Bronchodilator (Moran et al 1989)</td>
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<tr>
<td><em>Boswellia serrata</em></td>
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MORINGA OLEIFERA LAM.

GENERAL INFORMATION

Regional and other Names

Beng. : Sajna
Eng. : Drumstick, Horse Radish tree
Guj. : Saragavo
Hindi : Soanjna
Kan. : Nugge
Mal. : Sigru
Mar. : Shevga
Punj. : Soanjna
Sans. : Shobhanjana
Tam. : Murungai
Tel. : Sajana, Munaga, Mulaga

Description

Distribution: A small or middle sized tree, about 10 m high. It is also cultivated throughout India.

Macroscopic

Seed- Dark brown in colour, 3 angled, 1.5 to 1.8 cm long, with thin papery cream to white coloured wings on the ridges.

Root - Bark Thick soft corky, deeply fissured. Young parts tomentose

Leaf - Usually tripinate, rhachis slender, thickened and articulated at the base. Pinnae and Pinnules opposite, deciduous. Leaflets elliptic. The terminal obovate.

Flower- White fragrant, in large puberulous panicles. Calyx- lobes linear-lanceolate reflexed, puberulous outside. Stamens 5 fertile, alternating with 5-7 antherless.

Pods - Pendulous, greenish 22.5 to 50 cm or more in length, triangular ribbed (Kirtikar and Basu 1975a).
Ayurvedic Description

Botanical name: *Moringa oleifera*

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<td>Therapeutic uses</td>
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Properties and Uses Ascribed

All parts of the tree are considered medicinal and used in the treatment of ascites, rheumatism, and venomous bites and as cardiac and circulatory stimulant. The root is laxative, expectorant, diuretic, and good for inflammations, throat, bronchitis, piles, cures stomatitis, urinary discharges, and obstinate asthma. Root bark is useful in heart complaints, eye diseases, ‘kapha’, ‘vata’, all ‘tridosha’ fevers, inflammation, dyspepsia, and enlargement of spleen. The leaves are anthelmintic, aphrodisiac, cures hallucinations, dry tumors, hiccough, and asthma. The flowers cure inflammations, muscle diseases, ‘kapha’, ‘vata’. The fruit cures ‘kapha’ and biliousness, pain, leucoderma, tumour. The seed cures eye diseases, head complaints, ‘vata’. Oil is useful in leprous ulcers and as external application for rheumatism. The roots and seeds are prescribed for the treatment of snakebites and scorpion stings. The root and bark are abortifacient (Satyavati et al 1987).

*Moringa oleifera* Lam is a multipurpose tree cultivated to use as vegetable, for spice, for cooking and cosmetic oil and as a medicinal plant. The mature seeds contained 332.5g crude protein, 412.0 g crude fat, 211.2 g carbohydrate and 44.3 g ash per kg dry matter. The essential amino acid profile compared with the FAO/WHO/UNU scoring pattern requirements for different age groups showed
Review of literature

deficiency of lysine, threonine and valine. The content of methionine + cysteine (43.6 g/kg protein), however, was exceptionally higher and close to that of human milk, chicken egg and cow's milk (Oliveira et al 1999).

Seed extracts of *M. oleifera* Lam. have been proposed as an environment-friendly alternative, due to their traditional use for the clarification of drinking water. Recombinant or synthetic forms of a cationic seed polypeptide mediate efficient sedimentation of suspended mineral particles and bacteria. The polypeptide was also found to possess a bactericidal activity capable of disinfecting heavily contaminated water.

The hexane-extracted oil content of *M. oleifera* seeds ranged from 38.00 to 42.00%. Protein, fiber, and ash contents were found to be 26.50-32.00, 5.80-9.29, and, 5.60-7.50%, respectively. Results of physical and chemical parameters of the extracted oil were as follows: iodine value, 68.00-71.80; refractive index (40°C), 1.4590-1.4625; density (24°C), 0.9036-0.9080 mg/ml; saponification value, 180.60-190.50; unsaponifiable matter, 0.70-1.10%; and color (1 in. cell), 0.95-1.10 R + 20.00-35.30 Y. Tocopherols (alpha, gamma, and delta) in the oil were up to 123.50-161.30, 84.07-104.00, and 41.00-56.00 mg/kg, respectively. The oil was found to contain high levels of oleic acid (up to 78.59%) followed by palmitic, stearic, behenic, and arachidic acid up to levels of 7.00, 7.50, 5.99, and 4.21%, respectively (Anwar and Bhanger 2003).

**Ethnobotanical Studies**

Plant is used in abortions (Tarafder et al 1983, Nath et al 1997), Diabetes (Gupta and Mishra 2002) and as an Antipyretic (Singh and Kumar 1999), Anthelmensis (Bondya et al 2002) anti-herpes simplex virus type 1 (HSV-1) activity (Lipipun et al 2003). Leaf extract is 100% abortive. A decoction of bark is abortive (Adjanohoun 1983). Seeds are used as a vermifuge.
Microscopic Studies
The T.S. of seed showed testa and cotyledon. The epidermis of testa consists of large cells, with thick cuticle. Endosperm is comprised of thick walled parenchymatous cells with group of sclerides. Cotyledon consists of epidermis, one layer of palisade cells followed by parenchymatous cells filled with starch grains and oil globules (Gupta 2003).

Chemical Studies
From the stems of *M. oleifera*, 4-hydroxymellein, vanillin, B-sitosterone, octacosanic acid and B-sitosterol have been isolated. The isolation of 4-hydroxymellein from a plant species is reported for the first time (Saluja et al 1978).


The mucilage from the pods of *M. pterygosperma* designated as drum stick polysaccharide revealed the presence of galactose, dextrose, xylose and sodium, potassium, magnesium, calcium salts of glucurinic acid. Contrary to the definition of mucilages, the presence of dextrose was an exception (Rao and Mishra 1991).

Khare et al (1997) has isolated a new leucoanthocyanin characterized as leucodelphinidin-3-O-B-D-galactopuranosy (1->4)-O-B-D-glucopyraniside from *M. oleifera* gum.

The aqueous extract of the mature flowers of *M. oleifera* contains free natural sugars, D-mannose and D-glucose in the ratio of 1:5 and two unidentified carbohydrate bearing materials along with proteins and ascorbic acid of the above materials with varying proportion along with polysaccharide (PS) which on
hydrolysis gives D-glucose, G-glactose and D-glucuronic acid in a molar ratio of 1:1:9:0.9 (Pramanik and Islam 1998).

**Seeds of** *M. Pteregrina* on treatment with myrosinase, produce 2- proply, 2-butyl and 2-methyloproply isothiocyanate in addition to 5, 5-dimethyl-oxazolidine-2-thione, all new to the family but known as natural derivatives from other sources (Kjaer et al 1979).

*M. oleifera seeds* contain 38.16 % oil which contain Vitamin E (0.01 %) and beta carotene( 0.014%), the precursor of Vitamin A. (Dahot and Memon 1985). A new glycoside having molecular formula C_{15}H_{20}O_{7}, provisionally named as moringyne, was isolated from an acidic extract of the **seeds** of *M. oleifera* (Memon et al 1985). Mono-palmitic, di-oleic triglyceride has been isolated from the benzene extract of semi-dried **seeds** of *M. oleifera* Lam (Memon and Khatri 1987).

4(α-L-Rhamnosyloxy) benzyl isothiocyanate and 4(α-L-Rhamnosyloxy) phenylacetonitrile were isolated from the raw seeds of *M. oleifera* by hot water extraction (Dayrit et al 1990). 4(α-L-Rhamnosyloxy) phenylacetonitrile was a thermal degradation product, produced from the parent compound 4(α-L-Rhamnosyloxy) benzyl glucosinolate during roasting (Villasenor et al 1990). Starting from L-rhamnose, the first synthesis of the major glucosinolate isolated from *M. oleifera* seeds was effected in seven steps (Gueyrard et al 2000).

Bioassay-guided analysis of an EtOH extract of *M. oleifera* leaves shows isolation of two nitrile glycosides, niazirin and niazirinin and three mustard oil glycosides, 4-[(4'-O-acetyl-α-L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A, and niaziminin B. This is the first report of the isolation of nitriles, an isothiocyanate, and thicarbamates from the same plant species (Faizi et al 1994, 1995). The same compounds were also isolated form the whole **pods** along with two new compounds 0-[2'-hydroxy-3'- (2''-heptenyloxy) propy undecanoate and
O-ethyl-4-[(\(\alpha\)-L-rhamnosyloxy)-benzyl] carbamate along with the known substances methyl p-hydroxybenzoate and \(\beta\)-sitosterol (Faizi et al 1998).

\(\alpha\)-L-Rhamnosides of 4-hydroxy-benzyl compounds with nitrile, carbamate, and thiocarbamate groups occurring in *M. oleifera* leaf extracts and the \(\alpha\)-L-rhamnoside of anisaldehyde derivatives were synthesized (Leuck and Kunz 1998).

*M. oleifera* and *M. stenopetala* were analyzed for glucosinolates and phenolics (flavonoids, anthocyanins, proanthocyanidins, and cinnamates). Roots and seeds of *M. oleifera* and *M. stenopetala* had high concentrations of both 4-(\(\alpha\)-L-rhamnopyranosyloxy)-benzylglucosinolate and benzyl glucosinolate. Leaves from both species contained 4-(\(\alpha\)-L-rhamnopyranosyloxy)-benzylglucosinolate and three monoacetyl isomers of this glucosinolate. Only 4-(\(\alpha\)-L-rhamnopyranosyloxy)-benzylglucosinolate was detected in *M. oleifera* bark tissue. *M. oleifera* leaves contained quercetin-3-O-glucoside and quercetin-3-O-(6"-malonyl-glucoside), and lower amounts of kaempferol-3-O-glucoside and kaempferol-3-O-(6"-malonyl-glucoside). *M. oleifera* leaves also contained 3-caffeoylquinic acid and 5-caffeoylquinic acid (Bennett et al 2003).

**PHARMACOLOGICAL STUDIES**

**Anti-inflammatory activity**

A crude ethanolic extract of dried seeds of *M. oleifera* Lam. was tested for anti-inflammatory activity using carrageenan-induced inflammation in the hind paw of mice. It was found to inhibit 85\% of inflammation at a dose of 3 mg/kg body weight, while the mature green seeds inhibited edema by 77\% at the same dose (Guevare et al 1999, Udupa et al 1994).

A crude methanol extract of the root of the plant *M. oleifera* Lam. was screened for anti inflammatory effect using the rat paw edema and the rat 6-days air pouch
inflammatory models. Following oral administration, the extract inhibited carrageenan-induced rat paw edema in a dose-dependent manner, with IC$_{50}$ (dose producing 50% inhibition) of 660 mg/kg. On the 6-day air pouch acute inflammation induced with carrageenan, the extract was much more potent, with IC$_{50}$ values of 302.0 mg/kg and 315.5 mg/kg, for the inhibition of cellular accumulation and fluid exudation, respectively. *M. oleifera* contains anti-inflammatory principle(s) that may be useful in the treatment of both the acute and chronic inflammatory conditions (Ezeamuzie et al 1996).

**Antioxidant activity**

The oil from the dried seeds of the *M. oleifera* showed higher antioxidant activity than BHT and alpha-tocopherol (Lalas and Tsaknis 2002). Water, aqueous methanol, and aqueous ethanol extracts of freeze-dried leaves of *M. oleifera* Lam. were examined for radical scavenging capacities and antioxidant activities. All leaf extracts were capable of scavenging peroxyl and superoxyl radicals. The major bioactive compounds of phenolics were found to be flavonoid groups such as quercetin and kaempferol. On the basis of the results obtained, Moringa leaves are found to be a potential source of natural antioxidants due to their marked antioxidant activity (Siddhuraju and Becker 2003).

**Antimicrobial Activity**

4(α-L-Rhamnosyloxy) benzyl isothiocyanate was identified as an active antimicrobial agent from seeds of *M. oleifera* and *M. stenopetala*. Defatted and shell free seeds of both species contain about 8-10% of 4(α-L-rhamnosyloxy) benzyl isothiocyanate, but this amount is produced from *M. oleifera* only when ascorbic acid is added during water extraction. The compound acts on several bacteria and fungi. The minimal bactericidal concentration in vitro is 40μmol/l for *Mycobacterium phlei* and 56μmol/l for *Bacillus subtilis* (Eilert et al 1981). The antimicrobial activity of leaves, root, bark and seeds were also investigated against bacteria, yeast, dermatophytes and helminthes pathogenic to man. The
fresh leaf juice and aqueous extract of seeds inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Caceres et al 1991).

Seeds of *M. oleifera* were studied in an experimental model of *S. aureus* pyodermia in mice. It was found out that *M. oleifera* could be an alternative treatment for infections (Caceres and Lopez 1991).

**Cardiovascular Activity**

The aqueous extract of stem bark of *M. oleifera* induced a positive inotropic effect at low concentration and negative inotropic effect at high concentration using isolated frog heart and it also produced a dose dependent hypotensive effect on dog blood pressure (Limaye et al 1995). Five compounds niazinin A, niazinin B, niazimicin, niaziminin A and niaziminin B isolated from the ethanolic extract of the leaves produced hypotensive and bradycardiac effect in anaesthetized rat at a dose of 1-10 mg/kg *i.v.* (Gilani et al 1994).

Ethanolic and aqueous extracts of *M. oleifera* whole pods and their parts, namely, coat, pulp and seed also showed hypotensive activity. The activity of the ethanolic extract of both the pods and the seeds was equivalent at the dose of 30 mg/kg (Faizi et al 1998). The alkaloids obtained by the fractionation of the water extract of the leaves of *M. oleifera*, converted into their salt form, were tested for their activity on the isolated frog heart. The total alkaloidal salts were found to have a negative inotropic effect on the frog heart. This activity was further characterized by testing it on the isolated guinea pig ileum (Dangi et al 2002).

**Antihyperlipidaemic activity**

It was found that administration of the crude leaf extract of *M. oleifera* along with high-fat diet decreased the high-fat diet-induced increases in serum, liver, and kidney cholesterol levels by 14.35% (115-103.2 mg/100 ml of serum), 6.40% (9.4-8.8 mg/g wet weight) and 11.09% (1.09-0.97 mg/g wet weight) respectively.
It was concluded that the leaves of *M. oleifera* have definite hypocholesterolemic activity (Ghasi et al 2000).

*M. oleifera* and lovastatin in hypercholesterolaemic rabbits were found to lower the serum cholesterol, phospholipid, triglyceride, VLDL, LDL, cholesterol to phospholipid ratio and atherogenic index, but were found to increase the HDL ratio (HDL/HDL-total cholesterol) as compared to the corresponding control groups. Lovastatin- or *M. oleifera*-treated hypercholesterolaemic rabbits showed decrease in lipid profile of liver, heart and aorta while similar treatment of normal animals did not produce significant reduction in lipid profile of heart. *M. oleifera* was found to increase the excretion of faecal cholesterol. Thus, the study demonstrates that *M. oleifera* possesses a hypolipidaemic effect (Mehta et al 2003).

**CNS activity**

Methanolic extract (ME) of the root of *M. oleifera* potentiated significantly the sleeping time induced by pentobarbitone sodium, diazepam and meprobamate, showed analgesic properties and also potentiated analgesia induced by morphine and pethidine. Pretreatment with ME caused significant protection against strychnine- and leptazol-induced convulsions. The behavioural studies on mice indicate the CNS depressant nature of ME (Gupta et al 1999). Effect of chronic treatment of standardized aqueous extract of *M. oleifera* (MO) root (100, 200, 300, 350, 400, 450 mg/kg; p.o.) on penicillin (PCN) induced convulsion, locomotor behaviour, brain serotonin (5-HT), dopamine (DA) and norepinephrine (NE) level was studied in Holtzman strain adult albino rats. MO showed central inhibitory effect in the disturbed balance between 5-HT, DA and NE (Ray et al 2003).

**Antifertility Activity**

Rao et al (1979) had screened bark of *M. oleifera* for its antifertility effect on early pregnancy in albino rats. It does not exert much antifertility activity while
the aqueous extract of root and bark of *M. oleifera* at a dose of 200 mg/kg and 400 mg/kg respectively showed post-coital antifertility effect in rat and also induced foetal resorption at late pregnancy (Prakash et al 1987). The aqueous or 90% ethanolic extract of root showed abortifacient and teratogenic effect in rat (Nath et al 1992). The aqueous extract of roots of *M. oleifera* possesses antioestrogenic and antiprostigestational activity (Shukla et al 1988a). The aqueous extract of root was found to induce biochemical alteration in female genital tract of ovariectomised rat (Shukla et al 1989) and exhibited biphasic effect on periodicity of oestrous cycle in adult intact rat (Shukla et al 1987). The aqueous extract of roots of both the species induced anti implantation activity in rats (Shukla et al 1988b).

**Anticancer Activity**
Paste of *M. oleifera* leaves has been screened for its influence on the carcinogen detoxifying glutathione-S-transferase (GST) in Swiss mice. It increased GST activity by more than 78% in the stomach, liver and oesophagus. The results indicated protective activity against carcinogenesis. The crude ethanolic extract of seeds exhibited antitumor activity against Epstein-Barr virus-early antigen (EBV-EA) (Guevara et al 1999).

A number of biosynthetically and chemically related compounds were isolated from the roasted seeds of *M. oleifera*. Structure-activity correlation studies showed that 4(α-L-rhamnosyloxy) phenylacetonitrile, 4-hydroxyphenylacetonitrile, and 4-hydroxyphenyl-acetamide exhibited mutagenic activity (Villasenor et al 1989).

**Anti-hepatotoxic activity**
Aqueous and alcoholic extracts of root and flower of *M. oleifera* were screened for antihepatotoxic activity in paracetamol treated albino rats. Liver function was assessed based on liver to body weight ratio, serum levels of transaminase (SGPT, SGOT), alkaline phosphatase (SLAP) and bilirubin. All extracts were
fount to have antihepatotoxic activity. The LD_{50} value of ethanolic (90%) extracts of roots and flowers of *M. oleifera* were calculated to be 1023, 1047 mg/kg *i.p.* in mice respectively. The corresponding values for aqueous were 1078, 1092 mg/kg (Ruckmani et al 1998).

Pari and Kumar (2002) and Kumar and Pari (2003) have evaluated the hepatoprotective effect of an ethanolic extract of *M. oleifera* leaves on liver damage induced by antitubercular drugs such as isoniazid (INH), rifampicin (RMP), and pyrazinamide (PZA) in rats. Extract appears to enhance the recovery from hepatic damage induced by antitubercular drugs.

**Antiulcer Activity**

The methanolic extract of leaves of *M. oleifera* inhibited gastric lesion formation induced by aspirin, serotonin or indomethacin in rats (Pal et al 1995).

**Miscellaneous activity**

Hot water infusions of flowers, leaves, roots, seeds and stalks of bark of *M. oleifera* showed antispasmodic activity using isolated duodenum, oral anti-inflammatory activity by carrageenan-induced hind paw edema and oral diuretic activity by urine output in metabolic cages. The seeds infusion showed a significant inhibition of acetylcholine-induced contraction with an ED_{50} of 65.6 mg/ml bath concentration, inhibition of carrageenam-induced edema at 1000 mg/kg and diuretic activity at 1000 mg/kg. (Caceres et al 1992)

Availability of carotene (vitamin A) from vegetables was studied in rats by the liver storage bioassay method. Carotene of *M. oleifera* was 49.1% active in producing vitamin A. When the rats were supplemented with pure carotene, the Hb level increased (Absar et al 1977). The bioavailability of thiamin and riboflavin was higher from *M. oleifera* leaves (Girija et al 1982).

One of the studies carried out at Saudi Arabia showed that *M. oleifera* increased the blood glucose by 15% in alloxanized mice (Mossa 1985). While in another
study, ethanolic extract of *M. oleifera* showed significant blood glucose lowering effect within 2 weeks in alloxan diabetic albino rats (Kar et al 2003). The blood glucose levels and the corresponding insulin levels in response to drumstick leaves in southern India were compared to the levels achieved in response to 75g of glucose in non-insulin dependent diabetes mellitus patients. The blood glucose response was 56% as compared to 75g of glucose. It is concluded that the reduced blood glucose response to drumstick leaves is not due to insulin secretion (William 1993).

The role of *M. oleifera* aqueous leaf extract in the regulation of thyroid hormone status was studied in adult Swiss rats. Other than the thyroid hormone concentrations, hepatic lipid peroxidation (LPO) and the activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were evaluated. Reduction in the serum T3 concentration (approx. 30%) and an increase in the T4 concentration were observed suggesting the inhibiting nature of *M. oleifera* leaf extract in the peripheral conversion of T4 to T3. It is suggested that the lower concentration of this plant extract may be used for the regulation of hyperthyroidism (Tahiliani and Kar 2000).
ACHYRANTHES ASPERA Linn.

GENERAL INFORMATION

Regional and other Names

Sans. : Apamarga  
Beng. : Apang  
Guj. : Aghedo, Aghedi  
Hindi : Chirchira, Chirchitta, Latjira  
Kan. : Utranigida, Uttaraanne  
Mal. : Katalati  
Mar. : Aghada, Aghara  
Ori. : Apamaranga, Apamargo  
Tam. : Naayurivi, Nayuruvi  
Tel. : Apamargamu, Uttareni

Description

Distribution: An erect or procumbent, annual or perennial herb often with a woody base, commonly found as a weed on wayside and waste places throughout India up to an attitude, 2100 m.

Macroscopic

Root —Cylindrical tap root, slightly ribbed, 0.1-1.0 cm in thickness, gradually tapering, rough due to presence of some root scars, secondary and tertiary roots present, yellowish brown; odour, not distinct.

Stem —0.3-0.5 cm. in cut pieces, yellowish brown, erect, branched, cylindrical, hairy, solid, hollow when dry.

Leaf — Simple, subsessile, extipulate, opposite, decussate, wavy margin, obovate, slightly acuminate and pubescent due to the presence of thick coat of long simple hairs.

Flower — Arranged in inflorescence of long spikes, greenish-white, numerous, in a small dense axillary heads or spikes. Bracts and bracteoles persisting, ending in a spine.

Seeds — Sub-cylindric, truncate at the apex and round at the base, black and shining (Kirtikar and Basu 1975b).
Ayurvedic Description

Botanical name: *Achyranthes Aspera*

<table>
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<th>Sanskrit name</th>
<th>Apamarga</th>
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<th>Karma (Actions)</th>
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Therapeutic uses

The dried plant is used in sula (colic), udararoga (diseases of the abdomen), apaci (lymphadenitis-cervical), arsa (haemorrhoids), kandu (itching), medroga (obesity) (A.P.I. 1999). The dried root of the plant is used in chardi (vomiting), adhmana (tympanitis), kandu (itching), sula (colic), apaci (lymphadenitis), granthi (tumor), bhagandara (fistula-in-ano), hrdaroga (disease of heart), jwara (pyrexia), swittra (leucoderma), vadhiyra (deafness), udararoga (diseases of the abdomen), yaktrtoga (disorders of the liver), dantaroga (disease of tooth), raktavikara (blood disorders) (A.P.I. 2001).

Properties and Uses Ascribed

The plant is reported to be pungent, astringent, pectoral and diuretic. It is used as an emmenagogue, in piles and skin eruptions. The decoction of the plant is useful in pneumonia and renal dropsy while the juice of the plant is used in ophthalmia and dysentery. The leaves are used as a cure for gonorrhoea and excessive perspiration; their extract is used for leprosy and the heated sap for tetanus. The root is astringent; the paste is applied to clear opacity of cornea, and to wounds as a haemostatic. The root is also reported to be useful in cancer. A decoction of the root is used for stomach troubles and an aqueous extract for stones in the bladder. The flowers, ground and mixed with curd and sugar, are
given for menorrhagia. The flower tops are employed for the treatment of rabies. Powdered seeds are soaked in buttermilk and given for biliousness. The seeds are said to be emetic and used in hydrophobia (Nadkarni 1954; Chopra et al 1958; Wealth of India 1985).

Ethnobotanical Studies
The plant is used in dropsy, piles, skin eruptions, colic, as a diuretic, astringent and purgative (Bhatnagar et al 1973; Raj and Patel 1978; Khanna et al 1994); as an antidote to snake bite (Selvanayagum et al 1995); in fractured bones (Singh and Ali 1989; Girach et al 1992; Anis and Iqbal 1994); whooping cough, respiratory troubles (Husain and Siddiqui 1987); for asthma (Reddy et al 1988, 1989); as a laxative (John 1984) and in leucoderma (Pal and Jain 1989). The inflorescence is used in cough (Sebastian and Bhandari 1984a) and in hydrophobia (Anis and Iqbal 1994). Fruit is used in hydrophobia (John 1984). The seeds are employed as an emetic, purgative, and cathartic, in gonorrhoea, for insect bite and in hydrophobia (Bhatnagar et al 1973; Raj and Patel 1978; Singh and Pandey 1980; Reddy et al 1988, 1989), cough including whooping cough (Singh and Pandey 1980), as an anti-asthmatic (Singh and Pandey 1980). The leaves are used in wounds, injuries (Neogi et al 1969); in intermittent fever, as an anti-asthmatic, for urination, dog bite (Singh and Ali 1989; Anis and Iqbal 1994) and in typhoid (Sebastian and Bhandari 1984b). The root is used in whooping cough, tonsilitis (Singh and Ali 1989; Anis and Iqbal 1994), haemorrhage (Pal and Jain 1989), cough and hydrophobia, as an anti-asthmatic (Singh and Pandey 1980), diuretic, diaphoretic, and antisypililitic (Bhatnagar et al 1973).

Pharmacognostical Studies
The T.S. of young stem shows 6-10 prominent ridges and collenchyma is present under each ridge. The epidermis is single layered, covered with thick cuticle. Trichomes arising from the epidermis are simple, covering, multicellular straight or somewhat spirally running, highly warty. The cortex is composed of 6 to 8
layers of parenchymatous cells containing cluster and rosette crystals of ca-oxalate. Xylem is composed of annular, spiral and pitted vessel, tracheids, fibres and parenchyma. The diagrammatic T.S. of the young root shows a layer of epiblema with long unicellular hairs. Cortex is 5-6 layered, parenchymatous and narrow. The stelar region shows anomalous growth. Upper epidermal cells of leaf are more or less straight walled while the lower ones are wavy walled. Both the upper and lower epidermal cells are traversed with anomocytic and few anisocytic stomata. Trichomes are simple, covering, uniseriate, multi-cellular and many, arising from the lower epidermis. Rosette crystals of ca-oxalate measuring 20-45 μm in diameters are embedded throughout the parenchymatous cells of the mesophylls and the ground tissue of the mid rib (Prasad and Bhattacharya 1961).

CHEMICAL STUDIES

The plant is reported to yield a water-soluble base and a chloroform soluble base. The former was earlier designated as achyranthine (Basu et al 1957a) and was characterized as a betaine derivative of N-methylpyrrolidine-3-carboxylic acid (Basu 1957). Later studies by Kapoor and Singh (1966) showed that the water-soluble base was betaine and not achyranthine. The chloroform soluble basic fraction was shown to be a mixer of two uncharacterized alkaloidal entities (Kapoor and Singh 1967). The ethanol extract of the plant contained alkaloids and saponins while flavonoids and tannins were found absent (Kumar et al 1990).

The shoot yielded a new aliphatic dihydroxyketone, characterized as 36,47-dihydroxyhenpentacontan-4-one together with tritriacontanol (Misra et al 1991); an essential oil; a new long chain alcohol characterized as 17-pentatriacontanol (Misra et al 1992); four new compounds characterized as 27-cyclohexylheptacosan-7-ol, 16-hydroxy-26-methylheptacosan-2-one (Misra et al 1993), 4-methylheptatriacont-1-en-10-ol and tetracontanol-2 (Misra et al 1996).

Various parts of the plant, viz., seeds, stem, leaves (Banerji et al 1971) and root (Banerji and Chadha 1970) were reported to contain ecdysterone. The chloroform extract of the stem led to the isolation of pentatriacontan, 6-
pentatriacontanone, hexatriacontane and triacontane (Ali 1993). The inflorescence was reported to contain flavonoids and alkaloids (Singh and Dogra 1985).

The food value of the seeds in terms of its protein quality was studied. The composition of the seeds showed close similarity to Bengal gram with a protein content of 24.8 and calorific value of 3.92/g. The hydrolysate contained the usual amino acids. The values obtained for ten essential amino acids and cystine showed that the seed protein compared favorably with Bengal gram in its leucine, isoleucine, phenylalanine and valine content, while its tryptophan and sulphur amino acid (methionine and cystine) content were higher than most of the pulses. It was however, deficient in arginine, lysine and threonine as compared to the whole egg protein (Satyanarayana et al 1964).

The defatted seeds were reported to yield a saponin in a yield of 2 %, which was identified as oleanolic acid- oligosaccharide. The sugar moiety of the saponin was composed of glucose, galactose, xylose and rhamnose (Gopalachari and Dhar 1952, 1958). Khastgir and associates (1958) isolated a crude sapogenin fraction from the seeds, which yielded oleanolic acid. Later, investigation led to the isolation of two oleanolic acid based saponins, saponin A and saponin B which were characterized as α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→4)-β-D-glucuronopyranosyl(1→3)-oleanolic acid and β-D-galactopyranosyl (1→28) ester of saponin A, respectively (Hariharan and Rangaswamy 1970). In another study, the total saponins were hydrolysed with acid and the genin was identified as oleanolic acid (Batta and Rangaswami 1973). A rapid procedure for the separation of triterpenoid saponin based on partition chromatography from the plant has been described (Sarkar and Rastogi, 1960). The seeds contained hentriacontane, 10-octacosanone, 10-triacosanone and 4-tritriacontanone (Ali 1993).
The unripe fruit yielded two new saponins (C and D), which were identified as β-D-glucopyranosyl ester of α-L-rhamnopyranosyl (1→4)-β-D-glucuronopyranosyl (1→3)-oleanolic acid and β-D-glucopyranosyl ester of α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl (1→4)-β-D-glucuronopyranosyl (1→3)-oleanolic acid (Seshadri et al 1981).

The chemical constituents of the root varied in different preliminary studies carried out. The root was found to contain oleanolic acid as the aglycone from the saponin fraction (Khastgir and Sengupta 1958). Both root and shoot of the plant were found to contain saponin and alkaloids but no flavonoids (Sinha and Dogra 1985). In another study, the root of the plant was found to contain alkaloids but indicated absence of saponin and tannins (Joshi and Sabnis 1989). In yet another preliminary chemical study, the root was reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids. Glycosides were found to be absent (Agrawal et al 1989). Isolation of β-sitosterol was also reported from the root (Misra et al 1993).

PHARMACOLOGICAL STUDIES

General pharmacological studies of the A. aspera did not elicit any exciting activity. However, the anti fertility activity need to be looked into.

Anti inflammatory activity

The water-soluble alkaloid achyranthine isolated from A.aspera was screened for its anti inflammatory and antiarthritic activity against carrageenin-induced foot oedema, granuloma pouch, formalin induced arthritis and adjuvant arthritis in rats. It showed significant anti-inflammatory activity in all the four models employed but was less active than phenylbutazone and betamethasone. Further, achyranthine significantly reduce the weight of adrenal gland, thymus and spleen and raised the adrenal ascorbic acid and cholesterol contents. The effects were qualitatively similar to betamethasone. All the three drugs tested reduced food intake but had no significant effect on urinary and faecal output and mortality.
rate. Incidence of gastric ulcers was maximum with betamethasone and minimum with achyranthine (Neogi et al 1969).

**Anti microbial activity**

The aqueous solution of the base achyranthine as well as the entire plant of *A.aspera* showed antibacterial activity against *Staphylococcus aureus*, *Streptococcus hemolyticus* and *Bacillus typhosus* (Basu et al 1957b) While the alcoholic and the aqueous extract of the leaves showed antibacterial activity against *S. aureus* and *E. coli* (George et al 1947)

The seeds growing on cattle dung revealed antibacterial activity against bacterial strains of *B. subtilis*, *Pseudomonas cichorii* and *Salmonella typhimurium* (Sushil Kumar et al 1997). In another study, the 80 percent ethanolic extract of the leaves and stem of the plant inhibited *B. subtilis* and *S. aureus* bacterial strains at a concentration of 25 mg/ml (Valsaraj et al 1997).

**Antihepatotoxic activity**

The alcoholic extract of the plant *A.aspera* at 100mg/kg dose lowered total serum cholesterol (TC) and phospholipid (PL), triglyceride (TG) and total lipids (TL) levels by 60, 51, 33 and 53 per cent, respectively in triton-induced hyperlipidemic rats. The chronic administration of the extract at the same doses to normal rats for 30 days, lowered serum TC, PL, TG, and TL by 56,62,68 and 67 %, respectively followed by significant reduction in the levels of hepatic lipids. The possible mechanism of action of cholesterol lowering activity of the plant might be due to rapid excretion of bile acids causing low absorption of cholesterol (Khanna et al 1992).

**Anti fertility activity**

The alkaloidal fraction obtain from the alcoholic extract of the root bark of *A.aspera* inhibited the response of oxytocin in isolated rat uterus. This fraction
Review of literature

did not inhibit the response to serotonin and acetylcholine in rat uterus and to histamine in g. pig uterus (Gupta and Khanijo 1970).

The crude benzene extract of the stem was found to have potent abortifacient effect in mice (Pakrashi et al, 1975a). In an attempt to locate the active principle, various chromatographic fractions were tested for anti fertility activity in female mice. The maximal activity was found to be located in the fraction eluted with 50 percent benzene in petroleum ether (Pakrashi et al 1975b). The methanolic extract of the root revealed 60 percent anti implantation activity in rats while the acetone extract of the root prevented implantation in 50 percent of rats (Prakash 1986). The ethanolic extract of the plant (excluding root) at a dose of 100-200 mg/kg body wt administered orally revealed 60 percent anti fertility activity on early pregnancy in rats. Further, the plant also showed potent activity at secondary testing level (Prakash et al 1987a).

The n-butanol fraction of the aerial parts prevented pregnancy in adult female rats when administered orally at a daily dose of 75 mg/kg or more on 1-5d post coitum, but was ineffective in hamsters up to 300 mg/kg dose. No anti fertility activity was observed in the aqueous fraction in either rats or hamsters. In ovariectomized immature female rats, the extract exhibited potent estrogenic activity at a dose of 75mg/kg. It induced a marked stimulation in uterine weight. Marked uterotrophic effect was discerned even at a dose of 3.75 mg/kg (Wadhwa et al 1986).

Diuretic activity
The saponin isolated from the seeds of A. aspera in 10-20mg/kg i.m. doses in rats caused significant increase in urine output after 2,6 and 24h as compared to untreated rats. The diuretic effect was comparable to that observed with 3mg/kg dose of mersalyl. The optimum dose of the saponin was 10 mg/kg. After oral administration of the saponin (5-10mg/kg) in rats, a significant increase in urine output was observed which was comparable to that of 10 mg/kg oral dose of
acetazolamide. The diuretic effect of saponin, like acetazolamide, was associated with an increase in the excretion of sodium and potassium in the urine (Gupta et al 1972b).

Miscellaneous activity
In a preliminary study, the aqueous and alcoholic extracts of the roots of A. aspera caused a sharp and transient fall in blood pressure without any significant action on the respiration of anaesthetized dogs. In higher doses, there was slight respiratory depression. Atropine sulphate blocked the hypotensive effect of the extracts. On frog's heart the extracts had a temporary negative inotropic and chronotropic effects. The extracts produced spasm of isolated rabbit's ileum, increased the tone and amplitude of contractions in gravid and non-gravid uteri of albino rats, g. pigs and rabbits. Oral administration of the drug significantly increases the urine output in rabbits (Gambhir et al 1965).

The total chloroform soluble basic fraction (alkaloidal residue) obtained from the plant A. aspera raised the blood pressure of anaesthetized dog, caused initial transitory stimulation of respiration and increased the amplitude of cardiac contractions of isolated g. pig heart. It showed spasmolytic action against various spasmogens on intestine and uterine muscles of g. pigs and a slight anti-diuretic action in rats. No specific CNS effects were observed in mice. The fraction did not possess analgesic activity in rats (Kapoor and Singh 1967). The water-soluble alkaloid, achyranthine isolated from the plant was found to lower blood pressure, depress the heart, dilate the blood vessels and increase the rate and amplitude of respiration anaesthetized dogs. It showed spasmogenic effect on frog's rectus muscle and diuretic as well as purgative action in albino rats. No effect was observed on isolated rabbit, g. pig and rat ileum and on CNS. The drug exerted a slight antipyretic effect (Basu et al 1957b; Neogi et al 1970). The mixture of saponins isolated from the seeds of A. aspera caused a significant increase in force of contraction of the isolated heart of frog, g. pig and rabbit. The stimulant effect of the lower dose (1 to 50 μg) of the saponins was
blocked by pronethol and partly by mepyramine. The effect of higher dose was not blocked by pronethol. The saponin increases the tone of the hypodynamic heart and also the force of contraction of failing papillary muscle. The effect was quicker in onset and shorter in duration in comparison to that exerted by digoxin (Gupta et al 1972a). The effect of saponin on the phosphorylase activity of the perfused rat heart has been investigated and compared with that of adrenaline. The saponin has been found to stimulate the phosphorylase activity of the heart and its effect was comparable to that of adrenaline (Ram et al 1971).

The ethanolic extracts of the plant (Dhar et al 1968) and leaves (Aswal et al 1996) were screened for preliminary biological activities. The former extract showed hypoglycemic activity in rat. It was devoid of anti bacterial, anti fungal, anti protozoal, anthelmintic, antiviral and anticancer activities and effects on isolated g.pig ileum, respiration, CVS and CNS in experimental animals. The MTD on the extract was found to be 1000mg/kg bw orally in mice (Dhar et al 1968). The leaf extract was found to be devoid of anti protozoal and antiviral activities and effects on respiration, preganglionically stimulated nictitating membrane, CVS and CNS in experimental studies. The LD50 of the latter extract was >1000mg/kg i.p. in mice (Aswal et al 1996).

**Toxicity**

The alkaloid isolated from the plant was tested for its acute, subacute and chronic toxicity in rats. During acute toxicity test, there was a slight increase in sedation and slight loss in righting region at 6.0mg/kg dose level, which became prominent at 7.0mg/kg dose level. At higher doses, significant depletion in righting region, depression in respiration, remarkable increase in sedation and diarrhoea was observed. Subacute toxicity test revealed (5.0 and 6.0 mg/kg) a significant increase in sedation and hypnosis, depletion in respiration and loss of righting reflexes. At 6.0mg/kg dose, it also caused remarkable increase in salivation and diarrhoea. Chronic toxicity showed (3.0 mg/kg) an increase in
sedation, hypnosis, salivation and diarrhoea. There was a significant depression of respiration and loss of body weight (Mali et al 1990).

**CLINICAL STUDIES**

The plant was subjected to wide clinical evaluation with special reference to its use in leprosy, bronchial asthma and fistula-in-ano. Diuretic activity could not be confirmed.

**Leprosy**

The effect of oral decoction of *A. aspera* in the treatment of leprosy was studied (uncontrolled) in 19 patients who were found to have positive stain smears at the S. S. Hospital, Varanasi. Fourteen patients were in stage of reaction and rest of them had active lesions but none of them was in quiescent stage. The study revealed encouraging results in both lepra reaction as well as the quiescent stage of lepromatous leprosy (Tripathi et al 1963).

In an attempt to get additional data on the efficacy of the decoction of *A. aspera*, it was observed that the decoction was useful in the treatment of reaction in leprosy particularly in subacute and mild type. When administered in conjunction with the antileprosy drug diaminodiphenylsulphone (DDS), it was found that the chance of reaction became less and rate of improvement was faster. No toxic manifestation, which could be attributed to *A. aspera* was noted during the trial (Ojha et al 1966, Ojha and Singh 1968).

**Fistula-in-ano**

The studies revealed that the longterm use of ‘Kshaarasootra’ (a medical thread prepared by coating the latex of *Euphorbia neriifolia*, alkaline powder of *A. aspera* and *Curcuma longa*) was quite effective in treatment of various fistulous tracks (Despande and Sharma 1973, 1976; Despande et al 1966, 1975; Raghavaiah 1976; Gangasatyam 1981; Varshney and Tyagi 1991).

The Indian Council of Medical Research has carried out a multicentric randomized controlled trial to evaluate the efficacy of ‘Kshaarasootra’ in the
Review of literature

management of fistula-in-ano (265 patients) in comparison with the conventional surgery (237 patients). The results have revealed that the long-term outcome with ‘kshaarasootra’ (recurrence 4 percent) was better than with the surgery (recurrence 11 percent), although the initial healing time was longer (8 wk with thread and 4 wk with surgery). “Kshaarasootra’ offered an effective, ambulatory and safe alternative treatment for patient with fistula-in-ano (ICMR 1991).

“Kshaarasootra’ has also been found to give encouraging results in 5 patients of chronic non healing milk-fistula ‘stannadi-vrana’ with additional local application of ‘jatyaditaila’ and oral administration of ‘ shigru guggulu’ (two tablets t.i.d.) during the course of treatment (Singh et al 1994).

Bronchial Asthma

A pilot study was carried out at the Central Research Institute for Siddha in Madras on 15 cases of bronchial asthma. The oil obtained from the root of A.aspera soaked in cow urine was smeared on betel leaf and administered thrice a day to these patients. In most of the cases symptoms like wheezing, gasping, dyspnoea, sneezing and cough disappeared. A fall in total WBC, eosinophil counts and ESR was observed (Suresh et al 1985).