2. Review of literature

2.1. Definition and classification of asthma

Asthma is a major chronic lung disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. Although the fundamental causes of asthma are not completely understood, numerous antigens or allergens are capable of triggering the acute attacks of asthma (WHO, 2012), its manifestations appear to result from the interaction between the genetic makeup of the individual and the environment to which patient is exposed. In the recent years, the morbidity and mortality of population due to asthma is increasing despite the advances being made in understanding of this disease and availability of improved medications and information on treatment (Shabaraya et al., 2008).

In ancient times, asthma was already recognized in many cultures, including the Chineses, Hebrews, Greeks and Romans. The Greek physician Hippocrates (460–377 BC) first described asthma, which is derived from the Greek word “asthmaino” (Panos) indicating “panting or gasping” which means to pant or to breathe with an open mouth (Diamant et al., 2007).

The National Heart, Lung, and Blood Institute’s Second Expert Panel on the Management of Asthma defined bronchial asthma as “a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T lymphocytes, and epithelial cells” (NAEPP, 2007; Moore and Peters, 2006). In asthma bronchi, bronchioles and alveoli undergo in changes when stimulated by allergens or other environmental triggers. These changes are results of two specific responses: hyperreactive response (also called hyperresponsiveness) and inflammatory response. This inflammatory process produces recurrent episodes of airway obstruction, characterized by coughing, wheezing (a high-pitched whistling sound, especially when breathing out), shortness of breath (dyspnea or breathlessness), and chest tightness that often is worse at night and in the early morning, which are the hallmarks of asthma. These episodes, which usually are reversible either spontaneously or with treatment, also cause an associated increase in bronchial responsiveness to a variety of stimuli (NAEPP, 2007). Airway hyperresponsiveness occurs due to various extrinsic (allergens such as pollens, foods, dusts, gases, fumes, vapours, etc.) and intrinsic factors (respiratory
infections, exercise, genetic factors, and emotional factors-stress). Airway inflammation arises due to interaction of a multitude of cells, including lymphocytes, mast cells, eosinophils, neutrophils, macrophages, epithelial cells, dendritic cells and various mediators, including chemokines, cytokines, leukotrienes, nitric oxide, and immunoglobulins.

Asthma is classified into several forms on the basis of cause of asthma symptoms, its severity, method of its control, and its prevalence among populations of different age groups (Moore and Peters, 2006; NYTC, 2012; USA, 2012). According to NAEPP and GINA guideline asthma can be classified into following category (Table 1) based on the severity and symptoms.

Table 1: Classification of asthma based on severity according to NAEPP, 2007 and GIA, 2011 guidelines.

<table>
<thead>
<tr>
<th>Classification of Asthma Severity</th>
<th>Symptoms</th>
<th>Nighttime Symptoms</th>
<th>Lung Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild intermittent</td>
<td>Symptoms ≤2 times a week</td>
<td>≤2 times a month</td>
<td>FEV₁₀, or PEF ≥80% predicted</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic and normal PEF between exacerbations</td>
<td></td>
<td>PEF variability &lt;20%</td>
</tr>
<tr>
<td></td>
<td>Exacerbations brief (from a few hours to a few days); intensity may vary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild persistent</td>
<td>Symptoms &gt;2 times a week but &lt;1 time a day</td>
<td>&gt;2 times a month</td>
<td>FEV₁₀, or PEF ≥80% predicted</td>
</tr>
<tr>
<td></td>
<td>Exacerbations may affect activity</td>
<td></td>
<td>PEF variability 20%-30%</td>
</tr>
<tr>
<td>Moderate persistent</td>
<td>Daily symptoms</td>
<td>&gt;1 time a week</td>
<td>FEV₁₀, or PEF &gt;60%&lt;80% predicted</td>
</tr>
<tr>
<td></td>
<td>Daily use of inhaled short-acting ß₂-agonist</td>
<td></td>
<td>PEF variability &gt;30%</td>
</tr>
<tr>
<td></td>
<td>Exacerbations affect activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exacerbations ≥2 times a week; may last days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe persistent</td>
<td>Continual symptoms</td>
<td>Frequent</td>
<td>FEV₁₀, or PEF ≤60% predicted</td>
</tr>
<tr>
<td></td>
<td>Limited physical activity</td>
<td></td>
<td>PEF variability &gt;30%</td>
</tr>
<tr>
<td></td>
<td>Frequent exacerbations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FEV₁₀, forced expiratory volume in 1 second; PEF, peak expiratory flow rate.

2.2. Etiological history of asthma

The first etiological link with bronchospasm was made by Galen (130–201 AD), who also described the association between upper and lower airways. From ancient times throughout the middle ages, the physicians considered the paradigms by Hippocrates and Galen as golden standard. In his Treatise on asthma, Maimonides (1135–1204), physician of sultan Saladin, suggested to treat asthma with rest, avoidance of opium, good personal
and environmental hygiene and emphasized the importance of dietary factors (Ellul-Micallef, 1976; Cserhati, 2004).

In the 16th century, the German physician Georgius Agricola (formerly Georg Bauer; 1494–1555) described the association between environmental factors and airway symptoms and was possibly the first to report occupational asthma. He suggested to prevent asthma in miners with protective masks to avoid the inhalation of dust (Cserhati, 2005). In the Renaissance period airway symptoms associated with exposure to seasonal allergens were already reported (Cserhati, 2004; Cserhati, 2005). At that time, avoidance of some allergenic factors and cold baths (once in 14 days or once a month) were the mainstay of asthma therapy (Cserhati, 2005).

Bronchial asthma in its modern definition dates back to the early 19th century, when it was recognized as a unique airways disorder characterized by bronchospasm following Rene Laennec’s (1781–1826) invention of the stethoscope (Cserhati, 2004; Cserhati, 2005). In addition, the familial clustering of asthma and allergy was appreciated.

In 1860, Henry Hyde Salter (1823–71) proposed a classification into extrinsic and intrinsic asthma, based on the nature and putative mechanism of various stimuli (e.g. animal dander or emotional stress) inducing episodes of bronchospasm (Salter, 1860). Some 30 years later, William Osler (1849–1919) described the link between various (non) specific stimuli causing paroxysmal airways dysfunction in asthma - later recognized as bronchial hyperresponsiveness. Osler considered asthma as an inflammatory disease, based on several pathological changes within the asthmatic airways including oedema, gelatinous mucus, and Charcot–Leyden crystals (‘asthma crystals’) in the sputum (Osler, 1892). The identification of the asthma crystals as eosinophil granulocytes came when Paul Ehrlich (1854–1915) discovered tetrabromofluorescein (eosin) (Schwartz, 2004). Using aniline stainings Ehrlich also identified mast cells and basophils.

In the beginning of the 20th century, the hereditary and heterogeneous nature of asthma, its relationship with several allergies and its neural, inflammatory, and vascular mechanisms gained interest (Holgate, 2004). Furthermore, during a long period of time, the neuro-psychogenic origin of asthma has been entertained (Crocket, 1959). This
concept has gained renewed interest in recent years (Virchow et al., 1998; Lommatzsch et al., 2005). Others felt that “the sensitive nerves of the diaphragm are stretched and irritated, resulting in shortness of breath and a feeling of oppression which occurs especially during the night” (Hofbauer, 1931). Although neuro-psychological aspects were still considered of pivotal importance, following the discovery of allergic mechanisms, the origin of asthma was largely regarded as allergic.

While at that time inhaled allergen provocation was the mainstay for diagnosis of allergic asthma, this changed with the discovery of IgE and the possibility to measure specific IgE antibodies. However, Francis Rackemann (1887–1973) described patients with asthma without any evidence of allergic triggering of their symptoms and hence coined the term ‘intrinsic asthma’ (Rackemann et al., 1950). Similarly, attempting to classify asthma on the basis of provoking agents in 1971, Margaret Turner-Warwick concluded that ‘there remains a group of patients in whom asthmatic symptoms are unrelated to any demonstrable agent and where prick skin tests remain negative even when challenged with a wide range of antigens’ (Turner, 1971).

Although previously reported by Osler, bronchial or airway hyperresponsiveness as a major pathophysiological characteristic of asthma was first quantified in 1946 by Curry, who examined the effects of increasing doses of inhaled histamine in subjects with and without asthma (Curry, 1946). Eventually, these experiments resulted in one of the most reliable diagnostic tools for asthma.

The concept, that asthma is an inflammatory disorder was firmly established in the 20th century, which also marked the advent of interventional randomized control trials, the development of invasive and non-invasive methodologies and emerging immunological technologies, which increasingly replaced personal experience and observations. Airway remodeling, another important feature of asthma, has first been reported in this journal by Ellul-Micallef in 1973 (Ellul-Micallef, 1973). Applying flexible bronchoscopy, Laitinen and colleagues were amongst the first to report the structural changes within the airways of asthmatics (Laitinen et al., 1985). From that time on, an expanding number of (interventional) studies applying sub mucosal and even transbronchial biopsies have been conducted that helped to define the immunohistopathological changes within the asthmatic airways. Presently, there are 2 major
hypothesis on airway remodeling: while the structural airway changes are mostly regarded as a consequence of chronic airway inflammation (Jeffery et al., 2000), some view airway remodeling and chronic airway inflammation as parallel processes, since airway wall changes can be present even in the absence of a long-standing history of asthma (Fig. 1). Presently, it is debated whether airway remodeling may account for the accelerated decline in lung function in severe persistent asthma or whether structural changes within the airways may serve a protective purpose (Jeffery et al., 2000; Niimi et al., 2003). During the last decade of the 20th century, another longstanding concept, namely the systemic features of the allergic–asthmatic inflammation including the concept of unified airways, which was introduced by Galen almost 2000 years ago, were reinvented (Lombardi, 2001).

Pathophysiology of Airway-Remodeling

Figure 1: Pathophysiological mechanisms of airway remodeling in asthma (Diamant et al., 2007).
2.3. Prevalence of asthma in World and India

Asthma is a common disease that is rising in prevalence worldwide, with the highest prevalence in industrialized countries. As per WHO estimates 2007, there are 300 million people currently suffering from asthma globally (a number that could increase by further 100 million by 2025) and 255,000 people died of asthma in 2005 (Bousquet et al., 2005; Aggarwal et al., 2006; Jindal, 2007; MHFW, 2009; GIA, 2011; WHO, 2011). Most asthma related deaths occur in low- and lower-middle income countries. Asthma occurs in all races, and its prevalence has increased more than 30% since the late 1970s (Drazen, 2000). In the United States, more than 20 million people report symptoms consistent with or a diagnosis of asthma, including more than 6 million children, and nearly 5000 people die each year with asthma reported as the underlying cause of death (Mannino et al., 2002; NCHS, 2005; Reed, 2006). Asthma can develop at any time throughout life, although most cases begin before age 25 years (Drazen, 2000). In 2003, in the United States the asthma prevalence rate for boys under 18 years of age was 27% higher than the rate among girls, but among adults, prevalence rates were 77% greater among women than men (NCHS, 2005; Ford, 2005).

Prevalence rates of asthma are higher in western countries than in Asian countries (Aggarwal et al., 2006). Although the prevalence of asthma in India is somewhat similar to that seen in other Asian countries, the asthma incidences have increased significantly over the years in the country (Jindal, 2007). As per National family health survey of India, 2468 persons per 100,000 population are reported to be suffering from asthma, which is considerably higher in rural areas (2649 per 100,000 population) than in urban areas (1966 per 100,000 population) (MHFW, 2009). According to the global burden of asthma report (GINA), over 50 million suffer from asthma in Central and Southern Asia and an absolute 2% increase in the prevalence of asthma in India would result in an additional 20 million people with this disease (Moore and Peters, 2006; GIA, 2011).

The economic effect imposed by asthma is considerable. The Centers for Disease Control and Prevention projection for the cost of asthma in the United States in the year 2000 was $14.5 billion (Bukstein et al., 2006). Direct medical expenses from hospital care and physicians’ services cost more than $8 billion. Indirect costs, including lost school and work days and deaths from asthma, are estimated at $4.6 billion (NCSL,
2006). In addition, asthma can seriously impinge on quality of life in terms of restricted activities, avoidance of triggers, and fear of exacerbations. The cost of these issues cannot be estimated for individual patients with asthma.

2.4. Pathophysiology and Pathogenesis of Asthma

Airflow limitation in asthma is recurrent and caused by a variety of changes in the airway. These include:

2.4.1. Bronchoconstriction. In asthma, the dominant physiological event leading to clinical symptoms is airway narrowing and a subsequent interference with airflow. In acute exacerbations of asthma, bronchial smooth muscle contraction (bronchoconstriction) occurs quickly to narrow the airways in response to exposure to a variety of stimuli including allergens or irritants. Allergen-induced acute bronchoconstriction results from an IgE-dependent release of mediators from mast cells that includes histamine, tryptase, leukotrienes, and prostaglandins that directly contract airway smooth muscle (Busse and Lemanske, 2001). Aspirin and other nonsteroidal anti-inflammatory drugs can also cause acute airflow obstruction in some patients, and evidence indicates that this non-IgE-dependent response also involves mediator release from airway cells (Stevenson and Szczeklik, 2006). In addition, other stimuli (including exercise, cold air, and irritants) can cause acute airflow obstruction. The mechanisms regulating the airway response to these factors are less well defined, but the intensity of the response appears related to underlying airway inflammation. Stress may also play a role in precipitating asthma exacerbations. The mechanisms involved have yet to be established and may include enhanced generation of pro-inflammatory cytokines.

2.4.2. Airway oedema. As the disease becomes more persistent and inflammation more progressive, other factors further limit airflow (Fig. 2). These include oedema, inflammation, mucus hypersecretion and the formation of inspissated mucus plugs, as well as structural changes including hypertrophy and hyperplasia of the airway smooth muscle. These latter changes may not respond to usual treatment.
2.4.3. Airway hyper-responsiveness. Airway hyperresponsiveness - an exaggerated bronchoconstrictor response to a wide variety of stimuli - is a major, but not necessarily unique, feature of asthma. The degree to which airway hyperresponsiveness can be defined by contractile responses to challenges with methacholine correlates with the clinical severity of asthma. The mechanisms influencing airway hyperresponsiveness are multiple and include inflammation, dysfunctional neuroregulation, and structural changes; inflammation appears to be a major factor in determining the degree of airway hyperresponsiveness. Treatment directed toward reducing inflammation can reduce airway hyperresponsiveness and improve asthma control.

**FIGURE 2: FACTORS LIMITING AIRFLOW IN ACUTE AND PERSISTENT ASTHMA**

![Diagram of factors limiting airflow in acute and persistent asthma](image)

Key: GM-CSF, granulocyte-macrophage colony-stimulating factor; IgE, immunoglobulin E; IL-3, interleukin 3 (and similar); TNF-α, tumor necrosis factor-alpha

Source: Adapted and reprinted from The Lancet, 368, Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults, 780–93. Copyright (2006). with permission from Elsevier.
2.4.4. **Airway remodelling.** In some persons who have asthma, airflow limitation may be only partially reversible. Permanent structural changes can occur in the airway ([Fig. 2](#)); these are associated with a progressive loss of lung function that is not prevented by or fully reversible by current therapy. Airway remodelling involves an activation of many of the structural cells, with consequent permanent changes in the airway that increase airflow obstruction and airway responsiveness and render the patient less responsive to therapy (Holgate and Polosa, 2006). These structural changes can include thickening of the sub-basement membrane, sub epithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, blood vessel proliferation and dilation, and mucous gland hyperplasia and hypersecretion. Regulation of the repair and remodelling process is not well established, but both the process of repair and its regulation are likely to be key events in explaining the persistent nature of the disease and limitations to a therapeutic response. There are five features of airway remodelling, which are

1. Inflammation,
2. Mucus hypersecretion,
3. Subepithelial fibrosis,
4. Airway smooth muscle hypertrophy, and
5. Angiogenesis.

2.4.5. **Pathophysiologic mechanisms in the development of airway inflammation**

   Inflammation has a central role in the pathophysiology of asthma. As noted in the definition of asthma, airway inflammation involves an interaction of many cell types and multiple mediators with the airways that eventually results in the characteristic pathophysiological features of the disease: bronchial inflammation and airflow limitation that result in recurrent episodes of cough, wheeze, and shortness of breath. The processes by which these interactive events occur and lead to clinical asthma are still under investigation. Moreover, although distinct phenotypes of asthma exist (e.g., intermittent, persistent, exercise-associated, aspirin-sensitive, or severe asthma), airway inflammation remains a consistent pattern. The pattern of airway inflammation in asthma, however, does not necessarily vary depending upon disease severity, persistence, and duration of
disease. The cellular profile and the response of the structural cells in asthma are quite consistent.

2.4.5.1. Inflammatory Cells

2.4.5.1.1. Lymphocytes: An increased understanding of the development and regulation of airway inflammation in asthma followed the discovery and description of subpopulations of lymphocytes, T helper 1 cells and T helper 2 cells (Th1 and Th2), with distinct inflammatory mediator profiles and effects on airway function (Fig. 3). After the discovery of these distinct lymphocyte subpopulations in animal models of allergic inflammation, evidence emerged that, in human asthma, a shift, or predilection, toward the Th2-cytokine profile resulted in the eosinophilic inflammation characteristic of asthma (Cohn et al., 2004). In addition, generation of Th2 cytokines (e.g., interleukin-4 (IL-4), IL-5, and IL-13) could also explain the overproduction of IgE, presence of eosinophils, and development of airway hyperresponsiveness. There also may be a reduction in a subgroup of lymphocytes, regulatory T cells, which normally inhibit Th2 cells, as well as an increase in natural killer (NK) cells that release large amounts of Th1 and Th2 cytokines (Akbari et al., 2006; Larche et al., 2003). T lymphocytes, along with other airway resident cells, also can determine the development and degree of airway remodelling. Although it is an oversimplification of a complex process to describe asthma as a Th2 disease, recognizing the importance of n families of cytokines and chemokines has advanced our understanding of the development of airway inflammation (Barnes, 2002; Zimmermann et al., 2003).

2.4.5.1.2. Mast cells: Activation of mucosal mast cells releases bronchoconstrictor mediators (histamine, cysteinyl-leukotrienes, prostaglandin D2) (Boyce, 2003; Galli et al., 2005; Robinson, 2004). Although allergen activation occurs through high-affinity IgE receptors and is likely the most relevant reaction, sensitized mast cells also may be activated by osmotic stimuli to account for exercise-induced bronchospasm (EIB). Increased numbers of mast cells in airway smooth muscle may be linked to airway hyperresponsiveness (Brightling et al., 2002). Mast cells also can release a large number
of cytokines to change the airway environment and promote inflammation even though exposure to allergens is limited.

**FIGURE 3: AIRWAY INFLAMMATION**

Inhaled antigen activates mast cells and Th2 cells in the airway. They in turn induce the production of mediators of inflammation (such as histamine and leukotrienes) and cytokines including interleukin-4 and interleukin-5. Interleukin-5 travels to the bone marrow and causes terminal differentiation of eosinophils. Circulating eosinophils enter the area of allergic inflammation and begin migrating to the lung by rolling, through interactions with selectins, and eventually adhering to endothelium through the binding of integrins to members of the immunoglobulin superfamily of adhesion proteins: vascular-cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). As the eosinophils enter the matrix of the airway through the influence of various chemokines and cytokines, their survival is prolonged by interleukin-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF). On activation, the eosinophil releases inflammatory mediators, such as leukotrienes and granule proteins, to injure airway tissues. In addition, eosinophils can generate GM-CSF to prolong and potentiate their survival and contribution to persistent airway inflammation. MCP-1, monocyte chemotactic protein; and MIP-1α, macrophage inflammatory protein.

Reprinted by permission from Busse WW, Lemanske RF. *Advances in Immunology* N Engl J Med 2001; 344: 350-62. Copyright © 2001 Massachusetts Medical Society. All rights reserved.
2.4.5.1.3. Eosinophils: Increased numbers of eosinophils exist in the airways of most, but not all, persons who have asthma (Chu and Martin, 2001; Sampson, 2000; Williams, 2004). These cells contain inflammatory enzymes, generate leukotrienes, and express a wide variety of pro-inflammatory cytokines. Increases in eosinophils often correlate with greater asthma severity. In addition, numerous studies show that treating asthma with corticosteroids reduces circulating and airway eosinophils in parallel with clinical improvement. However, the role and contribution of eosinophils to asthma is undergoing a re-evaluation based on studies with an anti-IL-5 treatment that has significantly reduced eosinophils but did not affect asthma control (Leckie et al., 2000). Therefore, although the eosinophil may not be the only primary effector cell in asthma, it likely has a distinct role in different phases of the disease.

2.4.5.1.4. Neutrophils: Neutrophils are increased in the airways and sputum of persons who have severe asthma, during acute exacerbations, and in the presence of smoking. Their pathophysiological role remains uncertain; they may be a determinant of a lack of response to corticosteroid treatment (Fahy et al., 1995). The regulation of neutrophil recruitment, activation, and alteration in lung function is still under study, but leukotriene B₄ may contribute to these processes (Jatakanon et al., 1999; Wenzel et al., 1997; Wenzel, 2006).

2.4.5.1.5. Dendritic cells: These cells function as key antigen-presenting cells that interact with allergens from the airway surface and then migrate to regional lymph nodes to interact with regulatory cells and ultimately to stimulate Th₂ cell production from naïve T cells (Kuipers and Lambrecht, 2004).

2.4.5.1.6. Macrophages: Macrophages are the most numerous cells in the airways and also can be activated by allergens through low-affinity IgE receptors to release inflammatory mediators and cytokines that amplify the inflammatory response (Peters-Golden, 2004).
2.4.5.1.7. **Resident cells of the airway**: Airway smooth muscle is not only a target of the asthma response (by undergoing contraction to produce airflow obstruction) but also contributes to it (via the production of its own family of pro-inflammatory mediators). As a consequence of airway inflammation and the generation of growth factors, the airway smooth muscle cell can undergo proliferation, activation, contraction, and hypertrophy - events that can influence airway dysfunction of asthma.

2.4.5.1.8. **Epithelial cells.** Airway epithelium is another airway lining cell critically involved in asthma (Polito and Proud, 1998). The generation of inflammatory mediators, recruitment and activation of inflammatory cells, and infection by respiratory viruses can cause epithelial cells to produce more inflammatory mediators or to injure the epithelium itself. The repair process, following injury to the epithelium, may be abnormal in asthma, thus furthering the obstructive lesions that occur in asthma.

2.4.5.2. **Inflammatory Mediators**

2.4.5.2.1. **Chemokines** are important in recruitment of inflammatory cells into the airways and are mainly expressed in airway epithelial cells (Zimmermann et al., 2003). Eotaxin is relatively selective for eosinophils, whereas thymus and activation-regulated chemokines (TARCs) and macrophage-derived chemokines (MDCs) recruit Th2 cells. There is an increasing appreciation for the role this family of mediators has in orchestrating injury, repair, and many aspects of asthma.

2.4.5.2.2. **Cytokines** direct and modify the inflammatory response in asthma and likely determine its severity. Th2-derived cytokines include IL-5, which is needed for eosinophil differentiation and survival, and IL-4 which is important for Th2 cell differentiation and with IL-13 is important for IgE formation. Key cytokines include IL-1β and tumor necrosis factor-α (TNF-α), which amplify the inflammatory response, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which prolongs eosinophil survival in airways. Recent studies of treatments directed toward single cytokines (e.g., monoclonal antibodies against IL-5 or soluble IL-4 receptor) have not shown benefits in improving asthma outcomes.
2.4.5.2.3. **Cysteiny1-leukotrienes** are potent bronchoconstrictors derived mainly from mast cells. They are the only mediator whose inhibition has been specifically associated with an improvement in lung function and asthma symptoms (Busse, 1996; Leff, 2001). Recent studies have also shown leukotriene B	extsubscript{4} can contribute to the inflammatory process by recruitment of neutrophils (Gelfand and Dakhama, 2006).

2.4.5.2.4. **Nitric oxide** (NO): The L-arginine are the physiological precursors of endothelium-derived NO (Sakuma et al., 1988). Endothelial cells contain a cytosolic enzyme, NO synthase (NOS), which appears to be dependent on the presence of Ca	extsuperscript{2+} (Mayer et al., 1989). Calmodulin is required for Ca	extsuperscript{2+} to exert this modulatory effect (Busse & Mulsch, 1990). The enzyme converts L-arginine into an EDRF-like compound (Sakuma et al., 1988) in a NADPH-dependent manner (Mayer et al., 1989). This enzyme is involved in the formation of NO and L-citrulline from L-arginine (Palmer et al., 1988; Robbins et al., 1993). L-Arginine, O	extsubscript{2}, and NADPH are co-substrates; flavin adenine dinucleotide, flavin adenine dinucleotide, heme, and tetrahydrobiopterin and calmodulin are cofactors or prosthetic groups of this enzyme (Nevin and Broadley, 2002). Isoforms of NOS have been reviewed by Forstermann et al. (1991). The endothelial NOS (eNOS), also known as NOS-3, is Ca	extsuperscript{2+}/calmodulin-dependent and is a constitutive enzyme (cNOS). cNOS and/or its isoforms may also be found in neuronal NOS (nNOS), also known as NOS-1 (Bredt et al., 1990) and epithelial cells (Asano et al., 1994). The cNOS enzyme is short lived and produces only picomolar quantities of NO. An inducible NOS (iNOS), also known as NOS-2, is found in neutrophils (McCall et al., 1991), eosinophils (Zanardo et al., 1997; Iijima et al., 2001), macrophages (Jorens et al., 1991), and epithelial cells (Asano et al., 1994). Unlike cNOS, it produces nanomolar quantities of NO, is long lived, and is Ca	extsuperscript{2+}/calmodulin independent. It is induced by cytokines [interleukin (IL)-1b, tumour necrosis factor (TNF)-a, interferon (IFN)-g] and endotoxin, and its induction is inhibited by glucocorticosteroids (Hamid et al., 1993; Moncada et al., 1991; Morris & Billiar, 1994; Guo et al., 2000). The level of NO in exhaled air is used as a measure of iNOS-derived NO in the airways. Nitric oxide is produced predominantly from the action of inducible NO synthase in airway epithelial cells; it is a potent...
vasodilator (Deykin et al., 2002; Strunk et al., 2003). Measurements of fractional exhaled NO (FeNO) may be useful for monitoring response to asthma treatment because of the purported association between FeNO and the presence of inflammation in asthma (Green et al., 2002).

The absence of constitutive iNOS-derived NO production in epithelial cells of cystic fibrosis airways may play a role in Na⁺ hyperabsorption (Kelley & Drumm, 1998; Meng et al., 1998), leading to reduced water secretion and increased mucus viscosity. Increased iNOS expression, however, has been demonstrated in the subepithelial tissues of cystic fibrosis lungs (Meng et al., 1998). Corticosteroid treatment has been shown to reduce exhaled NO levels in cystic fibrosis patients, suggesting that iNOS-derived NO is produced in cystic fibrosis (Linnane et al., 2001). In the guinea-pig tracheal preparation, however, it has been demonstrated that TNF-α, histamine, O₂⁻, and PAF stimulated the secretion of mucin (a glycoprotein), the principal constituent of mucus. This effect was inhibited by L-NMMA, but not D-NMMA, indicating that mucin secretion may be increased by NO (Adler et al., 1995). An increase in eosinophil numbers in the bronchoalveolar lavage (BAL) of asthmatics and after antigen challenge has been demonstrated (Wardlaw et al., 1988). Airway hyper reactivity (AHR) and an increase in eosinophil numbers in the BAL is also noted following antigen challenge (Danahay & Broadley, 1998). The antigen-challenged guinea-pig, therefore, provides a useful model of human asthma. It is used to determine the under-lying physiology of asthma and to elucidate the pharmacology and efficacy of anti-asthma drugs.

2.4.5.2.5. Immunoglobulin E: IgE is the antibody responsible for activation of allergic reactions and is important to the pathogenesis of allergic diseases and the development and persistence of inflammation. IgE attaches to cell surfaces via a specific high-affinity receptor. The mast cell has large numbers of IgE receptors; these, when activated by interaction with antigen, release a wide variety of mediators to initiate acute bronchospasm and also to release pro-inflammatory cytokines to perpetuate underlying airway inflammation (Boyce, 2003; Sporik et al., 1995). Other cells, basophils, dendritic cells, and lymphocytes also have high-affinity IgE receptors.
The development of monoclonal antibodies against IgE has shown that the reduction of IgE is effective in asthma treatment (Busse et al., 2001; Holgate et al., 2005). These clinical observations further support the importance of IgE to asthma.

2.4.6. Pathogenesis

What initiates the inflammatory process in the first place and makes some persons susceptible to its effects is an area of active investigation. There is not yet a definitive answer to this question, but new observations suggest that the origins of asthma primarily occur early in life. The expression of asthma is a complex, interactive process that depends on the interplay between two major factors - host factors (particularly genetics) and environmental exposures that occur at a crucial time in the development of the immune system (Fig. 4).

**Figure 4: Host Factors and Environmental Exposures**

- **Genetic Factors**
  - Cytokine response profiles

- **Environment**
  - Allergens
  - Pollution
  - Infections
  - Microbes
  - Stress

- **Age**

- **Altered Innate and Adaptive Immune Responses**

- **Lower Airway Targeting**
  - LRI
  - RSV/PIV
  - Adenovirus
  - Chlamydia
  - Mycoplasma

**Persistent wheezing and asthma**

Key: LRI, lower respiratory illnesses; RSV, respiratory syncytial virus; PIV, parainfluenza virus

2.4.6.1. Host Factors

2.4.6.1.1. Innate immunity. There is considerable interest in the role of innate and adaptive immune responses associated with both the development and regulation of
inflammation (Eder et al., 2006). In particular, research has focused on an imbalance between Th1 and Th2 cytokine profiles and evidence that allergic diseases, and possibly asthma, are characterized by a shift toward a Th2 cytokine-like disease, either as overexpression of Th2 or under expression of Th1 (Fig. 5). Airway inflammation in asthma may represent a loss of normal balance between two “opposing” populations of Th lymphocytes. Two types of Th lymphocytes have been characterized: Th1 and Th2. Th1 cells produce IL-2 and interferon-γ (IFN-γ), which are critical in cellular defence mechanisms in response to infection. Th2, in contrast, generates a family of cytokines (IL-4, -5, -6, -9, and -13) that can mediate allergic inflammation. The current “hygiene hypothesis” of asthma illustrates how this cytokine imbalance may explain some of the dramatic increases in asthma prevalence in westernized countries.

**FIGURE 5: CYTOKINE BALANCE**

Numerous factors, including alterations in the number or type of infections early in life, the widespread use of antibiotics, adoption of the Western lifestyle, and repeated exposure to allergens, may affect the balance between Th1-type and Th2-type cytokine responses and increase the likelihood that the immune response will be dominated by Th2 cells and thus will ultimately lead to the expression of allergic diseases such as asthma.

Reprinted by permission from Busse WW, Lemanske RF. *Advances in Immunology N Engl J Med* 2001; 344: 350-62. Copyright © 2001 Massachusetts Medical Society. All rights reserved.

This hypothesis is based on the assumption that the immune system of the newly born is skewed toward Th2 cytokine generation. Following birth, environmental stimuli
such as infections will activate Th\(_1\) responses and bring the Th\(_1\)/Th\(_2\) relationship to an appropriate balance. Evidence indicates that the incidence of asthma is reduced in association with certain infections (\textit{M. tuberculosis}, measles, or hepatitis A), exposure to other children (e.g., presence of older siblings and early enrolment in childcare), and less frequent use of antibiotics (Eder \textit{et al.}, 2006; Gern \textit{et al.}, 1999; Gern and Busse, 2002; Horwood \textit{et al.}, 1985; Sears \textit{et al.}, 2003). Furthermore, the absence of these lifestyle events is associated with the persistence of a Th\(_2\) cytokine pattern. Under these conditions, the genetic background of the child who has a cytokine imbalance toward Th\(_2\) will set the stage to promote the production of IgE antibodies to key environmental antigens, such as house-dust mite, cockroach, \textit{Alternaria}, and possibly cat. Therefore, a gene-by-environment interaction occurs in which the susceptible host is exposed to environmental factors that are capable of generating IgE, and sensitization occurs. Precisely why the airways of some individuals are susceptible to these allergic events has not been established.

There also appears to be a reciprocal interaction between the two subpopulations in which Th\(_1\) cytokines can inhibit Th\(_2\) generation and vice versa. Allergic inflammation may be the result of an excessive expression of Th\(_2\) cytokines. Alternatively, recent studies have suggested the possibility that the loss of normal immune balance arises from a cytokine dysregulation in which Th\(_1\) activity in asthma is diminished. The focus on actions of cytokines and chemokines to regulate and activate the inflammatory profile in asthma has provided on-going and new insight into the pattern of airway injury that may lead to new therapeutic targets.

\textbf{2.4.6.1.2. Genetics.} It is well recognized that asthma has an inheritable component to its expression, but the genetics involved in the eventual development of asthma remain a complex and incomplete picture (Holgate, 1999; Ober, 2005). To date, many genes have been found that either are involved in or linked to the presence of asthma and certain of its features. The complexity of their involvement in clinical asthma is noted by linkages to certain phenotypic characteristics, but not necessarily the pathophysiologic disease process or clinical picture itself. The role of genetics in IgE production, airway hyperresponsiveness, and dysfunctional regulation of the generation of inflammatory
mediators (such as cytokines, chemokines, and growth factors) has appropriately captured much attention. In addition, studies are investigating genetic variations that may determine the response to therapy. The relevance of polymorphisms in the beta-adrenergic and corticosteroid receptors in determining responsiveness to therapies is of increasing interest, but the widespread application of these genetic factors remains to be fully established.

2.4.6.1.3. Sex. In early life, the prevalence of asthma is higher in boys. At puberty, however, the sex ratio shifts, and asthma appears predominantly in women (Horwood et al., 1985). How specifically sex and sex hormones, or related hormone generation, are linked to asthma has not been established, but they may contribute to the onset and persistence of the disease.

2.4.6.1.4. Environmental factors: Two major environmental factors have emerged as the most important in the development, persistence, and possibly severity of asthma: airborne allergens and viral respiratory infections. In the susceptible host, and at a critical time of development (e.g., immunological and physiological), both respiratory infections and allergens have a major influence on asthma development and its likely persistence. It is also apparent that allergen exposure, allergic sensitization, and respiratory infections are not separate entities but function interactively in the eventual development of asthma.

2.4.6.1.5. Allergens. The role of allergens in the development of asthma has yet to be fully defined or resolved, but it is obviously important. Sensitization and exposure to house-dust mite and Alternaria are important factors in the development of asthma in children. Early studies showed that animal danders, particularly dog and cat, were associated with the development of asthma. Recent data suggest that, under some circumstances, dog and cat exposure in early life may actually protect against the development of asthma. The determinant of these diverse outcomes has not been established. Studies to evaluate house-dust mite and cockroach exposure have shown that the prevalence of sensitization and subsequent development of asthma are linked (Huss et al., 2001; Sporik et al., 1990; Wahn et al., 1997). Exposure to cockroach allergen, for
example, a major allergen in inner-city dwellings, is an important cause of allergen sensitization, a risk factor for the development of asthma (Rosenstreich et al., 1997). In addition, allergen exposure can promote the persistence of airway inflammation and likelihood of an exacerbation (Fig. 6).

**Figure 6:** Simplified view of allergic inflammation in the airways: Asthma is an episodic narrowing of the bronchi thought to be caused by an underlying chronic inflammatory disorder. In allergic asthma, inhaled allergen initiates the inflammatory response by interacting with IgE bound to mast cells and basophils. This leads to a cascade of events involving other immune cells and resulting in the release of numerous inflammatory mediators into the interstitial space, where they influence the growth and function of cell types within the airway wall. The drugs available for the treatment of asthma are targeted at inhibiting the inflammatory responses and/or relaxing the bronchial smooth muscle. Letters denote the putative sites of action for the various classes of drugs used in treating asthma. β, β₂ adrenergic agonists; cs, corticosteroids; l, leukotriene modifiers; m, muscarinic receptor antagonists; cr, cromolyn; t, theophylline; aI, anti-IgE therapy. The sunburst (🌞) symbolizes an allergen.
2.4.6.1.6. **Respiratory infections.** During infancy, a number of respiratory viruses have been associated with the inception or development of the asthma. In early life, respiratory syncytial virus (RSV) and parainfluenza virus in particular, cause bronchiolitis that parallels many features of childhood asthma (Gern and Busse, 2002; Sigurs et al., 2000). A number of long-term prospective studies of children admitted to hospital with documented RSV have shown that approximately 40 per cent of these infants will continue to wheeze or have asthma in later childhood (Sigurs et al., 2000). Symptomatic rhinovirus infections in early life also are emerging as risk factors for recurrent wheezing. On the other hand, evidence also indicates that certain respiratory infections early in life - including measles and even RSV (Stein et al., 1999) or repeated viral infections (other than lower respiratory tract infections) (Illi et al., 2001; Shaheen et al., 1996) - can protect against the development of asthma. The “hygiene hypothesis” of asthma suggests that exposure to infections early in life influences the development of a child’s immune system along a “nonallergic” pathway, leading to a reduced risk of asthma and other allergic diseases. Although the hygiene hypothesis continues to be investigated, this association may explain observed associations between large family size, later birth order, day-care attendance, and a reduced risk of asthma (Eder et al., 2006; Illi et al., 2001).

The influence of viral respiratory infections on the development of asthma may depend on an interaction with atopy. The atopic state can influence the lower airway response to viral infections, and viral infections may then influence the development of allergic sensitization. The airway interactions that may occur when individuals are exposed simultaneously to both allergens and viruses are of interest but are not defined at present.

2.4.6.1.7. **Other environmental exposures.** Tobacco smoke, air pollution, occupations, and diet have also been associated with an increased risk for the onset of asthma, although the association has not been as clearly established as with allergens and respiratory infections (Malo et al., 2004; Strachan and Cook, 1998a; Strachan and Cook, 1998b).
In utero exposure to environmental tobacco smoke increases the likelihood for wheezing in the infant, although the subsequent development of asthma has not been well defined. In adults who have asthma, cigarette smoking has been associated with an increase in asthma severity and decreased responsiveness to inhaled corticosteroids (ICSs) (Dezateux et al., 1999).

The role of air pollution in the development of asthma remains controversial and may be related to allergic sensitization (American Thoracic Society, 2000). One recent epidemiologic study showed that heavy exercise (three or more team sports) outdoors in communities with high concentration of ozone was associated with a higher risk of asthma among school-age children (McConnell et al., 2002). The relationship between increased levels of pollution and increases in asthma exacerbations and emergency care visits has been well documented.

An association of low intake of antioxidants and omega-3 fatty acids has been noted in observational studies, but a direct link as a causative factor has not been established. Increasing rates of obesity have paralleled increasing rates in asthma prevalence, but the interrelation is uncertain (Ford, 2005). Obesity may be a risk factor for asthma due to the generation of unique inflammatory mediators that lead to airway dysfunction.

In summary, our understanding of asthma pathogenesis and underlying mechanisms now includes the concept that gene-by-environmental interactions are critical factors in the development of airway inflammation and eventual alteration in the pulmonary physiology that is characteristic of clinical asthma.

2.5. Pharmacotherapy for Asthma

Early anti-asthma regimens largely aimed at relief of symptoms or modification of external factors, applying plant extracts, life-style adaptations, surgery, or hypnosis for the relief of asthma (Hofbauer, 1931; Belcher, 1961). Apart from these treatment options, in the pre-inhaler era, early pharmacotherapy consisted of inhaling the smoke of the so-called “asthma cigarettes”, containing various relieving compounds including atropine, belladonna, menthol, morphine or cocaine (Brown, 1917). Furthermore, since asthma was considered to have a psychological origin, psychopharmacca such as chlorpromazine were
prescribed (Crocket, 1956). However, none of these ‘‘control’’-aiming therapies proved effective, while some treatment options such as early immunotherapeutic vaccines and the use of opiates appeared hazardous (Brown, 1917). While at that time asthma therapy was predominantly based on trial and error, targeting inflammatory mechanisms came in the course of mainly the second half of the 20th century with the discovery of pathological, pathophysiological and immunological substrates following the invention and/or refinement of spirometry, flexible bronchoscopy and immunological techniques.

Aerosol Delivery of Drugs

Topical delivery of drugs to the lungs can be accomplished by use of aerosols. In theory, this approach should produce a high local concentration in the lungs with a low systemic delivery, thereby significantly minimizing systemic side effects. The drugs used most commonly in the treatment of asthma, $\beta_2$ adrenergic receptor agonists and glucocorticoids, have potentially serious side effects when delivered systemically. Since the pathophysiology of asthma appears to involve the respiratory tract alone, the advantages of aerosol treatments with limited systemic effects are substantial. Indeed, in clinical practice, probably >90% of asthmatic patients who are capable of manipulating inhaler devices can be managed by aerosol treatments alone. Because of the specialized nature of aerosol delivery and the substantial effects that these systems have on the therapeutic index, the principles of this delivery method are important to review. Even under ideal circumstances, only a small fraction of the aerosolized drug is deposited in the lungs, typically 2–10%. Most of the remainder is swallowed. To minimize systemic effects, an aerosolized drug should be either poorly absorbed from the gastrointestinal (GI) system or rapidly inactivated via first-pass hepatic metabolism. Furthermore, any manoeuvres that increase deposition in the lungs or decrease the percentage of drug reaching the GI system should enhance the desired effects and reduce undesired systematic effects. Because >50% of patients using inhalers do not use proper technique and thus deposit too small a fraction of inhaled drug into the lungs, patients should be counselled in the proper use of an inhaler (Brunton et al., 2008).
2.5.1. β2-adrenergic receptor agonists

Although the use of adrenal substances in asthma dates back to 1900 (Solis-Cohen, 1900), in the 1940s epinephrine (or adrenaline) became the standard bronchodilator therapy for the treatment of acute asthma (Sears and Lotvall, 2005). However, due to its non-specific mechanism of action, the use of epinephrine was complicated by several—mostly cardiovascular—side effects (Waldeck, 2002). A breakthrough came in the beginning of 1960s with the discovery of the adrenergic receptor subsets, yielding the α and β receptor, with a further subdivision into the β₁ receptor, mainly located in the heart and intestinal smooth muscle, and into the β₂ subset on bronchial and uterus smooth muscle (Lands et al., 1967; Ahlquist, 1948). Isoprenaline was the first agonist interacting with β adrenergic receptors, while salbutamol, and later on terbutaline, were the first agonists with a higher specificity for β₂ adrenergic receptors (Bergman et al., 1969; Brittain et al., 1968). Soon after its development in 1968, salbutamol rapidly became—and still is—the most widely used fast-acting reliever for asthma (GINA, 2005). The success of salbutamol initiated the development of several other short acting β₂ agonists (SABAs), like carbuterol, clenbuterol and fenoterol, with a duration of action up to 6 h (Engelhardt, 1976; O’Donnell, 1970). The final step for this class of drugs came in 1980s with the development of long-acting β₂ agonists (LABAs). Salmeterol was first launched with a duration of action up to 12 h (Johnson, 1995), followed by formoterol. The latter drug combines long lasting bronchodilator effects (>12 h) with a fast onset of action, similar to salbutamol (Fig. 7) (Bartow et al., 1998). Currently, several novel LABAs are being developed with a duration of action up to 24 h, creating the possibility of once daily dosing (Cazzola et al., 2005).

The mechanism of action of β₂ agonists is predominantly bronchodilator through airway smooth muscle relaxation, despite modest anti-inflammatory activity encountered in some studies (Li et al., 1999; Reid et al., 2003). Formerly prescribed as “4–6 times daily” maintenance therapy, current guidelines now recommend SABAs on “as needed” basis (GINA, 2005). This change of view came with the growing understanding that airway inflammation is a key feature of asthma and that targeting airway inflammation should be the primary goal of asthma treatment, as different from “symptoms control” only. Moreover, several studies in asthma provided evidence that maintenance therapy
with both SABAs and LABAs — in or without combination with ICS (inhaled corticosteroids) — is associated with potential masking of the airway inflammation (McIvor, 1998; Sears, 1990). In addition, despite concomitant use of ICS, maintenance therapy with LABAs has been shown to induce tolerance to its broncho-protective effects and cross-tolerance to the bronchodilator effects of SABAs (Cheung et al., 1992; Haney and Hancox, 2005; Van der Woude, 2001; Van Veen, 2003). Finally, data from several studies show that patients who are homozygous for arginine (Arg/Arg) as opposed to glycine (Gly/Gly) at the 16th amino acid position of the β2 adrenergic receptor have an impaired therapeutic response to β2 agonists (Israel et al., 2004; Wechsler, 2006). In these patients, long-term treatment with albuterol has been found to be associated with significant decrease in lung function over time (Israel et al., 2004). Some of these deleterious effects during long-term use of LABAs with or without an adequate dose of ICS may have resulted in an increased morbidity and even asthma deaths in the recently reported SMART study (Nelson et al., 2006). Present guidelines, therefore, favour maintenance therapy with LABAs only in combination with appropriate doses of corticosteroids in the more severe disease (treatment steps 3–5) (GINA, 2005; Hasford and Virchow, 2006).

**Figure 7:** Structure and mechanism of action of the most widely used β-agonists developed in the last century.

<table>
<thead>
<tr>
<th>Name</th>
<th>Adrenaline</th>
<th>Isoprenaline</th>
<th>Salbutamol</th>
<th>Salmeterol</th>
<th>Formoterol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specificity</strong></td>
<td>n and β agonist</td>
<td>β1 and β2 agonist</td>
<td>Selective β2 agonist</td>
<td>Selective β2 agonist</td>
<td>Selective β2 agonist</td>
</tr>
<tr>
<td><strong>Mechanism of action</strong></td>
<td>Fast onset and short duration</td>
<td>Fast onset and short duration</td>
<td>Fast onset and short duration</td>
<td>Slow onset and long duration</td>
<td>Fast onset and long duration</td>
</tr>
<tr>
<td><strong>Preferred route of administration</strong></td>
<td>Injected or nebulized</td>
<td>Sublingual or nebulized</td>
<td>MDI or nebulized</td>
<td>MDI or DPI</td>
<td>MDI or DPI</td>
</tr>
<tr>
<td><strong>Side-effects</strong></td>
<td>Cardiotoxicity, Hypertension, Tachycardia, Tremor</td>
<td>Tachycardia, Palpitations, Tremor</td>
<td>Tremor</td>
<td>Tremor</td>
<td>Tremor</td>
</tr>
</tbody>
</table>
**Mechanism of action and use in asthma:** The β adrenergic receptor agonists available for the treatment of asthma are selective for the β₂ receptor subtype. With few exceptions, they are delivered directly to the airways via inhalation. The agonists can be classified as short- or long-acting. Short-acting agonists are used only for symptomatic relief of asthma, whereas long acting agonists are used prophylactically in the treatment of the disease (Brunton *et al.*, 2008).

The mechanism of the anti-asthmatic action of β adrenergic receptor agonists is undoubtedly linked to the direct relaxation of airway smooth muscle and consequent bronchodilation. Although human bronchial smooth muscle receives little or no sympathetic innervation, it nevertheless contains large numbers of β₂ adrenergic receptors. Stimulation of these receptors activates the GS-adenyl cyclase–cyclic AMP pathway with a consequent reduction of in smooth muscle tone. β₂ Adrenergic receptor agonists also increase the conductance of Ca²⁺-sensitive K⁺ channels in airway smooth muscle, leading to membrane hyperpolarization and relaxation (Brunton *et al.*, 2008).

There are β₂ adrenergic receptors on cell types in the airways other than bronchial smooth muscle. Of particular interest, stimulation of β₂ adrenergic receptors inhibits the function of numerous inflammatory cells, including mast cells, basophils, eosinophils, neutrophils, and lymphocytes. In general, stimulating β₂ adrenergic receptors in these cells increase intracellular cyclic AMP, ultimately inhibiting the release of inflammatory mediators and cytokines (Brunton *et al.*, 2008).

Long-term exposure to β₂ agonists may desensitize some of these receptor-response pathways; thus, there is little evidence that these drugs, used chronically, reduce airway inflammation (Brunton *et al.*, 2008).

**Long-Acting β Adrenergic Receptor Agonists:** Salmeterol xinafoate (SEREVENT) and formoterol (FORADIL) are long-lasting adrenergic agents with very high selectivity for the β₂ receptor subtype; bronchodilation lasts over 12 hours. The mechanism underlying the extended duration of action of salmeterol appears related to its high lipophilicity. After binding the receptor, the less lipophilic, short-acting agonists are removed rapidly from the receptor environment by diffusion in the aqueous phase, while salmeterol
persists in the membrane and only slowly dissociates from the environment of the receptor (Brunton et al., 2008).

**Oral Therapy with β Adrenergic Receptor Agonists**: The use of orally administered β adrenergic agonists for bronchodilation has not gained wide acceptance largely because of the risk of adverse effects, especially tremulousness, muscle cramps, cardiac tachyarrhythmias, and metabolic disturbances. Brief courses of oral therapy (albuterol or metaproterenol syrups) are well tolerated and effective in young children (<5 years old) who cannot manipulate metered-dose inhalers yet have occasional wheezing with viral upper respiratory infections. In some patients with severe asthma exacerbations, any aerosol, whether delivered via a metered-dose inhaler or a nebulizer, can worsen cough and bronchospasm owing to local irritation. In this setting, oral therapy with β₂ adrenergic agonists (e.g., albuterol, metaproterenol, or terbutaline tablets) can be effective. However, the frequency of adverse systemic effects with orally administered agents is higher in adults than in children (Brunton et al., 2008).

2.5.2. Glucocorticoids

The first reports of corticosteroids in the treatment of asthma date back some 50 years ago. At that time, these drugs were administered either intravenously or orally with good therapeutic results (Bordley and Carey, 1949; Schwartz, 1951). However, the initial enthusiasm was dampened by the serious side effects accompanying long-term use of systemic corticosteroids, confining their systemic application to severe cases and exacerbations only (GINA, 2005). In the early 1970s, the first topically active, aerosolized corticosteroid, beclomethasone dipropionate (BDP), was introduced into clinical practice (Brown et al., 1972; Clark, 1972). This ICS showed effectivity in the treatment of asthma without the adverse effects associated with prednisone (Anonymous, 1975; Anonymous, 1976).

Interestingly, the widespread use of ICS started some 20 years later, most likely as a result of the increasing evidence that asthma is an inflammatory disease and the effect of this paradigm-switch on the concurrent guidelines for asthma treatment (GINA, 2005; Stafford et al., 2003; McFadden, 2004).
Corticosteroids are currently the most effective anti-inflammatory drugs for the treatment of persistent asthma. Especially, prolonged treatment with ICS has been shown to result in sustained improvement of symptoms and lung function in combination with a decrease in rescue medications, exacerbations and airway hyperresponsiveness in adults and children (Yeadon and Diamant, 2000; Sont et al., 1999). These effects are mediated through the intracellular glucocorticoid receptor in a large variety of (inflammatory) cells, resulting in both suppression of inflammatory gene transcription and activation of anti-inflammatory gene transcription (Barnes, 2002; Barnes, 2006).

In the past two decades, modification of the initial compounds and inhalers increased the potency and first-pass metabolism in combination with an improved lung deposition. Presently, available ICS differ little in clinical efficacy and side effects, fluticasone and budesonide being the most widely used alone or in combination with a LABA in one inhaler device (Fig. 8). Being a pro-drug, the recently launched ciclesonide combines the advantages of a prolonged activity (once daily use) with still less (local and systemic) side effects (Nave et al., 2005; Richter et al., 2005; Zietkowski et al., 2006) (Fig. 8).

However, despite their established clinical effectivity, even prolonged treatment with high doses of ICS can neither fully reverse all chronic aspects of the airway inflammation nor cure the disease (Dworski and Sheller, 1992; Ward and Walters, 2005).

Systemic glucocorticoids long have been used to treat severe chronic asthma or severe acute exacerbations of asthma. The development of aerosol formulations significantly improved the safety of glucocorticoid treatment, allowing it to be used for moderate asthma. Asthmatic subjects who require inhaled β2 adrenergic agonists four or more times weekly are viewed as candidates for inhaled glucocorticoids (Brunton et al., 2008).

**Mechanism of glucocorticoid action in asthma:** Glucocorticoids do not directly relax airway smooth muscle and thus have little effect on acute bronchoconstriction. Their anti-inflammatory effects in asthma include modulation of cytokine and chemokine production; inhibition of eicosanoid synthesis; marked inhibition of accumulation of basophils, eosinophils, and other leukocytes in lung tissue; and decreased vascular
permeability. Because of their profound and generalized anti-inflammatory actions, glucocorticoids are the most effective drugs used in the treatment of asthma (Brunton et al., 2008).

**Figure 8:** Structure and major advantages and disadvantages of the most widely used corticosteroids.

<table>
<thead>
<tr>
<th>Name</th>
<th>Beclomethasone</th>
<th>Triamcinolone</th>
<th>Budesonide</th>
<th>Fluticasone</th>
<th>Ciclesonide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preferred administration</strong></td>
<td>2-4 dd via MDI/DPI</td>
<td>2-4 dd via MDI/DPI</td>
<td>1-2 dd via MDI/DPI</td>
<td>1-2 dd via MDI/DPI</td>
<td>1 dd via MDI</td>
</tr>
</tbody>
</table>
| **Advantages** | -Proven clinical efficacy 
-Low-priced 
-Moderate oral bioavailability | -Proven clinical efficacy 
-Low-priced | -Proven clinical efficacy 
-Moderate oral bioavailability 
-Available as combination therapy | -Proven clinical efficacy 
-Low oral bioavailability 
-Available as combination therapy 
-Most potent | -Low oral bioavailability 
-On site activation 
-Once daily dosing |
| **Disadvantages** | -Not available as combination therapy | -High oral bioavailability 
-Not available as combination therapy 
-Least potent | -Short lung residence time | -Greatest risk of HPA axis suppression | -Limited clinical experience 
-Not available as combination therapy |

**Inhaled Glucocorticoids:** A major advance in asthma therapy was the development of inhaled glucocorticoids that targeted the drug directly to the relevant site of inflammation. These formulations greatly enhance the therapeutic index of the drugs, substantially diminishing the number and degree of side effects without sacrificing clinical utility. There are five glucocorticoids available in the U.S. for inhalation therapy: beclomethasone dipropionate (BECLOVENT, VANCERIL), triamcinolone acetonide (AZMACORT), flunisolide (AEROBID), budesonide (PULMICORT), and fluticasone propionate (FLOVENT); all are effective in controlling asthma at the appropriate doses.
Inhaled glucocorticoids are used prophylactically to control asthma rather than acutely to reverse asthma symptoms. As with all prophylactic therapies, compliance is a significant concern. Issues relating to drug compliance, therefore, become relevant when choosing among the various steroid formulations. The newer, highly potent drugs (e.g., fluticasone, flunisolide, and budesonide) can be effective with as little as 1–2 puffs administered twice or even once daily. Many patients prefer this more convenient dosage regimen, providing improved compliance and better asthma control. The appropriate dose of steroid must be determined empirically. Important variables that influence the effective dose include the severity of disease, the particular steroid used, and the device used for drug delivery, which determines the actual quantity of drug delivered to the lungs. Maximal improvement in lung function may require several weeks of treatment (Brunton et al., 2008).

**Systemic Glucocorticoids:** Systemic glucocorticoids are used for acute asthma exacerbations and chronic severe asthma. Substantial doses of glucocorticoids (e.g., 40–60 mg prednisone or equivalent daily for 5 days; 1–2 mg/kg/day for children) often are used to treat acute exacerbations of asthma. Although an additional week at somewhat reduced dosage may be required, the steroids can be withdrawn once control of the symptoms by other medications has been restored; any suppression of adrenal function dissipates within 1–2 weeks. More protracted bouts of severe asthma may require longer treatment and slower tapering of the dose to avoid exacerbating asthma symptoms and suppressing pituitary/adrenal function. Previously, alternate-day therapy with oral prednisone was employed commonly in persistent asthma. Now, most patients with asthma are better treated with inhaled glucocorticoids (Brunton et al., 2008).

2.5.3. Leukotriene receptor antagonists and leukotriene synthesis inhibitors

In parallel with the discovery of other components of the airway inflammation in asthma, in 1940 Kellaway and Trethewie discovered the “slow reacting substance of anaphylaxis”, which appeared to constitute of leukotrienes (Feldberg et al., 1938; Samuelsson, 1983). In the last two decades of the 20th century, a large array of studies on leukotrienes have been conducted both in healthy volunteers and in patients with asthma.
Apart from their broncho-active properties, leukotrienes appeared to mimic several other features of asthma, including airway hyperresponsiveness, airway inflammation and airway remodeling (Diamant and Sampson, 1999). The discovery of leukotrienes introduced a new focus in asthma research and the subsequent development of anti-mediator drugs. Unlike other anti-mediator drugs (including antihistamines, platelet activating factor and prostaglandin inhibitors), potent anti-leukotrienes effectively reduced several features of asthma in both adults and children (Bjermer and Diamant, 2004; Lane, 1998). In the second half of the 1990s, the leukotriene synthesis inhibitor, zileuton, and the leukotriene receptor antagonists (LTRAs), pranlukast, zafirlukast and montelukast entered into clinical practice: a novel class of anti-asthma therapy since 25 years (Lane, 1998). Although not quite as potent as corticosteroids, LTRAs combine anti-inflammatory mainly anti-eosinophilic activity with mild, bronchodilator properties, based on antagonism of cysteinyl leukotrienes (CysLTs) at the CysLT1-receptor within the airways and on inflammatory cells (Diamant and Sampson, 1999; Bjermer and Diamant, 2004; Lane, 1998; Ind, 1996). Presently, application of LTRA has been approved for both adults and children in most steps of the asthma management plan, mainly as add-on medication, with a recent extension to virally induced bronchoconstriction and asthma with allergic rhinitis (GINA, 2005; Bisgaard et al., 2005; Bjermer et al., 2000; Diamant and Van der, 2005; Virchow and Bachert, 2006). Another more specific application for LTRA is aspirin-induced asthma, recently referred to as Aspirin-Exacerbated Airway Disease-AERD (Ind, 1996). Interestingly, in this journal Szczechlik and Nizankowska reported not only patients with an increased sensitivity to aspirin (aspirin-induced asthma), but also a small number of patients with asthma who had a bronchodilator response to aspirin (Szczechlik and Nizankowska, 1983).

Zafirlukast (ACCOLATE) and montelukast (SINGULAIR) are leukotriene receptor antagonists. Zileuton (ZYFLO) is an inhibitor of 5-lipoxygenase, which catalyses the formation of leukotrienes from arachidonic acid (Brunton et al., 2008).

**Mechanism of action in asthma:** Leukotriene-modifying drugs act either as competitive antagonists of leukotriene receptors or by inhibiting the synthesis of leukotrienes (Brunton et al., 2008).
Leukotriene-Receptor Antagonists: Cysteiny1 leukotrienes (CysLTs) include leukotriene C4 (LTC4), leukotriene D4 (LTD4), and leukotriene E4 (LTE4). All the CysLTs are potent constrictors of bronchial smooth muscle. On a molar basis, LTD4 is \(~1000\) times more potent than is histamine as a bronchoconstrictor. The receptor responsible for the bronchoconstrictor effect of leukotrienes is the CysLT1 receptor. Although each of the CysLTs is an agonist at the CysLT1 receptor, LTE4 is less potent than either LTC4 or LTD4. Zafirlukast and montelukast are selective high-affinity competitive antagonists for the CysLT1 receptor. Pranlukast is another CysLT1-receptor antagonist used in some countries in the treatment of asthma, but it is not approved for use in the U.S. Inhibition of CysLT-induced bronchial smooth muscle contraction likely is involved in the therapeutic effects of these agents to relieve the symptoms of asthma (Brunton et al., 2008).

Leukotriene-Synthesis Inhibitors: The formation of leukotrienes depends on lipoxygenation of arachidonic acid by 5-lipoxygenase. Zileuton is a potent and selective inhibitor of 5-lipoxygenase activity and thus blocks the formation of all 5-lipoxygenase products. Thus, in addition to inhibiting the formation of the cys-LTs, zileuton also inhibits the formation of leukotriene B4 (LTB4), a potent chemotactic autacoid, and other eicosanoids that depend on leukotriene A4 (LTA4) synthesis. Logically, the therapeutic effects of a 5-lipoxygenase inhibitor would include all those observed with the CysLT-receptor antagonists, as well as other effects that may result from inhibiting the formation of LTB4 and other 5-lipoxygenase products (Brunton et al., 2008).

2.5.4. Anti-IgE therapy

Omalizumab (XOLAIR) is the first biological agent approved for the treatment of asthma. Omalizumab is a recombinant humanized monoclonal antibody of the IgG1k subclass, targeted against IgE. IgE bound to omalizumab cannot bind to IgE receptors on mast cells and basophils, thereby preventing the allergic reaction at a very early step in the process (Brunton et al., 2008).
Mechanism of action of Omalizumab: Omalizumab is a monospecific anti-IgE antibody. Specific B lymphocytes produce IgE antibodies. The Fc region of IgE heavy chains binds with high affinity to receptors (FcεRI) in the plasma membranes of mast cells and basophils (and other cells). Allergen interacts with the antigen-binding site of cell-bound IgE, causing FcεRI cross-linking and cell activation. Omalizumab neutralizes the free IgE in the serum by binding to the Fc regions of the heavy chains to form high-affinity IgE-anti–IgE complexes. This prevents the IgE from binding to FcεRI, thereby blocking allergen-induced cell activation (Brunton et al., 2008).

2.5.5. Cromolyn sodium (disodium cromoglycate) and nedocromil sodium

Since mast cells have been thought to play a key role in the pathophysiology of asthma, in the 1970s these cells and their pro-inflammatory products became major focus of anti-asthma pharmacotherapy (Orr, 1973; Kuzemko, 1989). Traditionally, cromones (Cromolyn and Nedocromil) have been termed “mast cell stabilizers”. Their mechanism of action has been based on inhibiting the release of pro-inflammatory mediators from mast cells following IgE-cross linking. However, sodium cromoglycate caused only a modest inhibition (of 10–20%) of the mast cell mediator release accompanied by a rapid onset tachyphylaxis in in vitro studies (Church and Hiroi, 1987; Pearce, 1993). In clinical studies of asthma, the overall efficacy of cromones was only marginally better than placebo, although clearly inferior to ICS (inhaled corticosteroids) (Sridhar and McKean, 2006; Anonymous, 2000; Carra et al., 2001; Kannisto et al., 2002; Hoshino et al., 1998). Presently, treatment with cromones is confined to very mild disease, as add-on therapy in severe chronic asthma or in special patient populations (Sridhar and McKean, 2006; Holgate, 1996; Spooner et al., 2002).

Although an important pro-inflammatory mediator of asthma, allergy and anaphylactic shock, pharmacotherapy targeting histamine, the major release product of mast cells, has been shown to be of little if any effect on asthma control (Price and Kemp, 1999; Canny et al., 1997; Eiser, 1991). Ketotifen, a drug inhibiting the release of pro-inflammatory mediators (histamine and leukotrienes) from mast cells and basophils combined with H1-antagonistic activity, showed inferior anti-inflammatory effect in asthma when compared with cromoglycate and ICS (Hoshino et al., 1998; Monie et al.,
Review of Literature

1982; Stratton et al., 1984). Therefore, current evidence does not support a predominant role for this category of drugs in the mainstay treatment of asthma (GINA, 2005).

**Mechanism of action:** Cromolyn and nedocromil inhibit mediator release from bronchial mast cells; reverse the increased functional activation in leukocytes obtained from the blood of asthmatic patients; suppress the activating effects of chemotactic peptides on human neutrophils, eosinophils, and monocytes; inhibit parasympathetic and cough reflexes; and inhibit leukocyte trafficking in asthmatic airways (Brunton et al., 2008).

2.5.6. Xanthines

For centuries, strong coffee and tea were recommended for the relief of dyspnoea due to bronchospasm. While at that time practitioners were most likely not aware of the pharmacological mechanism, i.e. bronchodilator effects through inhibition of phosphodiesterase (PDE); this was probably the first application of xanthines in the treatment of asthma. The anti-asthmatic effect of theophylline was first described by Hirsch in 1922. Subsequently, theophylline followed by its more soluble derivate aminophylline in 1937, became the most widely prescribed drugs for asthma for about four decades (Terr and Bloch, 1996; Barnes, 1997). However, in clinical practice, theophylline showed limited efficacy with serious side-effects at higher doses due to its narrow therapeutic window (Barnes, 1997; Eason and Markowe, 1989). These disadvantages and the advent of the superior sympathicomimetics finally led to its relegation to second/third line anti-asthma treatment in developed countries during the 1980s (GINA, 2005; BGMA, 2003; Davies et al., 1998; Reed et al., 1998). In recent years, interest revived in xanthine derivates due to their oral formulation and low cost. Moreover, circumstantial evidence pointed to some anti-inflammatory properties (Price and Kemp, 1999; Barnes, 2003), through the suppression of the inflammatory gene transcription by activation of histone deacetylase (HDAC), which is the key target for corticosteroids (Ito et al., 2002). This mechanism may explain the beneficial effects on asthma control reported by several investigators when combining (low dose) theophylline with inhaled corticosteroids (ICS) (Evans et al., 1997; Ukena et al., 1997).
Recently, additional anti-inflammatory effects have been reported, including the acceleration in eosinophil apoptosis and the decrease in recruitment of lymphocytes and neutrophils into the airways (Barnes, 2003; Yasui et al., 1997). These properties may be promising in the treatment of severe asthma or COPD (Barnes, 2003). Although initially classified as a PDE inhibitor, the pharmacological effects of theophylline appear much broader and largely not yet identified.

In parallel with the renewed interest in theophylline, there has been development of several more specific PDE inhibitors in the last decade. Despite a better tolerability of these drugs, the gastrointestinal side effects are still substantial (Boswell-Smith et al., 2006). Targeting PDE-3 has been shown to produce bronchodilation (Myou et al., 2003). Alternatively, targeting the major isoform within airway inflammatory cells, specific PDE-4 inhibitors (e.g. roflumilast and cilomilast) have been developed for the treatment of asthma and COPD, although with varying success (Fan, 2006; Torphy, 1998; Lipworth, 2004). Future studies in asthma applying combined PDE-3/4 inhibitors should demonstrate their putative superior effectivity (Myou et al., 2003).

**Theophylline**

Theophylline, a methylxanthine, still is commonly used for asthma pharmacotherapy in many countries. In developed countries, the advent of inhaled glucocorticoids, b adrenergic receptor agonists, and leukotriene-modifying drugs has diminished theophylline use significantly, and it has been relegated to a third- or fourth-line treatment in patients whose asthma is otherwise difficult to control (Brunton et al., 2008).

**Mechanism of action:** Theophylline inhibits cyclic nucleotide phosphodiesterases (PDEs), thereby preventing hydrolysis of cyclic AMP and cyclic GMP to 5’-AMP and 5’-GMP. Inhibition of PDEs leads to an accumulation of cyclic AMP and cyclic GMP, thereby increasing signal transduction through these pathways. Theophylline and related methylxanthines are relatively nonselective in PDE inhibition. Cyclic nucleotide production is regulated by endogenous receptor–ligand interactions leading to activation of adenylyl cyclase and guanylyl cyclase. Inhibitors of PDEs therefore can be thought of
as drugs that enhance the activity of endogenous autacoids, hormones, and neurotransmitters that signal via cyclic nucleotides (Brunton et al., 2008).

Theophylline is also a competitive antagonist at adenosine receptors. Adenosine can act as an autacoid and transmitter with myriad biological actions. Of particular relevance to asthma are the observations that adenosine can cause bronchoconstriction in asthmatics and potentiate immunologically induced mediator release from lung mast cells. Inhibition of the actions of adenosine therefore also must be considered when attempting to explain the mechanism of action of theophylline (Brunton et al., 2008).

Theophylline also may owe part of its anti-inflammatory action to its ability to activate histone deacetylases in the nucleus. In theory, the deacetylation of histones could decrease the transcription of several pro-inflammatory genes and potentiate the effect of corticosteroids (Brunton et al., 2008).

2.5.7. Anticholinergic agents

During several centuries, the most controversial modality of asthma treatment has probably been the “asthma cigarette”. The active ingredient of these cigarettes consisted of alkaloids from the Belladonna plant, delivered to the lung by smoking. This therapy has been advocated for the treatment of asthma until the middle of the 20th century. At that time empirically based, today we know that the mechanism of action was largely caused by the ingredients’ anticholinergic properties. In the late 1970s, this knowledge resulted in the development of ipratropium, a synthetic anticholinergic, for the treatment of asthma. Ipratropium, and the later developed, long-acting tiotropium, both antagonize the effect of acetylcholine at the muscarinic M₁ and M₃ receptor. Despite still a limited role in the treatment of asthma, anticholinergics may benefit patients with genetically determined adverse responses to β₂ agonists - up to 20% of the asthma population (Wechsler et al., 2006). In addition, during an acute exacerbation when response to SABAs is poor, addition of an anticholinergic may provide a faster-onset relief (Gross, 2006; Teale et al., 1992).

With the advent of inhaled β adrenergic agonists, use of anticholinergic agents declined. Renewed interest in anticholinergic agents paralleled the realization that parasympathetic pathways are important in bronchospasm in some asthmatics and the
availability of *ipratropium bromide* (ATROVENT), a quaternary muscarinic receptor antagonist that has better pharmacological properties than prior drugs. A particularly good response to ipratropium may be seen in the subgroup of asthmatic patients who experience psychogenic exacerbations (Brunton *et al*., 2008).

The cholinergic receptor subtype responsible for bronchial smooth muscle contraction is the muscarinic M₃ receptor. The bronchodilation produced by ipratropium in asthmatic subjects develops more slowly and usually is less intense than that produced by adrenergic agonists. Some asthmatic patients may experience a useful response lasting up to 6 hours. The variability in the response of asthmatic subjects to ipratropium presumably reflects differences in the strength of parasympathetic tone and in the degree to which reflex activation of cholinergic pathways participates in generating symptoms in individual patients. Hence, the utility of ipratropium must be assessed on an individual basis by a therapeutic trial (Brunton *et al*., 2008).

Combined treatment with ipratropium and β₂ adrenergic agonists results in slightly greater and more prolonged bronchodilation than with either agent alone in baseline asthma. In acute bronchoconstriction, the combination of a β₂ adrenergic agonist and ipratropium is more effective than either agent alone and more effective than simply giving more β₂ adrenergic agonist. Thus, the combination of a selective β₂ adrenergic agonist and ipratropium should be considered in acute treatment of severe asthma exacerbations. Ipratropium is available in metered-dose inhalers and as a nebulizer solution. A metered-dose inhaler containing a mixture of ipratropium and albuterol (COMBIVENT) also is available in the U.S. In Europe, metered-dose inhalers containing a mixture of ipratropium and fenoterol are available (DUOVENT, BERODUAL) (Brunton *et al*., 2008).

Recently, tiotropium (SPIRIVA), a structural analogue of ipratropium, has been approved for the treatment of chronic obstructive pulmonary disease (COPD) and emphysema. Like ipratropium, tiotropium has high affinity for all muscarinic receptor subtypes, but it dissociates from the receptors much more slowly that ipratropium. In particular, binding and functional studies indicate that tiotropium dissociates from muscarinic M₃ receptors more slowly than from muscarinic M₂ receptors. The high affinity of tiotropium for muscarinic receptors, combined with its very slow dissociation
rate, permits once-daily dosing. The slow dissociation rate also provides a theoretical advantage in that it limits the capacity of large concentrations of the endogenous agonist acetylcholine to surmount the receptor blockade. Tiotropium is provided as a capsule containing a dry-powder formulation that is intended only for oral inhalation using the Handi Haler inhalation device (Brunton et al., 2008).

2.6. Animal model for screening of anti-asthmatic activity

2.6.1. In vitro methods

2.6.1.1. Histamine (H₁) receptor binding

Histamine is considered to play a major role in asthmatic attacks. H₁-antagonists have been used since decades as therapeutic agents. This assay is used to determine the affinity of test compounds to the histamine H₁ receptor by measuring their inhibitory activities on the binding of the H₁ antagonist ³H-pyrilamine to a plasma membrane preparation from guinea pig brain.

2.6.1.2. Spasmolytic activity in isolated trachea

The isolated tracheal chain of guinea pigs can be used to test for β-blocking activity. In addition, this model can be used to test compounds which inhibit bronchospasms. It is used to detect β-sympathomimetic, H₁-receptor blocking and leukotriene receptor blocking properties of test drugs.

Carbachol is a cholinergic agonist that produces contraction of bronchial smooth muscle by muscarinic stimulation.

Histamine is an important mediator of immediate allergic (type 1) and inflammatory reactions. It causes bronchoconstriction by activating H₁-receptors.

Calcium ionophores induce the release of leukotrienes via the 5-lipoxygenase pathway. Leukotrienes are powerful bronchoconstrictors that appear to act on smooth muscle via specific receptors.

To assess a compound’s ability to inhibit carbachol induced bronchospasm via β-receptor activation, a β-receptor blocking agent (for example propranolol) must be added. If relaxation of bronchial smooth muscle is brought about by β-receptor activation, the spasmolytic effect will decrease following propranolol administration.
The effect of bradykinin can be abolished by bradykinin antagonists (Hock et al., 1991). The effects of potassium channel openers can also be studied in this test (Englert et al., 1992).

**Guinea pig tracheal chain:** The isolated tracheal chain of guinea pig is used for testing the bronchodilating activity of test drugs. Carbachol or histamine is used to increase the tone of tissue. Trachea is removed from euthanized guinea pig and mounted by suitable methods. It is then tested for action of drugs. The most common mounting method for tracheal chain includes:

1. **Castillo J.C & DeBeer E.J. method (1947):** In this method trachea is removed from freshly sacrificed guinea pig and is sectioned with scissors into 6 to 8 approximately equal rings connected in series by short loop of silk thread, the chain being set up in organ bath, each ring being oriented so that the dorsal smooth muscle band is vertical. In this method often the magnitude of response is small and preparation of chain is laborious.

2. **Constantine J.W Method (1965):** This method is described the spirally cut tracheal strip preparation. The excised trachea is soaked in Krebs-Henseleit solution and cleaned of extraneous tissue. Then it is cut from one end to the other, in a spiral fashion such that 2-3 segments of cartilage separated in each turn of spiral. The entire strip could be used or it can be cut into half thus providing two preparations from one donor. This method is quick and simple and gives reproducible response.

**2.6.1.3. Isolated perfused lungs:** The isolated perfused rat lungs allow the simultaneous registration of pulmonary vascular and airway responses to various drugs. Changes (increase or decrease) in pulmonary arterial pressure after injection of the test compounds are measured in mm Hg and compared with baseline values.
2.6.2. *In vivo* methods

2.6.2.1. Broncho-spasmolytic activity in anesthetized guinea pigs (Konzett-Rössler method)

The principle was first described by Kiese, 1935. Konzett and Rossler, 1940 published a method suitable for screening procedures which found worldwide acceptance. A survey on the history and further modifications was given by Doring and Dehnert, 1997.

The method is based on registration of air volume changes of a living animal in a closed system consisting of the respiration pump, of the trachea and the bronchi as well as of a reservoir permitting measurement of volume or pressure of excess air. Bronchospasm decreases the volume of inspired air and increases the volume of excess air. Thus, the degree of bronchospasm can be quantified by recording the volume of excess air. Administration of spasmogens like acetylcholine, histamine, bradykinin, serotonin, ovalbumin, PAF, substance P, methacholine or leukotrienes, results in contraction of bronchial smooth muscle.

The method permits the evaluation of a drug’s bronchospasmolytic effect by measuring the volume of air, which is not taken up by the lungs after bronchospasm.

2.6.2.2. Effect of arachidonic acid or PAF on respiratory function in vivo

Platelet-activating factor, also known as a PAF, is a potent phospholipid activator and mediator of many leukocyte functions, including platelet aggregation, inflammation, and anaphylaxis. It is produced in response to specific stimuli by a variety of cell types, including neutrophils, basophils, platelets, and endothelial cells. PAF is biosynthesized from lysophosphatidylcholine (LPC) and acetyl CoA by the enzyme LPC acetyltransferase (LPCAT). It is degraded by a group of enzymes called PAF acetylhydrolases (PAFAHs) which are related to phospholipase A2. Based on the classical method of Konzett and Rössler, 1940; Lefort and Vargafting, 1978; Vargafting *et al.*, 1979 studied the effects of arachidonic acid and PAF on respiratory function of guinea pigs *in vivo*.

Arachidonic acid is metabolized into thromboxane (TXA2) and prostacyclin (PGI2). TXA2 produced in the lung leads to bronchoconstriction, which is independent from circulating platelets and leukotrienes; TXA2 produced intracellularly in platelets
induces a reversible thrombocytopenia. PGI₂ produced in the vessel wall leads to the reduction of systolic and diastolic blood pressure. All three effects are inhibited by drugs which block cyclooxygenase. In contrast, agents which block thromboxane synthetase inhibit bronchoconstriction and thrombocytopenia, but lead to a potentiation of blood pressure reduction.

In contrast to arachidonic acid, PAF as inducer leads to bronchoconstriction, which is platelet-dependent. In addition, PAF induces thrombocytopenia, leukocytopenia, reduction of blood pressure and increase of hematocrit. These effects are also reversible, but more persistent than those induced by arachidonic acid, and quickly result in tachyphylaxis. The test allows to evaluate the sites of action of drugs, which interfere with the mechanisms of broncho-constriction and thrombocytopenia; in an in vivo-model guinea pigs are challenged with the spasmogens and platelet-aggregating substances arachidonic acid or platelet activating factor (PAF).

2.6.2.3. Body plethysmography and respiratory parameters after histamine-induced bronchoconstriction in anesthetized guinea pigs

Guinea pigs can be placed in a plethysmograph for measurement of respiratory parameters. Respiratory frequency and respiratory amplitude are recorded. The decrease of respiratory amplitude (diminished respiratory volume due to bronchoconstriction) and the reflectory increase of respiratory frequency after histamine inhalation are attenuated by bronchodilatatory drugs. Additional respiratory parameters can be recorded using a Fleisch tube and a catheter inserted into the pleural cavity (Englert et al., 1992). The method can be used for various purposes, e.g., to evaluate the antagonism against bradykinin-induced bronchoconstriction (Wirth et al., 1991 & 1993) or the bronchodilator effects of potassium channel openers (Englert et al., 1992) or to measure the effect of morphine on respiration in rats (Kokka et al., 1965).
2.7. Radnoti single chamber organ bath

Features

The Radnoti single chamber organ bath is a high quality bath ideal for conducting physiological measurements on a single tissue or organ sample in teaching or research applications.

It is supplied with a 70 mL High Tech tissue bath, which is individually mounted via an adjustable ring-clamp to a stainless steel support rod that is anchored to a sturdy steel base. The base is coated to resist corrosive salt solutions. Tissue and transducer holders, micropositioner are also supplied.

The perfusate flows from a water jacketed, temperature controlled 1-liter reservoir then enters the tissue bath through a pre-heating coil built within the water jacket of tissue baths. The baths is emptied via a bottom drain that is controlled by a stopcock. The bath may also be used in a constant flow mode with the excess perfusate exiting an overflow outlet at the top of the bath. Both overflow and drain may be routed to permit monitoring the release of endogenous substances, drug metabolites, or to design a cascade system configuration. Gas regulation is finely controlled via a removable Teflon needle valve inserted at the side of the chamber.

Instrument is supplied with a suitable isometric or isotonic Force Transducer. To maintain a constant temperature in the tissue chamber, the thermoregulated water pump is available.

- Modular in design allows easy customization
- Coated steel base is resistance to corrosive salt solutions
- Water jacketed glassware provide constant temperature maintenance
- High-Tech tissue bath design provides constant flow mode with the waste perfusate exiting an overflow outlet at the top of the bath
- Gas regulation is finely controlled via a removable Teflon needle valve Oxy-tube

Applications

Radnoti Organ Baths are used for in vitro dose response experiments, used extensively to investigate the physiology and pharmacology of tissue preparations from various species (chick, toad, rabbit, rat, guinea-pig, etc.) Some of the tissues that may be studied with an organ bath system include:
Smooth Muscle:
- Vascular, e.g. arterial/aortic rings
- Guinea-pig tracheal strip
- Vas Deferens (secretory duct of the testicle)
- Uterine
- Colon

Skeletal Muscle:
- Toad Abdominus Rectus
- Chick Biventer Cervis
- Mammalian Diaphragm

Figure 9: Figure showing Radnoti single chamber organ bath system.
The Radnoti transducer operates on the principle of converting picofarad capacitance changes into an amplified DC output voltage by means of a patented circuit. The transducer consists of a stiff beam suspended between two capacitor plates. This forms a differential capacitor. Using this principal, the beam can be exceptionally stiff, approaching the ideal of measuring force without motion. As an example, for a force of 2 grams, the beam deflection is a maximum of only 5 microns in either the 0.2 or 2.0 gram range mode. The linearity is within +1% with a high DC voltage output and freedom from drift.

The transducer has a short tissue mounting rod with a groove that projects from the front. One end of a non-stretching string or wire must be formed into a loop and fastened into the groove on the shaft. The other end of the string or wire is subsequently attached to the muscle preparation. The string or wire must be at right angle to the transducer rod and be pulled straight down once the final setup has been completed. The transducer is equipped with a transparent, removable plastic shield that helps to prevent build-up of salts (i.e. corrosives) which may otherwise tend to accumulate on the transducer rod and migrate into the transducer circuit. In time, this salt build-up can deteriorate performance. The wire or string from the beam must be directed through the slot in the splash shield without touching the edge.

**Radnoti Thermal Circulator Pump**

**Organ bath experiments: Methods**

Dose response studies are typically conducted to assess concentration-response relationships in isolated tissue preparations. Traditionally, tissue-[Organ Baths](organ_baths) are used
for *in vitro* dose response experiments to investigate the physiology and pharmacology of tissue preparations from various species (e.g. chick, toad, rabbit, rat, guinea-pig, etc.). Tissue-Organ Baths are used to maintain the integrity of the tissue for several hours, in a temperature-controlled environment, while physiological measurements are performed. Typical experiments involve the addition of drugs to the organ bath or direct/field stimulation of the tissue. The tissue reacts by contracting/relaxing and an isometric or isotonic transducer is used to measure force or displacement, respectively. From the experimental results dose-response curves are generated (tissue response against drug dosage or stimulus potency).

Isolated tissue-organ experiments can generally be run in groups of 2, 4, 8 or more sample preparations, thereby enabling a high throughput in most laboratories. These in vitro preparations are also more readily instrumented and can be easily subjected to controlled changes in perfusate, oxygen availability, drug administration etc than is otherwise possible in the intact animal.

A water-jacketed organ bath provides a stable and easily adjustable way of temperature control. Substrates and other nutrients that are required to sustain tissue function are provided via a physiological solution, which allows the study of evoked tissue responses to:

- Pharmacological drug/agents (Dose Response studies)
- Electrical stimulation
- Both pharmacological and electrical stimulation

Tissues are usually prepared in a petri-dish containing physiological solution (i.e. Kreb’s solution). The ends of the tissue are then attached to the mounting hook and transducer using silk or fine wire (non-compliant) to mount the tissue. The tissue placement may vary depending on the tissue type such as:

- Ring/Vessel Preparation
- Strip Preparation
Some of the more common tissues that are studied with an organ bath system include:

Cardiovascular
- Aorta rings
- Heart tissue (papillary muscle, left ventricles)
- Arteries (mesenteric arteries)

Gastrointestinal
- Ileum
- Colon
- Gastric antral muscle
- Sphincter

Respiratory
- Tracheal rings
- Phrenic diaphragm
- Pulmonary arterial smooth muscle
- Lung parenchyma

Skeletal Muscle
- Soleus
- Gastrocnemius

Other isolated tissue preparations include urinary bladder, penile muscle strips and prostate.
2.8. Plant profile


**Figure 10:** Photograph of *Achillea millefolium* Linn.

---

**Biological Source**

Drug consists of the aerial parts of *Achillea millefolium* Linn. Family: Compositae (Asteraceae).
Common names

Yarrow, Milfoil (Eng.); Gandana (Hind.); Roojamari (Kan.); Biranjasipha (San.).

Distribution

Yarrow is a perennial herb that produces one to several stems (8 to 16 inches tall) from a fibrous underground horizontal rootstock (rhizome). It is known to be both native and introduced. Leaves are evenly distributed along the stem, with the leaves near the middle and bottom of the stem being the largest. The leaves have varying degrees of hairiness (pubescence). Leaf blades are lance-shaped in outline, but are finely divided. Overall leaf dimensions range from ¼ to 1¼ inch wide by 1¼ to 6 inches long. The flower heads (inflorescence) have a flattened dome shape (with approximately 10-20 ray flowers. The flowers are whitish to yellowish-white. The plant commonly persists from May through June. It is distributed in the Western Himalayas from Kashmir to Kumaon at an altitude between 2400 to 3400 m.

Description

Macroscopic

Stem—Dried pieces are cylindrical, occasionally branched, nodes distinct, often attached with leaf traces, especially on young stem; internodes measuring 0.5 to 1.5 cm in length, and 1 to 5 mm in diameter, longitudinally furrowed and ridged, pubescent, fractures short outer somewhat splintery in young stem, older stem splits into onto longitudinal pieces; externally yellowish green to pale brown, internally whitish, spongy, old stems are hollow in the centre. Taste slightly bitter and mucilaginous, odour fragrant.

Leaf—Compound, 3-pinnatisect, about 4 to 8 cm long consisting of hairy rachis and numerous linear, finely pointed pinnules, green in colour.

Flower—Numerous, small capitulum, up to 0.5 cm long and broad, arranged at the top in densely packed corymbose clusters. Each flower head composed of ray florets and disc florets. Ray florets bracteate, unisexual, consisting of 5 gamopetalous white corolla and a gynoecium with inferior ovary, long style and bifid stigma. Disc florets are bracteates,
bisexual consisting of 5 gamopetalous, camanulate corolla, 5 stamens and a gynoecium with inferior ovary, long style and bifid stigma; fruits, very minute, one seeded indehiscent cypsela, conical, about 1 to 1.5 mm in length, smooth, light greyish in colour.

**Microscopic**

**Stem-TS** of stem is irregularly circular in outline showing a layer of epidermis, narrow zone of cortex and a ring of fibro vascular bundles encircling the wide central pith.

The detail TS of stem shows a layer of epidermis is present with striated cuticle with very few stomata and bearing multicellular trichomes with long, unicellular, thin-walled terminal cell and short 4 to 5 squarish basal cells; 2 to 3 rows of collenchymatous hypodermis lies underneath the epidermis except at the ridges where it is many more layered; cortex composed of 4 to 6 rows of parenchymatous cells. Endodermis is distinct, enclosing a ring of conjoint, collateral vascular bundles each capped with an arc of 8 to 15 rows of pericyclic fibres; phloem is narrow, crossed with few phloem fibres; xylem consists of radially arranged vessels, associated with parenchyma and thin-walled fibres; pith is very wide, parenchymatous and contains prismatic and rosette crystals of calcium oxalate.

**Leaf-TS** of leaf showing pinnule passing through midrib is protruding at the lower side and flat or slightly elevated at the upper side with a broad short winged lamina extensions on its either of the lateral sides, each embedded with isolated meristele smaller in size than that of the midrib.

The detailed TS of leaf shows upper and lower epidermis covered with thin cuticle, bearing simple, multicellular trichomes and crossed with few stomata; a well-developed conjoint, collateral meristele consisting of radially arranged xylem vessels, an arc of phloem and sheath of dorsiventrally located sclerenchymatous band, encircled by an endodermis, embedded in the centre of parenchymatous tissue of the midrib. Lamina is dorsiventral, 2 to 3 layers of ill developed palisade cells lie underneath the upper epidermis discontinuous over the midrib, the place being occupied by few rows of collenchyma, remaining cells of lamina being of spongy parenchyma; trichomes are just like that of stem but are longer and whip like.
Powder

Shows abundant fragment of bracts exhibiting groups of spindle-shaped cells from the margin; lignified sclereids of various sizes and shapes; microrosette crystals of calcium oxalate embedded in the parenchyma cells; spirally cut xylem vessels; fragments of lignified reticulate parenchyma of corolla in surface view; ray florets with striated papillate epidermis and cells with microrosette crystals; spinulate pollen grains; simple, multicellular, long trichomes from leaf and stem; epidermal cells of leaf and stem with anomocytic stomata, cells of stem being elongated, striated and with very few stomata; longitudinally cut pith cells of the stem with cluster and prismatic crystals of calcium oxalate, and long wide lumened irregularly running fibres, inflated at many places from the stem.

Chemical Constituents (Anonymous-A, 2008)

Major

Apigenin. Luteolin and their 7-O-glucosides. (Kaloshina and Neshta, 1973; Hoerhammer, 1964; Guedon et al., 1993)

Volatile oil (up to 1%) forms the other major component of the plant. The content and composition of volatile oil show large variations attributable to several factors including the plant part and age. The volatile oil of octaploid plants is linalool rich and that of hexaploid is rich in camphor, sabinene and 1,8-cineole. The oil of tetraploid plants contains up to 25% azulene, 23% β-pinene and 11% caryophyllene (Anonymous-A, 2008).

Others

Apigenin-7-malonylglucoside, artemetin, casticin, cirsiliol, cosmosin, hispidulin, 6-hydroxyflavones, 6-hydroxyflavonols, 6-hydroxyluteolin 6,7,3’,4’-tetramethylether, 5-hydroxy 3,6,7,4’-tetramethoxyflavone, luteolin-7-malonylglucoside, luteolin-7-O-β-glucopyranoside, nepetin, O-glycosides of kaempferol and quercetin, rutin, salvigenin; acetylbalchanolide, 8-acetylegelolide, achillicin (8α-acetoxy-10-epi-artabsin), achillifolin, achillin, achimillic acid A lactone, achimillic acids A, B & C, 8α-angeloxy-
Review of Literature

Achillicin

$p$-hydroxy-phenethylamide

Feruloylcaffeoylquinic acid
$R_1-R_3 = H$ or caffeoyl/feruloyl

Proline

Stachydrine

Betonicine

Betaine

Choline
achillin, 8α-angeloxy-leucodin, 10-angeloyldesacetylisoapressin, 8-angeloyl-egelolide, aristeanin, austricin, balchanolid, Benedictine, benedictonlide, cnicin, cnicinolide, desacetylmartricarin, dihydroparthenenolide, dihydroreynosin, estafiatin, hydroxyachillin, 7β-hydroxy-α-longipin-2-en-1-one, isoachifolidiene, isoapressin, 10-isovaleroyldesacetylisoapressin, leucodin, leucomyacin, α-longipin-2-en-1-one, millefin, millefolide, α-peroxyachifolid, β-peroxyisoachifolid, 5β-tigloyl-achillifolin, 8-tigloyldesacetylezomontanin; achilleine (betonicine), betaine, choline, stachydrine; folic acid, vitamin PP, vitamin C, vitamin K; alkamide; N-(2-methylpropyl)-(E,E)-2,4-decadienamide; proline; fatty acids; triacontane; β-sitsosterol, stigmasterol, campesterol, cholesterol, β-amyrin, α-amyrin, taraxasterol, pseudotaraxasterol; volatile oil constituents, aesculetin, artemazulene, α & β-carotene, prunasin, (+)-sesamin,
triacylglycerol, scopoletin, umbelliferone, viburnitol, adenine, ferulic acid, salicylic acid, chlorogenic acid (Anonymous-A, 2008).

**Quantitative Standards**

*Foreign matter:* Not more than 2.0 per cent. *Ash:* Not more than 12.5 per cent. *Acid-insoluble ash:* Not more than 4.5 per cent. *Ethanol-soluble extractive:* Not less than 3.0 per cent. *Water-soluble extractive:* Not more than 10.0 per cent.

**Medicinal Uses**

Several tribes of the Plains region of the United States including the Pawnee and Chippewa tribes used common yarrow. The Pawnee used the stalk in a treatment for pain relief. The Chippewa used the leaves in a steam inhalant for headaches. They also chewed the roots and applied the saliva to their appendages as a stimulant. The Cherokee drank a tea of common yarrow to reduce fever and aid in restful sleep.

*Achillea*, a genus of Asteraceae family, has long been used in traditional medicine for treatment of fever, asthma, bronchitis, cough, skin inflammation, jaundice, other liver ailments (Zargari, 1989), dysentery and diarrhoea (British Herbal Medicine Association, 1983). Aqueous and alcoholic extracts of yarrow are used in traditional European medicine internally in the treatment of gastro-intestinal and hepato-biliary disorders and externally in case of skin inflammations and for wound healing (Willuhn, 2002).

**Pharmacology** (Saeidnia et al., 2011; Anonymous, 2009; Anonymous-A, 2008)

**Anti-cancer activity:** Achimillic acids A, B, & C, isolated from the flowers showed *in vivo* anti-leukemic activity in mice (Toyo et al., 1994).

**Anti-fungal, anti-microbial and anti-oxidant activity:** The essential oil exhibited *in vivo* antifungal (Fiori et al., 2000), antimicrobial (Karamenderes et al., 2003; Kedzia et al., 1992), antioxidant (Yatsyuk, 1990) and *in vivo* CNS depressant activity in mice (Kudrzycka-Bieloszabska and Glowniak, 1967).
Anti-hyperglycaemic activity: The decoction of *A. millefolium* showed anti-hyperglycaemic activity against epinephrine- and alloxan-induced hyperglycaemia without affecting blood insulin level in rats and mice (Molokovskii *et al*., 2003).

Anti-pyretic, anti-inflammatory and antiphlogistic activity: The plant showed mild antipyretic activity (Nikonorow, 1941). An aqueous extract of the dry flower heads showed anti-inflammatory activity in mice, attributed to be a mixture of protein carbohydrate complexes (Goldberg *et al*., 1969). Sesquiterpene and sesquiterpene lactones (Lyss *et al*., 2000), choline (Bauer *et al*., 1957), N-(2-methylpropyl)-(E,E)-2,4-decadienamide (LaLonde *et al*., 1980) and α-peroxyachifolid (Rucker *et al*., 1991) isolated from the plant showed anti-inflammatory, hypotensive, larvicidal and antimalarial activities, respectively. Yarrow is also used in the treatment of wounds and chronic gastro-intestinal and skin inflammations as an antiphlogistic drug similar to chamomile (Madaus, 1976; Fischer and Krug, 1984; Willuhn, 2002). While a topical anti-inflammatory activity of the sesquiterpenes was already shown being caused by inhibition of the arachidonic acid metabolism (Kastner *et al*., 1993), other mechanisms of action might also contribute to the antiphlogistic activity of the drug. As various proteases, for instance human neutrophil elastase (HNE) and matrix metalloproteinases (MMP-2 and -9), are associated with the inflammatory process. Benedek *et al*., 2007, screened 20% methanolic extract, the dicafeoylquinic acid (DCQA) fraction (48.8% DCQAs), and the flavonoid fraction (11.1% flavonoids with apigenin-7-O-glucoside, rutin and luteolin-7-O-glucoside) of yarrow for the *in vitro*-antiphlogistic activity. Results revealed that antiphlogistic activity is at least partly mediated by inhibition of HNE and MMP-2 and -9. Anti-inflammatory activity has been described for the azulene components documented for the volatile oil of yarrow (Chandler *et al*., 1982).

Anti-Spermatogenic activity: The ethanolic (96 and 80 %) extracts of flowers, leaves and stem showed antispermatogenic effect in albino mice (Montanari *et al*., 1998).

Hepatoprotective activity: The leaf and flower extract demonstrated hepatoprotective activity (Popovic *et al*., 2002).
**Immunosuppressive activity:** Methanol extract and some other fractions of *A. millefolium* were studied on humoral immunity in BALB/c mice by microhaemagglutination test. Only two fractions showed a significant decrease in the anti-SRBC titer of mice. The immunological properties may be related to presence of glycosylated derivatives of caffeic acid, because caffeic acid glucoside XXII was isolated and identified from the active fractions. Some known compounds including, luteolin 7-O-glucoside XXIV and apigenin 7-O-glucoside XXV have also been reported from this species (Yassa *et al.*, 2007). Effects of the essential oils of *A. talagonica* and *A. millefolium* have been studied on humoral immune responses in BALB/c mice. The oil isolated from *A. millefolium* ssp. *millefolium* possessed a high percentage of sesquiterpenes (55.4%) in which bisabolol XXVI was the main compound. The volatile oil of *A. millefolium* decreased the anti-SRBC antibody titer, but the oil of *A. talagonica* was not effective. High percentage of sesquiterpenes and presence of proazulene in *A. millefolium* together with the lack of these compounds in *A. talagonica* could account for the different immunological effects of these plants (Saeidnia *et al.*, 2004).

**Spasmolytic activity:** Achillicin, isolated from stem showed direct cardiac depressant effect on frog heart, spasmolytic activity on rabbit ileum and hypothermia in rats (Tewari *et al.*, 1974). The flavonoid containing fraction of the plant exhibited anti-spasmodic activity (Hoerhammer, 1964). Besides essential oil and sesquiterpenes phenolic compounds such as flavonoids and phenolcarboxylic acids are a major group of plant constituents present in yarrow. Due to their high solubility in water and ethanol, those polar substances are completely extracted into teas and tinctures which are the traditional application forms of yarrow. Recently, Lemmens-Gruber *et al.*, (2006), demonstrated the spasmolytic activity of the flavonoids and the choleretic activity of the dicafeoylquinic acids (DCQAs) from yarrow (Benedek *et al.*, 2006). Moreover, the safety of the plant after chronic exposure was previously reported (Cavalcanti *et al.*, 2006).

**Others:** The ethanolic extract of flowers, leaves and stem showed mosquito repellent and larvicidal activity (Tunon *et al.*, 1994).

**Figure 11:** Photograph of *Rubia cordifolia* Linn.

---

**Biological Source**

Drug consists of dried root and stem of *Rubia cordifolia* Linn. Family: Rubiaceae.

**Common names**

Indian madder (Eng.); Manjistha, Manjith (Beng.); Manjitha (Guj.); Manjitha, Manjit (Hind.); Manjustha (Kan.)
Distribution

It is a perennial, herbaceous prickly climber distributed in the Himalayas from Kashmir eastwards and Nilgiris and other hilly district of India up to an altitude of 2500 m.

Description (Anonymous, 2001; Anonymous-A, 2005)

Macroscopic

Root-Cylindrical, often surmounted by a knotty crown about 1.5 to 4 cm in diameter; about 2 to 9 cm in length and 0.2 to 0.6 cm in width; surface smooth finely longitudinally striated and rarely grooved, often exhibiting lateral root scars; dark reddish brown externally and internally. Fracture short, taste sweetish, acrid and disagreeable, odour characteristic pleasant.

Stem-Slender, more or less cylindrical, occasionally branched, surface scabrous, stiff and grooved often longitudinally cracked, nodes distinct and swollen. Brown to reddish brown in colour. Fracture, taste and odour are similar to root.

Microscopic

Root & Stem-Diagrammatic sketch of smoothly cut TS of root and stem circular in outline shows a well-developed central wood occupying two third area of the section in root and small pith in stem surrounded by narrow phloem and wide periderm.

TS of root shows well developed cork, consisting of 3 to 8 layered suberized radially arranged cells, occasionally filled with reddish brown content; followed by 3 to 10 cells broad parenchymatous cortex. Some cortical cells filled with acicular, clusters and sandy crystals of calcium oxalate which are more in number toward periphery region. Phloem 8 to 12 layered broad, consists of sieve tubes, companion cells and phloem parenchyma. Secondary xylem consists of vessels, fibres, tracheids and xylem parenchyma. Vessels are broader towards the peripheral region of the xylem. The size of vessels varies from 30 to 270 µm in length and 18 to 90 µm in breadth. Medullary rays are uni- to multiseriate and oval to circular starch grains are present in cortical and phloem parenchyma cells.
All the anatomical characters of stem are similar to root except phloem which is 15 to 20 celled broad, with phloem parenchyma smaller towards inner side, gradually becoming larger and tangentially elongated towards periphery, a few cells contain sandy crystals of calcium oxalate. Central parenchymatous pith present, cells towards periphery are smaller and towards central region are large. At maturity the pith cells crushed in the centre.

**Powder**

**Root & Stem**-Shows numerous fragments of cork, lignified xylem vessels, tracheids and fibres, raphides, clusters and sandy crystals of calcium oxalate, parenchyma with red content, starch grains are observed under microscope.

**Chemical Constituents** (Anonymous, 2001; Anonymous-A, 2005)

**Major**

Anthraquinones: rubiadin (1,3-dihydroxy-2-methylantraquinone) (Tripathi et al., 1997; Itokawa et al., 1983; Dosseh et al., 1981), alizarin (Murti et al., 1972; Itokawa et al., 1983), purpurin (Murti et al., 1972).
Others

Purpuroxanthin (Murti et al., 1972), ruberythric acid (Murti et al., 1972; Itokawa et al., 1983), 1-acetoxy-6-hydroxy-2-methylanthraquinone-3-O-α-rhamnosyl(1→4)-α-glucoside (Varma et al., 1985), mollugin (Ho et al., 1996; Itokawa et al., 1993), 1,3-dihydroxy-2-ethoxymethyl-9,10-anthraquinone, lucidin primeveroside, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-O-(6′-O-acetyl)-α-rhamnosyl(1→2)-β-glucoside (Itokawa et al., 1983), furomollugin, rubilactone (Ho et al., 1996), 2-carboxymethyl-3-prenyl-2,3-epoxy-1,4-napthoquinone (Itokawa et al., 1993), 1-hydroxy-2-hydroxymethyl-9,10-anthraquinone, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone (Itokawa et al., 1983; Itokawa et al., 1993), rubioncolin B (Itokawa et al., 1993), 1-hydroxy-2-methylanthraquinone (Tessier et al., 1981), nordamnacanthal, physcion, 1,4-dihydroxy-6-methylanthraquinone, 1,4-dihydroxy-2-methylanthraquinone, 1,5-dihydroxy-2-methylanthraquinone, 3-prenyl-5-methoxy-1,4-napthoquinone (Dosseh et al., 1981), 1-hydroxy-2-methoxyanthraquinone, 1,4-dihydroxy-2-methyl-5-(or 8)-methoxyanthraquinone, 1,3-dimethoxy-2-carboxyanthraquinone (Dosseh et al., 1981), cyclic hexapeptides: RA-VII, RA-V, RA-IV & RA-III (Itokawa et al., 1984); RA-IV, RA-III, RA-II, RA-I (Itokawa et al., 1986); RA-XV, RA-XVI (Takeya et al., 1993).

Quantitative Standards

Foreign matter: Not more than 1.0 per cent. Ash: Not more than 10.0 per cent. Acid-insoluble ash: Not more than 0.8 per cent. Ethanol-soluble extractive: Not less than 23.0 per cent. Water-soluble extractive: Not more than 47.0 per cent.
Medicinal Uses

Anti-convulsant activity: The triterpene isolated from root and stem was found to possess anticonvulsant and anxiolytic activities (Kasture et al., 2000).

Anti-hyperglycaemic activity:

Anti- Spermatogenic activity:
Anti-tumour activity: The napthohydroquinone, carboxymethyl-3-prenyl-2,3-epoxy-1,4-napthoquinone and a napthohydroquinone dimer possessed moderate cytotoxic activity in Chinese hamster and rubioncolin B showed antitumor activity against sarcoma 180 ascites in mice (Itokawa et al., 1993). The cyclic hexapeptides isolated from the root showed significant activity against leukaemias and ascites tumours (Itokawa et al., 1984; Itokawa et al., 1986; Takeya et al., 1993). The mollugin isolated from the root showed inhibition of passive cutaneous anaphylaxis, degranulation of mast cells and also lymphoid leukaemia (P388) in mice (Gupta et al., 1999) and showed promising activity against experimental tumours (Adwankar and Chitnis, 1982).

Hepatoprotective and antioxidant activity: The alcoholic extract of root and its constituent rubiadin were found to inhibit lipid peroxidation (Tripathi and Sharma, 1998) and had antioxidant property (Tripathi et al., 1997). The two napthohydroquinones i.e. furomollugin and mollugin isolated from the root strongly supressed the secretion of hepatitis B surface antigen in human Hep3B-cells (Ho et al., 1996).

Immunomodulatory activity: The alcoholic extract of root possesses immunomodulatory activity (Joharapurkar et al., 2003).
2.8.3. _Saussurea costus_ (Falc.) Lipsch./ _Saussurea lappa_ (Decne.) C. B. Clarke
(Anonymous-A, 2006; Kapoor, 2001)

**Figure 12:** Photograph of _Saussurea costus_ (Falc.) Lipsch. (syn. _Saussurea lappa_ (Decne.) C. B. Clarke).

**Biological Source**
Drug consists of the dried root of _Saussurea costus_ (Falc.) Lipsch. (syn. _Saussurea lappa_ (Decne.) C. B. Clarke). Family: Compositae (Asteraceae).

**Common names**
Costus (Eng.); Kudo (Beng.); Kutha (Hind.); Postkhai, Kuth (Kash.); Kuth (Punj.)
Distribution

The plant is perennial herb about 1 to 2 m in height, distributed in Himalayas, Kashmir eastward at an altitude between 2500 and 3000 m and also cultivated in Himachal Pradesh, Uttaranchal and Sikkim.

Description (Kapoor, 2001)

Macroscopic

Dried root pieces stout, thick or slender up to 15 cm long and 0.5 to 5 cm wide; thick pieces mainly representing the primary roots which are fusiform, somewhat conical or gradually tapering in shape, often exhibiting remains of aerial stem or collapsed central scars left by them at the apex and the remnant of rootlets or their scars appearing as white points throughout the surface; slender pieces representing mostly the lateral roots or the lower parts of the main roots are more or less cylindrical; surface straight or spirally longitudinally wrinkled or finely ridged, dark brown. Fracture short pale brown to somewhat whitish in colour. Taste bitter and pungent; odour characteristic and aromatic.

Microscopic

Diagrammatic TS of the root shows irregularly circular outer margin, a well-marked dark brown cambium ring separating a narrow outer light yellowish bark from a wide inner porous xylem, traversed by medullary rays and reddish resin canals.

Detailed TS of the roots shows cork, the outermost tissue of the section, consisting of 3 to 5 layers of tangentially elongated yellowish brown suberized cells followed by 3 to 6 rows of thin-walled parenchymatous cortex; phloem, the wider zone of the section consisting of 40 to 60 or more rows of parenchymatous cells interspersed with small groups of sieve tissues and lignified fibres all being arranged in radial rows, in more or less concentric manner, cambium is distinct in strips of 2 to 3 layers; the secondary xylem is composed of strands of radially arranged 1 to 10 small vessels associated with tracheids, fibres and parenchyma separated by wide wedge-shaped medullary rays; subspherical to ellipsoidal resin canals filled with yellowish-brown masses and oil globules and bound by 2 to 3 layers of elongated parenchyma traversed throughout the section.
Powder

Shows abundant irregular yellowish to orange resin masses, oil globules and colourless, irregular, large crystalline masses of inulin scattered as such throughout; longitudinally cut fragments of isolated or groups of scalariform and reticulate xylem vessels; pitted fibrous tracheids and moderately thick-walled fibres with sharp, blunt or occasional branching tips; few, long thread like, thick-walled, narrow lumened phloem fibres; fragment of oval to circular resin canals encircled by 2 to 3 layers of parenchymatous sheath and fragments of thick-walled cork cells in surface view.

Chemical Constituents (Anonymous-A, 2006)

Major

Costunolide (Rao et al., 1958; Rao et al., 1960) and dehydrocostus lactone (Ukita, 1939; Romanul et al., 1956; Mathur et al., 1965).
Mokko Lactone  Dehydrocostus Lactone  Eremanthin

Dihydrocostanolide  (+)-costunolide

Isodihydrocostunolide  B-cyclo costunolide

Cynaropicrin
Others

Alantolactone (Govindan and Bhattacharyya, 1977), aploptaxene (Romauk et al., 1959), (-)-(E)-trans-bergamota-2,12-dien-14-al, (-)-α-trans-bergamotene (Maurer and Grieder, 1977), camphene (Semmler and Feldstein, 1914), (-)-caryophyllene (Romauk et al., 1959), (-)-caryophyllene oxide (Maurer and Grieder, 1977; Mathur, 1972), cedrene, cedrol (Romauk et al., 1959), (-)-α-costal, (+)-β-costal, (+)-γ-costal (Maurer and Grieder, 1977), α-costene, β-costene (Semmler and Feldstein, 1914), costic acid (Bawdekar and Kelkar, 1965), costol (Romauk et al., 1959), (-)-α-costol, (+)-β-costol, (+)-γ-costol, (-)-ar-curcumene (Maurer and Grieder, 1977), α-cyclocostunolide, β-cyclocostunolide, cyanaropicrin (Cho et al., 1998), dihydro-α-ionone (Maurer and Ohloff, 1977), 4β,15-dihydro-3-oxo-trans-germacran-6α,12-olide (Chhabra et al., 1998), dihydroaplotaxene (Klein and Thomel, 1976), dihydrodehydrocostus lactone (Semmler and Feldstein, 1914; Mathur, 1972), 11β,13-dihydroglucozaluzanin (Yang et al., 1997), 1β,6α-dihydroxy costic acid ethyl ester (Sun et al., 2003), (-)-elem-1,3,11(13)-trien-12-al (elemental), (-)-elem-1,3,11(13)-trien-12-ol (Maurer and Grieder, 1977), (-)-β-elemene (Romauk et al., 1959; Maurer and Grieder, 1977), (-)-elemol (Maurer and Grieder, 1977), 11,13-epoxy-3-ketodehydrocostus lactone (Chhabra et al., 1998a), 11,13-epoxydehydrocostus lactone (Chhabra et al., 1998a; Chhabra et al., 1997), 4α,15-epoxyisozaluzanin C (Chhabra et al., 1998), 11,13-epoxydehydroisozaluzanin C, 11,13-epoxyisozaluzanin C (Chhabra et al., 1998a; Chhabra et al., 1997), (E)-geranylacetone (Maurer and Ohloff, 1977), germacra-1(10),4,11(13)-trien-12-al, germacra-1(10),4,11(13)-trien-12-oic acid, germacra-1(10),4,11(13)-trien-12-ol, (+)-germacrene A (De Kraker et al., 2001), 3,9,11-guaiatriene-12-acid (Klein and Thomel, 1976), guaianolide α,β-unsaturated aldehydes (Kalsi et al., 1995), humulene (Romauk et al., 1959), 4β-hydroxy-11(13)-eudesmene-12-al (Yang et al., 1997), 15-hydroxy dehydrocostus lactone (Saxena and Dixit, 1992), α-ionone (Klein and Thomel, 1976), β-ionone (Romauk et al., 1959), isoalantolactone, isocostic acid (Yang et al., 1997), isodehydrocostus lactone (Kalsi et al., 1983), isodehydrocostuslactone-15-aldehyde (Kumar et al., 1995), (E)-9-isopropyl-6-methyl-5,9-ocadien-2-one, isozaluzanin C (Maurer and Ohloff, 1977), lappadilactone, lappalone (Sun et al., 2003), 4β-methoxydehydrocostus lactone (Kumar et al., 1995), 12-methoxydihydrodehydrocostus
lactone, 3-oxodehydrocostus lactone, picriside B, reynosin, santamarine (Cho et al., 1998), saussureal, saussurealdehyde, saussureamine A, saussureamine B, saussureamine C, saussureamine D, saussureamine E, (+)-selina-4,11-diene, (-)-α-selinene, (+)-β-selinene (Maurer and Grieder, 1977), 4β,15,11β,13-tetrahydro-3-oxo-trans-germacran-6α, 12-olide (Chhabra et al., 1998), p-cymene, myrcene (Romauk et al., 1959), 1- pentadecene (Klein and Thomel, 1976), phellandrene (Semmler and Feldstein, 1914), α- amyrin, friedelin (Maurer and Grieder, 1977), 3β-acetoxy 9(11)-baccharane, 1- hydroxypinoresinol 1β-D-glucopyranoside, (-)-massoniresinol 4”-O-β-D-glucopyranoside, olivil 4”-O-β-D-glucopyranoside, syringing I and II, sassurine, acetic acid, α-amorphenic acid, 3-methylbutyric acid, behenic acid (Bawdekar and Kelkar, 1965), 4-ethyloctanoic acid, heptanoic acid, hexamoic acid, 3-isopropylpentanoic acid, (Z,Z)-9,12-octadecadienoic acid 2-hydroxy-1,3-propanediyl ester, (Z,Z)-9,12-octadecadienoic acid, octanoic acid, 7-octenoic acid, oleic acid (Maurer and Grieder, 1977), palmitic acid (Bawdekar and Kelkar, 1965), daucosterol, 22-dihydrostigmasterol, pregnenolone, β-sitosterol (Maurer and Grieder, 1977) and inulin.

Quantitative Standards

**Foreign matter:** Not more than 2.0 per cent. **Ash:** Not more than 4.5 per cent. **Acid-insoluble ash:** Not more than 1.5 per cent. **Ethanol-soluble extractive:** Not less than 10.0 per cent. **Water-soluble extractive:** Not more than 25.0 per cent.

Adulterants / Substituents

Roots of *Arctium lappa* Linn., *Inula racemosa* DC., *I. racemosa* Hook.f. and *Carduus edelbergii* Rech.f. ssp. *Lanatus* Kazmi (syn. *C. nutans* auct. Non Linn.) are reported adulterants of the drug, which can easily be distinguished by the absence of characteristic odour and taste of the genuine drug.

Medicinal Uses

Anti-fungal (Barrero et al., 2000; Chunekar and Pandey, 2002; Sharma, 2001), anthelmintic (Seki et al., 1992; Sharma, 2001), anti-asthmatic (Gupta and Ghatak, 1967; Sharma, 2001) and anti-diabetic (Upadhyay et al., 1996; Singh and Sharma, 1990).
Pharmacology

**Anti-diabetic activity:** The alcoholic extract lowered the blood glucose levels in rats without affecting the plasma insulin. It slightly stimulated the thyroid gland (Chaturvedi *et al.*, 1993). The drug in powder form, solid extract or decoction was effective in the treatment of diabetes in obese patients (Upadhyay *et al.*, 1996; Singh and Sharma, 1990).

**Anti-tumour activity:** The crude extract of root showed cytotoxicity with significant lethality to brine shrimp larvae and moderate cytotoxicities against human tumour cell lines (Jung *et al.*, 1998). The drug, mokko lactone and dehydrocostus lactone were shown to inhibit killing function of cytotoxic T lymphocytes (Yuuya *et al.*, 1999). Costunolide promoted cell apoptosis (Lee *et al.*, 2001) and showed cancer preventive activity against hamster check pouch carcinogenesis (Ohnishi *et al.*, 1997).

**Cardiac activity:** Pre-treatment of root powder of *S. costus* syn. *S. lappa* reversed the isoproterenol induced decrease in aortic PGE$_2$ like activity in ischaemic rabbit aorta model (Dwivedi *et al.*, 1987). The crude root powder was shown to reduce the frequency of angina in patients (Dwivedi *et al.*, 1989). Methanolic extract of drug and costunolide showed inhibitory activity against bovine aortic endothelial cell proliferation (Jeong *et al.*, 2002).

**Hepatoprotective activity:** The extract and components costunolide and dehydrocostus lactone showed significant anti-hepatitis B surface antigen activity (Chen *et al.*, 1995).

**Spasmolytic activity:** Aqueous and alcoholic extracts and fractions containing essential oil and alkaloids exhibited spasmolytic activity in isolated intestine, uterus, respiratory muscles and tracheal model; and also increase blood pressure and heart rate in dog (Bose *et al.*, 1961). The delactonized root oil and lactone fractions showed hypotensive effect in anaesthetised dogs, spasmolytic activity in various smooth muscles and bronchodilatory effect in guniea pigs (Gupta and Ghatak, 1967).