

Asthma is one of the globally concerned chronic diseases and is a major cause of morbidity and death rate. It is the 14th most important disorder in the world in terms of extent and duration of disability. The Global Asthma Report (2014) noted that asthma affected as many as 334 million people worldwide today and its prevalence has increased considerably over past 20 years, especially in children. 14% of the world's children and 8.6% of young adults (aged 18-45 years) experience respiratory and asthmatic symptoms. 4.5% of young adults have been diagnosed with asthma and/or are taking treatment for asthma. Disability, premature death and hospital admissions, is more for children aged 10-14 years and for the elderly aged up to 75-79 years. Unfortunately the World Health Organization (WHO) is not undertaking any future global asthma monitoring work; however the Global Asthma Network (GAN) continue its work with worldwide studies to ascertain out how the pattern of asthma is changing in children and adults (The Global Asthma Report, 2014). In late reports of 2007, WHO estimated that asthma accounts for approximately 500,000 hospitalizations each year, in addition to 250,000 deaths annually attributed to the disease. The rate of asthma increases as communities adopts western lifestyles and become urbanized, and there may be an additional 100 million persons diseases with asthma by 2025 (WHO, 2007). The economic burden of asthma is difficult to quantify, but estimates for various countries and regions are tremendously high particularly in developed countries. A study in the United States of America estimated that the total cost of asthma to society was \$56 billion in 2007 and \$3,259 per person per year in 2009. A further European study in 2011 has estimated the entire cost of asthma in 2011 to be €19.3 billion among Europeans aged from 15 to 64 ages. In a separate study in Asia-Pacific region, the sum of direct and indirect costs of asthma per person per year ranged from \$184 in Vietnam to \$1,189 in Hong Kong in 2000. The attempts to reduce the economic burden of asthma should move towards better management of asthma, improving access to care and adherence to evidence-based treatment, in locations where prevalence is rising at high rates (The Global Asthma Report, 2014). In low and middle income countries, prevalence of asthma is estimated to be increasing faster. Low-income countries face extra challenges to achieve good asthma management compared to other countries because of more difficulty in getting an uninterrupted supply of quality-assured, affordable essential asthma medicines, well-trained health professionals and well-organized health services to provide

long-term care. In many low-income countries, the huge majority of asthma patients is only being treated on an emergency basis during an acute attack of asthma. Health services need to be organized for the long-term management of asthma. In India, asthma imposes a big substantial burden; there is a lack of appropriate epidemiological data to determine prevalence of asthma or the allergic asthma. In a multicenter study by the Asthma Epidemiology Study Group of Indian Council of Medical Research found the prevalence of bronchial asthma in Indian adults to be 2.38%. In India, 20 to 26% people suffer from allergic rhinitis and this is reported in 75% of children and 80% of adults. Despite imposing substantial burden of asthmatic diseases and significant prevalence worldwide in children and adults, the pathological state still remains underdiagnosed, undertreated and underestimated (Aggarwal et al., 2006).

The term “asthma” comes from the Greek meaning, “to breathe hard”. The Global Initiative on Asthma (GINA) as a collaborative endeavor between the National Heart, Lung, and Blood Institute (NHLBI) of the United States Department of Health and Human services and World Health Organization has published guidelines “Global Strategy for Asthma Management and Prevention update 2011” and “Global Strategy for Asthma Management and Prevention 2006”. GINA defines asthma as a disorder by its clinical, physiological and pathological characteristics as a chronic inflammatory disorder in which airways become narrow excessively and extremely sensitized to the response to various stimuli referred as airway hyper-responsiveness (AHR) and eosinophilic airway inflammation. The inflammatory condition due to hyper responsiveness leads to persistent episodes of recurrent wheezing (especially in children), recurrent breathlessness, recurrent chest tightness, and recurrent cough (worse particularly at night). These episodes are associated with airflow obstruction within the lungs that is often reversible either spontaneously or with the treatment. Chronically inflamed airways are hyperresponsive; obstructed and hence airflow is limited due to bronchoconstriction, mucus plugs and increased inflammation. Asthma can often be diagnosed on the basis of a patient’s symptoms and medical history. A number of common factors that influence a person’s risk of developing asthma include exposure to allergens (house dust mites, animals with fur, cockroaches, pollens, and molds), occupational irritants, tobacco intake, smoking, respiratory (viral)

infections, exercise, strong emotional expressions, chemical irritants, and use of drugs like aspirin and beta blockers (GINA Guide, 2006 & 2011; Tarek, 2007).

The degree of symptoms, airflow limitation, and lung function variability allows asthma to be classified as intermittent, mild persistent, moderate persistent, and severe persistent. Asthma severity classification is represented in Figure 1.1. Clinical manifestations of asthma can be controlled with appropriate treatment. When asthma is controlled, there should be no more than occasional flare-ups and severe exacerbations. The medication for asthma in adults can be through inhalation, oral, and parenteral (subcutaneous, intramuscular or intravenous injection) routes. Inhalation is preferably used for its major advantage that the drug is delivered into airways directly. As per these guidelines medication includes Controller and Relievers. The Controllers stand for the medication meant to be taken daily for long term, to clinically control asthma by anti-inflammatory effect. The Relievers are meant to be taken during acute asthmatic attacks for bronchodilation (GINA Guide, 2006 & 2011).

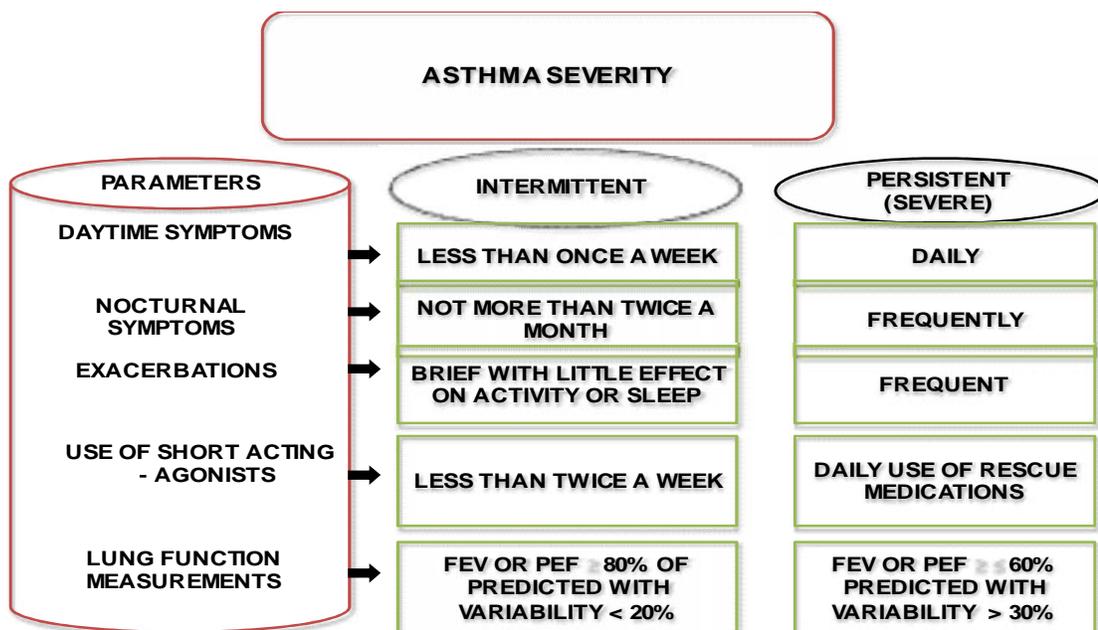


Figure 1.1: Classification of severity due to asthma (FEV: forced expiratory volume in one second; PEF: peak expiratory flow).

Global Strategy for Asthma Management and Prevention update 2011 gives the outline for the treatment available for asthmatic disorders includes: β_2 -agonists (metaproterenol and salbutamol), Anticholinergics (ipratropium and tiotropium), methylxanthines (theophylline, aminophylline and acepihylline), corticosteroids (prednisolone, dexamethasone and fluticasone), anti-leukotrienes (montelukast and zafirlukast), and mast cell stabilizers (cromolyn sodium and nedocromil sodium) (GINA Guide, 2011). Long term exposure of medication for asthma is associated with severe side effects in the form of hypothalamic-adrenal axis suppression & adrenal insufficiency, decreased bone mineral density, dysphonia (corticosteroids) (Kelly and Nelson, 2003), tachycardia, skeletal muscle tremor, hypokalemia, prolongation of QTc interval (β_2 adrenergic agonists) (Guhan et al., 2000), seizures and arrhythmias (theophylline) (Hendeles et al., 1977) etc.

Asthma involves a complex pathophysiological cascade including permanent airway obstruction, airway hyper-responsiveness, and multicellular inflammation. Inflammatory reactions of asthma involve multiple cells and cellular mediators which play major role making airway epithelium to become fragile and denuded, epithelial subbasement membranes, thicken with increased mucus production, consistency and endothelial leakage leading to mucosal edema.

Immunological reactions in asthma involve activation of mast cells initiate the acute responses to allergens and also to other indirect stimuli, such as exercise, hyperventilation and fog. Macrophages traffic into the airways and gets activated by allergens via low affinity IgE receptors, initiating an inflammatory response via the release of cytokines. Alveolar macrophages normally have a suppressive effect on lymphocyte function, but this may be impaired in asthma due the exposure of antigen. Dendritic cells (specialized macrophage) in airway epithelium are very effective antigen-presenting cells and therefore initiate allergen-induced responses in asthma. Eosinophil infiltration is a characteristic feature of asthmatic airways. Allergen inhalation result in a marked increase in eosinophils in bronchial alveolar lavage fluid, thus there is a close relationship between eosinophil counts in peripheral blood or bronchial lavage and airway hyper responsiveness (through release of basic proteins and oxygen-derived free radicals during asthma). B-lymphocytes differentiate into antibody producing plasma cells in the bone marrow, when exposed to

specific antigens, simultaneously T-cells, under the influence of thymic hormones, migrate to the thymus, which on appropriate stimulus by antigen presenting cells (APC) acquire T-cell receptor (TCR) and get differentiated to helper T-cells (with specific protein cluster of differentiation CD4+) and cytotoxic T-cells (with specific protein cluster of differentiation CD8+). The CD4+ (TH cell) subtypes of T-cells differentiate further outside the thymus into several phenotypes: TH1, TH2 and TH3 which are distinguished by the different cytokines (IL-2 and IFN- γ), they synthesize. TH1 T-cells produce cytokines that stimulate proliferation and differentiation of T-lymphocytes and NK cells. These cytokines play an important role in cell mediated immunity (CMI). Type 2 T-helper cells (TH2) release cytokine (IL-4, IL-5, IL-10 and IL-13) that stimulate B-lymphocytes production for humoral immunity. TH2-helper cells activate immunogenic cascade that results inflammation leading to the late asthmatic response. During these immunological reactions, cytokines, interleukins-1, IL-6, and tumor necrosis factor- α (TNF- α) stimulate the HPA axis with IL-1 having the broadest range of actions. IL-1 stimulates the release of CRH from hypothalamic neurons, interacts directly with the pituitary to increase the release of ACTH and may directly stimulate the adrenal gland to produce glucocorticoids. Factors, that are inhibited include components of the cytokine network, including interferon- γ (IFN- γ), granulocyte- macrophage colony-stimulating factor (GM-CSF), interleukins (IL-1, IL-2, IL-3, IL-6, IL-8, and IL-12), and TNF- α (Goodman & Gilman's, 2008; Alamgir and Uddin, 2010; Patil et al., 2011). Mediator-induced abnormalities in the parasympathetic and nonadrenergic, noncholinergic nervous systems also lead to increased bronchial hyperresponsiveness. The mediators like histamine, prostaglandins and leukotrienes contract airway smooth muscle, increase microvascular leakage, increase airway mucus secretion and attract other inflammatory cells. The cysteinyl-leukotrienes LTC₄, LTD₄ and LTE₄ are potent constrictors of human airways and reported to increase hyperresponsiveness. Cytokines play a critical dominant role in orchestrating the type of inflammatory response. Cytokines synthesized by macrophages, mast cells; eosinophils, lymphocytes and structural cells such as epithelial cells and endothelial cells participate in chronic inflammatory response. Inflammatory mediators like histamine and leukotrienes play role in the acute, sub-acute inflammatory responses and in exacerbations of asthma.

Inflammatory reactions are result of nitric oxide (NO) which is produced by several cells in airway by activation of NO synthases (NOS). An inducible form of the enzyme (iNOS) is expressed in epithelial cells of asthmatic patients and can be further induced by cytokines in airway epithelial cells. This may account for increased concentration of NO in the exhaled air of asthmatic patients. NO itself is a potent vasodilator, may increase plasma exudation in airways, and may also amplify the Th2-lymphocyte mediated response (Jeffrey, 2007; Barnes 1996).

Oxidative stress in epithelial lining covering respiratory system also plays a major role. It is vulnerable to oxidant damage because of its large surface area, role in gas exchange and host defense. The toxicity caused by oxidants which are directly inhaled, such as cigarette smoke and air-pollution or generated through an inflammatory process such as responses to allergens and viral infections, are normally balanced by the protective activity of an array of endogenous antioxidant defense system which may be functionally dependent on an adequate supply of nutritional antioxidants. Reactive oxygen species, released from eosinophil, alveolar macrophages, and neutrophils, directly contract airway smooth muscles; stimulate histamine release from mast cells and mucus secretion. Asthma is therefore associated with oxidative-antioxidative imbalance. Antioxidant status may affect asthma risk by influencing the development of the asthmatic immune phenotype, the asthmatic response to antigen provocation, or the inflammatory response during and after an asthma attack (Gupta and Verma, 2007). Thus, the cellular and physiological events like immunogenic responses: immunostimulatory and immunosuppressive due to hypersensitivity of the airway; release of mediators like histamine, TNF-alpha, ILs, LTs, NO etc. resulting pro-inflammatory sequences; and dysregulation of oxidative-antioxidative balance due to increased production of free radicals is responsible for the vitiated situation of impaired respiratory responses in the form of asthma.

The concept of Herbalism through complementary and alternative medicine has been emerged as a tool to explore potent pharmacological interventions for asthmatic reactions with nil or less side effects. Rigveda, of India and Hippocrates, the Greek physician of Western medicine believed that diseases have natural causes and used various herbal remedies for treatments. Herbalism defines the role of medicinal herbs to prevent, treat the diseases and to promote well-being (maintaining health and healing). Present scenario for

R&D is reverse pharmacology correlating the three fields of modern medicine, Indian systems of medicine and pharmaceutical science (Vaidya, 2007). WHO estimates that 80% of the world populations presently use herbal medicine for primary health care (WHO technical report series, 1996). The herbal medicine and Ayurvedic drugs are widely preferred because of less cost, strength, effectiveness, better tolerance, safety with less side-effect (Atmakuri, 2010). Over the past decade, herbal and Ayurvedic drugs have become a subject of world importance, with both medicinal and economic implications. A regular and widespread use of herbs throughout the world has increased serious concerns over their quality, safety and efficacy. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims (Malviya et al., 2011). Most of the plant-derived principles have been procured as medicine on the basis of their traditional/folklore knowledge in the health care system. There is a correlation between the indications of pure herbal substances and those of respective crude extracts used in traditional medicine. The potential efficacy of the traditional medications comprising medicinal plants has stimulated the interest of scientists and doctors to turn on the use of traditional or herbal medications for the cure from some chronic and difficult diseases. Herbal medication for asthma includes the use of various medicinal plants like *Glycyrrhiza glabra* (Glycyrrhizin), *Adhaotda vasica Nees* (Vasicine and Vasicinol), *Albizia lebbek* (Saponins), *Ephedra sinica* (Ephedrine), *Ocimum sanctum* (Myrcenol, Nerol and Eugenol), *Solanum xanthocarpum* (Solasodine), *Aegle marmelos* (Aegelin, Aegelemine), *Cinnamomum zeylanicum* (Eugenol, Cinnamic aldehyde), *Calotropis procera* (Alpha and beta amyryn), *Asystasia gangetica* (Isoflavone glycosides and Dalhorinin), *Allium cepa* (unsaturated thiosulphinates), *Achyranthes aspera* (Oleanolic acid), *Terminalia chebula* (Tannins), *Poncirus trifoliata* (flavonoids), *Clausena excauata* (Phenolic compounds, Furanocoumarins), *Magnifera indica* (Magniferin) and *Boerhaavia diffusa* (Alkaloids) etc. (Sharma et. al., 2014; Kale et. al., 2010; Jalwa, 2010; Savithramma, 2007). There is an urgent need for redefining research in the field of pharmacotherapy to counter asthma as it involves multicomponent and complex pathophysiology, with low toxicity and minimize side effects in the prevention and management in particular for children. Several herbal products/constituents which have been suggested to use for management of asthma but their pharmacologic/therapeutic basis have not yet been documented. Moreover, the

mechanisms of action are also partially explored or unexplored. Thus, herbal principles are used for asthma should be supported by scientifically validated evidences, including pre-clinical and clinical assessments which have become the criteria for acceptance of herbal health claims.

The authenticity, quality and purity of herbal drugs are the important aspects of the potential herbal medications which can be established in reference to the standard database, monograph and pharmacopoeia. The standardization is essential in order to assess the quality of herbal drugs and to prove acceptability, monographic evaluation. Safety, efficacy and quality of the plants should be ensured as an objective of the research oriented for pharmacological evaluation of anti-asthmatics. Even there are many limitations or difficulties to develop procedures for standardization of an herbal or Ayurvedic polyherbal formulation used in asthma e.g. Chyavanaprash which is an admixture of several medicinal plants (more than 20) due to their multi components with varying concentrations. The processes of botanical identification, taxonomic authentication, pharmacognostic standardization and minimizing adulteration become complex and tedious. The difference in chemical nature by virtue of individual chemical constituents in various plant drugs present in a polyherbal formulation may lead to over dosage as all chemical constituents may not be active or can be toxic on accumulation on chronic usage. Also to evaluate herbal drugs in terms of specificity, sensitivity and precision may be difficult in the absence of marker compounds.

The literature for chemical composition of these medicinal plants showed the presence of polar constituents like alkaloids, steroids, glycosides, flavonoids, saponins etc. assumed to be only effective and thus ignoring nonpolar or volatile constituents which may be of great concern if handles precisely. Every plant part exhibits more than one medicinal property so difficult to ascertain which molecules or class of compound is responsible. The volatile components are usually ignored, which may have potential therapeutics due to their higher systemic effects via inhalation routes for immediate effect, as mandatory in asthma. Perhaps pharmacological literature addresses use of many terpenoids like 1, 8-cineole, borneol, alpha terpineol, myrcene and limonene etc. which have been reported for their use as anti-asthmatic are still to be explored for drug development.

Himachal Pradesh in North West of India, where nature comprises of a good heritage of ethno botanical flora and natural wealth supporting the wide biodiversity of plants belonging to European, Tibetan, Chinese and Indian traditional medicine in the different subtropical, temperate, alpine and cold desert regions. The two plants *Hedychium spicatum* var. *acuminatum* and *Pistacia integerrima* J. L. Stewart, ex Brandis are also native of Himachal Pradesh and reported to contain flavonoids, alkaloids, steroids, tannins, essential oils and terpenoids. These plants have been suggested for the treatment of asthma and constituted into various formulations (Chauhan, 2006). Terpenoids and volatile principles have substantial potential which has to be evaluated in plants like *H. spicatum* and *P. integerrima*. The two plants have not yet been scientifically explored irrespective of their wide therapeutic uses in cough and asthma related disorders.

Thus, for the management of asthmatic syndrome, the need of standardized and scientifically evaluated herbal based products with nil or less side effects in rationalized dosage should be ascertained, which should possibly have immunomodulatory, anti-inflammatory, anti-oxidative effects with enhanced bronchodilator activity.

1.1 OBJECTIVES OF THE RESEARCH

Aim:

The present study was hypothesized to evaluate the pharmacological potential of rhizomes of *Hedychium spicatum* var. *acuminatum* and galls of *Pistacia integerrima* J. L. Stewart ex Brandis against experimentally induced asthmatic syndrome in Guinea pigs.

Objectives:

- 1 Standardization of selected plants by establishing their botanical identification, taxonomic authentication, pharmacognostical evaluation and standardization through physiochemical evaluation.
- 2 Phytochemical evaluation of the constituents to insight mechanism of the therapeutic action in selected plant drugs.
- 3 Safety and acute oral toxicity studies of prepared extracts.
- 4 Pharmacological evaluation of therapeutic potential of selected two plants against asthmatic disorders and *in-vitro* biological evaluation of plant drug extracts for their probable mechanism of actions.