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6. DISCUSSION

Bronchial asthma is a syndrome, characterized by increased responsiveness of trachea and bronchi to various stimuli and manifested by acute, recurrent and chronic attacks of widespread narrowing of airways. Clinically, asthma is expressed by airway obstruction that involves inflammation of the pulmonary airways and bronchial hyperresponsiveness that is usually reversible (Fireman P., 2003). Ayurveda has recommended number of drugs from indigenous plants sources for the treatment of bronchial asthma and other allergic disorders and have been successful in controlling the disease as well.

*Moringa oleifera* (*M. oleifera*) is one such drug, used by many Ayurvedic practitioners for the treatment of asthma and chronic rheumatism. The plant is also reported to elicit good clinical response in children suffering from upper respiratory tract infection. *Moringine*, an alkaloid obtained from this plant relaxes bronchioles (Kirtikar and Basu, 1975). Further, it is also reported that alkaloid from *M. oleifera* closely resembles ephedrine in action. However, no systemic scientific studies have been carried out to investigate the efficacy of *M. oleifera* in the treatment of bronchial asthma. The present study was aimed at evaluation of *M. oleifera* seed kernels for detailed preclinical and clinical studies to assess its mechanism of action and therapeutic potential. Further, pharmacognostical evaluation such as macroscopy, microscopy, ash value and extractive values and phytochemical studies of seed kernels were also carried out to establish its authentication and quality parameters.

Seed kernels used in the study were buff coloured ovoid and somewhat triangular in shape with one end acutely pointed. Its surface was smooth and showing three prominent longitudinally running furrows. It did not have any odour and its taste was bitter. The T.S. of seed kernel showed outer reticulated parenchymatous cells followed by cotyledon. Cotyledon consisted of epidermis, one layer of palisade cells followed by parenchymatous cells filled with starch.
grains and oil globules. Procambium was found to be thin parenchymatous elongated cells throughout the endosperm. Phloem was found to be developing from this tissue. The powder study of seed kernel characteristically showed presence of oil globules, lignified reticulated parenchyma and parenchymatous endosperm. None of these characteristics have been reported earlier. Hence it can be taken as one of the tool for the standardization of the plant material of *M. oleifera* seed kernels.

Quantitative limit tests like ash and extractive values are the parameters used to standardize the herbal drugs (Bhutani 2000). *M. oleifera* seed kernels used in the study showed that it contained 2.04 % of foreign matter, 4.128 % of total ash and 0.52% of acid-insoluble ash. It showed 48.4 % of ethanol soluble and 31.2 % of water soluble extractive values. Ethanol soluble extractive and water soluble extractive values were found to be much higher due to the fact that, seed kernels are reported to contain about 38-42% of oil and this oil also got extracted in ethanolic extract.

Phytochemical evaluation is another tool for the quality assessment. In the present study, the qualitative chemical examination of alcoholic extract of *M. oleifera* showed the presence of alkaloids, flavanoids, glycosides, tannins and terpenoids. The presence of alkaloids in the seeds (Memon et al 1985) and roots (Chopra and Chopra 1932) of *M. oleifera* have already been reported. Leaves and stem showed the presence of terpenoids (Nadkami et al 1954). Ethanolic extract of leaves and stem gum has been reported to contain nitrile glycosides (Faizi et al 1994). The results of our study suggest that all these constituents were present in alcoholic extract of seed kernels.

*M. oleifera* seed kernels were subjected to a clinical study in asthmatic patients. The preliminary study on clinical efficacy using peak flow meter was determined in 25 patients. The peak flow meter provides a simple, quantitative, reproducible and objective measurement of airflow obstruction in the larger airways. PEFR is
a simple index of pulmonary function used in both research and clinical practice. PEFR is reported to be reduced by more than 40% in asthma where the resistance of the airways is increased owing to bronchial constriction. Response to asthma treatment is usually accompanied by an increase in PEFR value and a decrease in its variability. The results of our study showed a significant increase in PEFR (42.7±5.51%). After confirming the efficacy of *M. oleifera*, the detailed study was carried out by measuring pulmonary-function tests (PFT) using spirometer in 20 patients. Spirometry provides objective, quantifiable measures of lung functions. Spirometry is the measurement of the movement of air into and out of the lungs during various breathing maneuvers. PFT is a useful tool in diagnosing and measuring the effect of drug in the treatment of pulmonary diseases.

Both the clinical studies were open label, noncomparative and conducted for 3 weeks in patients of either sex, satisfying inclusion and exclusion criteria. The results of this study of *M. oleifera* revealed that the habit of smoking was found to be prevalent to the extent of 71% and 86% respectively among asthmatic male patients. Tobacco burning, which is a ubiquitous source of indoor irritant, produces a large and complex mixture of gases, vapors, and particulate matter. More than 4,500 compounds and contaminants have been identified in tobacco smoke, among them, reparable particles, polycyclic hydrocarbons, carbon monoxide, carbon dioxide, nitric oxide, nicotine and acrolein increases the risk of respiratory tract illness (Nafstad 1997, Gilliland 2000). The present study supports the previous finding that smoking is a one of the major risk factor for asthma. It has been reported that, for many patients, the disease has its roots in infancy, and both genetic factors (Cookson 1999; Prescott et al 1998) and environmental factors like viruses, allergens and occupational exposures (Stein et al 1999; Halonen et al 1999; Venables and Chan-Yeung 1997) contribute to its inception and evolution. This indicates that asthma may be heritable disease. The family history of the asthmatic patients of both the clinical study revealed that they had such a background (20 % and 25%).
Further, the treatment with *M. oleifera* in both studies did not produce any change in general physical parameters like temperature, heart rate and blood pressure after 3 weeks of treatment. The results of hematological parameters revealed that majority of patients showed significant increase in their hemoglobin (Hb) values. These observations support earlier report that *M. oleifera* increases Hb levels in rats (Absar et al 1977). ESR was significantly reduced by treatment with *M. oleifera*. Eosinophils are considered to be the major inflammatory cells in asthma as they damage the epithelial cell lining in bronchial tree (Holgate et al 1997). In the present study, eosinophil count was not found to be significantly decreased by *M. oleifera* as most of the patients were found to have normal eosinophil count. However, in patients with eosinophil count higher, the treatment with *M. oleifera* was found to reduce the eosinophil count to normal value.

The results of the effect of *M. oleifera* on four basic symptoms of bronchial asthma like dyspnoea, wheezing, chest tightness and cough revealed that score of all symptoms was reduced significantly by *M. oleifera*. According to Unani medical theory, obstructive breathing may be due to a phlegmatic (thick sticky sputum) condition and it is produced mainly in those patients who have phlegmatic temperament. *M. oleifera* fruit is reported to cure *kapha* (Satyavati et al 1987). Our results support the effectiveness of *M. oleifera* in ameliorating the symptoms of bronchial asthma.

The another set of clinical study conducted using spirometry tests on 20 patients also revealed positive effects after 3 weeks treatment with *M. oleifera*. FVC (Forced Vital Capacity) is clinically useful as an index of lung function. FEV1 (Forced Expired Volume in one sec) is a useful measure of how fast one can breath out and full lungs can be emptied. It is a best measure of lung function for assessing airflow limitation or asthma severity (Pierce and David 2002). In the present study, a significant increase in FVC (32.97 ± 6.03 %) and FEV1 (30.05 ± 8.12 %) was observed. Out of total 20 patients, 25% of patients showed the
predicted values of FVC above 80% before treatment with *M. oleifera*. After treatment, 40% of patients were found to have the predicted values of FVC above 80%. Out of total 20 patients, 45% of patients were having FVC less than 60% of predicted values before treatment with *M. oleifera*. After treatment, only 30% of patients were found to have FVC less than 60% of predicted values. Further, 50% of patients were having predicted values of FEV₁ below 60%, which was reduced to 35% after the treatment with *M. oleifera*. Out of total 20 patients, 30% of patients were having predicted values of FEV₁ above 80% before treatment with *M. oleifera*. After treatment with *M. oleifera*, 40% of patients were found to have predicted values of FEV₁ above 80%. However, the ratio of FEV₁/FVC was not significantly changed by treatment with *M. oleifera*.

We also observed an increase in PEFR by 32.1 ± 11.7%. Out of total 20 patients, 40% of patients were having PEFR in range of 20-40% of predicted values before treatment with *M. oleifera* and this was decreased to 20% in patients having PEFR less than 40% of predicted values. The Forced Expiratory Flow between 25 and 75% (FEF₂₅-₇₅%) was also significantly increased by *M. oleifera* with a mean increase by 20.04 ± 10.64%. Out of total 20 patients, 45% of patients were having FEF₂₅-₇₅% less than 60% of predicted values before treatment with *M. oleifera*. After treatment, this was decreased to 40% of patients having FEF₂₅-₇₅% less than 60% of predicted values. Maximum ventilatory volume (MVV) was also significantly increased (34.95 ± 8.44%) by *M. oleifera*. Out of total 20 patients, 40% of patients were having MVV in range of 20-40% of predicted values before treatment with *M. oleifera* and this was decreased to 25% in patients having MVV less than 40% of predicted values.

The significant increase in lung volumes (FVC and FEV₁) and lung flow rates (PEFR, FEF₂₅-₇₅% and MVV) suggest the usefulness of *M. oleifera* in the treatment of bronchial asthma. We did not find any change in general physical parameters or hematological parameters of the patients. Further no untoward
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Effect was observed during the course of the study. All these results suggest the safety of this drug in the dose used.

*The results from the clinical study on M. oleifera suggest that, there was appreciable decrease in severity of symptoms of asthma and also simultaneously improvement in lung function parameters. Also, none of the patients showed change in any general parameters or any adverse effect suggest safety of drug in dose used. Further, the hematological profile showed enhancement in the Hb level. Considering the availability along with convenience and efficacy in oral administration, the drug offers a good future in treatment of asthma.*

Since *M. oleifera* was found to be effective in reducing the symptoms of bronchial asthma and improving the lung function parameters of asthmatic subjects, further studies were carried out to correlate the results of clinical studies with experimental studies. Several experimental studies were done on the alcoholic extracts of *M. oleifera* to reveal the possible mechanism of action of anti-asthmatic activity. Since bronchodilators, mediator release inhibitors, anti-inflammatory drugs and anti-bacterials are the different classes of drugs used conventionally in the treatment of bronchial asthma, various animal models and experimental protocols were used to determine the mechanisms of anti-asthmatic activity of *M. oleifera*.

Bronchial asthma is characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators (e.g. Histamine, Tryptase, Leukotrienes and prostaglandins). Some of these mediators directly cause acute bronchoconstriction, airway hyperresponsiveness and bronchial airway inflammation.

In present study, significant increase in preconvulsion time was observed due to pretreatment with *M. oleifera*, when the guinea pigs were exposed to either Ach
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or histamine aerosol. This bronchodilating effect of *M. oleifera* was comparable to ketotifen. It has been reported that *Albizia lebbeck* (Tripathi and Das 1977) and *Ocimum sanctum* (Singh and Agarwal 1991), which are well known anti-asthmatic herbal drugs have similar mechanism of action.

Spasmolytic effect of *M. oleifera* was also evaluated by observing the effect of its alcoholic extract on histamine, Ach, 5HT and BaCl$_2$. *M. oleifera* produced dose dependent inhibition of ileal contractions induced by histamine, Ach, 5-HT and BaCl$_2$. These indicate that *M. oleifera* has a non-specific spasmolytic activity on smooth muscle. *Tylophora asthmatica* has also been shown to possess non-specific spasmolytic activity (Harnath and Shyamalakumari 1975). These effects of *M. oleifera* correlate the improvement in the symptoms and lung function parameters of asthmatic subjects.

In addition to bronchodilating activity, a significant number of therapeutic approaches for bronchial asthma have been designed based on the antagonism of specific mediators released from mast cells. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Mast cells are found throughout the walls of the respiratory tract, and increased numbers of these cells have been described in the airways of asthmatic with an allergic component. These cells are activated as a result of an antigen or chemical and cause subsequent mobilization of calcium and degranulation of the cell (Conard 1975). Degranulated cells liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors for eosinophils, neutrophils etc. from mast cells. They play a significant role in airway inflammatory response such as airway eosinophilia, late asthmatic response and airway hyperresponsiveness as well as in immediate hypersensitivity reaction like bronchial contraction. Degranulation of mast cells has been taken as the criteria of positive anaphylaxis. Anaphylaxis and Compound 48/80 induced secretion from mast cell share a common requirement as far as the presence of calcium is concerned. However, compound 48/80 can
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utilize intracellular calcium stores to initiate the release process, even in the absence of calcium from the extracellular medium (Burka 1984). On the other hand, anaphylaxis requires the presence of calcium in the extracellular medium which moves into the cell via calcium gates in the membrane (West 1983). Ketotifen fumarate, a well-known mast cell stabilizer, reduces synthesis of prostaglandins E₂, thromboxane A₂, leukotriene C₄, and B₄. It also inhibits release of histamine, serotonin and other inflammatory mediators from mast cells. Simultaneously it blocks H₁ receptors. Khellin is a compound isolated from *Ammi visnaga* and its structural analogue furanochromone khelin. Cromolyn sodium, which is developed from the structural modification of Khellin (Cox et al 1970) is the mast cell stabilizer used in the treatment of mild to moderate asthma. *Adhatoda vasica*, *Albizzia lebbeck*, *Colesus forskohlii*, *Tylophora asthmatica* etc. are several well known drugs from indigenous plant sources used in asthma and have been reported to have mast cell stabilizing activity (Tripathi et al 1979; Atal 1980; Marone et al 1987; Geetha et al 1981). A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by alcoholic extract of *M. oleifera* points towards its ability to interfere the release and/or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity.

Further, airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The degree of bronchial hyperresponsiveness and airway obstruction is closely linked to the extent of inflammation (Bousquet et al, 2000). Thus inflammatory mediators have been implicated in the pathogenesis of allergic and inflammatory disorders like bronchitis (West 1983). Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release of mediators and the effects of inflammatory mediators. The carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors. The time course of
edema development in carrageenan induced paw edema model in rats is generally represented by a biphasic curve (Winter et al 1962). The first phase occurs within an hour of injection and is partly due to trauma of injection and also to serotonin component. Prostaglandins play a major role in the development of second phase of reaction which is measured around 3 hr time and thereafter (Vinegar et al 1969).

Alcoholic extract of *M. oleifera* possess potent anti-inflammatory activity, which was comparable to that of standard Diclofenac Sodium. Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial asthma and inflammation, the beneficial effect of alcoholic extract of *M. oleifera* could be due to inhibition of their release possibly due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

In India, the patients with bronchial asthma are commonly prescribed with antibiotics. It has been reported that 78.4% of asthmatic patients receive different antibiotics (Goyal and Patel, 2003). On further investigation, it has been reported that these patients are resistant to many antibiotics prescribed (Goyal and Patel, 2003). It is possible that these patients are suffering from bronchial infection but have been diagnosed as asthmatic patients because of their symptoms like breathlessness. It is not unusual to accept that infection may lead to bronchial hypersecretion and hence the congestion. So doctors consider it as bronchial asthma. In allopathy, multidrug approach is there where patients receive bronchodilators, corticosteroids along with antibiotics.

Further, acute exacerbations in asthma associated with production of purulent sputum usually are due to infection with virus, bacteria and fungi. These infections should be treated promptly with an appropriate antibiotic. Sometimes, nonpathogenic bacteria accumulate due to the bronchial obstruction and plugging, causing serious infection. Plants produce a range of chemical substance to protect themselves from the attack of various pathogenic
microorganisms. The substances that can either inhibit the growth of microorganisms or kill them are considered for developing new drugs for various infectious diseases. Use of these medicinal plants can substitute antibiotics to treat associated infection.

We have studied anti microbial activity of cold-water extract, hot water extract and alcoholic extract of *M. oleifera* which was tested against organisms like *E. coli*, *P. aeruginosa*, and *S. aureus* and Minimum inhibitory concentration (MIC) was found out. In the present study, cold-water extract of *M. oleifera* was found to be more active against Gram-positive bacteria, while alcoholic extract was found to be active against Gram-negative bacteria. Boiling of seeds for a short time in water diminishes anti microbial activity.

*Results of the above studies suggest that anti-asthmatic activity of M. oleifera could be due to its bronchodilator, mast cell stabilizing and anti-inflammatory property.*

However, considering that this is a crude extract, the possibility that the active principles, when eventually isolated pure, could be of high potency cannot be ruled out, especially, if such principles are present in small amounts. As a part of developing fingerprinting of the active extract responsible for anti-asthmatic activity from *M. oleifera*, we isolated a major compound resolving at Rf 0.55 under the conditions of EtoAc/MeOH/H2O (95:4:1) as mobile phase and silica gel as the stationery phase using by column chromatography. It was subjected to spectral analysis for structure evaluation by HPTLC, UV, IR, LC-Mass and NMR. The possible structure of this marker compound was found to be a derivative of benzyl isothiocyanate.

Another drug evaluated in the present study for anti-asthmatic drug is *Achyranthes aspera Linn.* (*A. aspera*). *A. aspera* is commonly found as a weed on way side and at waste places throughout India. It is widely used for asthmatic
cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhea and abdominal pain (Bhattari 1993; Reddy et al 1989; Singh 1986; Jain and Puri 1984; John 1984). A powder of dried leaf mixed with honey is useful in the early stages of asthma (Singh 1995). One of the drugs from Siddha system of medicine, Naayuruvi kuzhi thallum has A. aspera as the primary constituent is reported to be quite effective in the management of asthma (Suresh et al 1985). Saad et al (2003) have studied the efficacy of salt obtained from A. aspera in bronchial asthma. However, no systemic scientific studies have been carried out to investigate the efficacy of A. aspera in the treatment of bronchial asthma. On this basis, A. aspera was selected as a drug in the present study for detailed preclinical and clinical evaluation.

A.aspera used in the study was an erect herb or under shrub up to 1 m high. Roots were cylindrical taproot, slightly ribbed, yellowish brown in colour, odour, not distinct. Stems were yellowish brown, erect, branched, cylindrical, hairy, solid, and hollow when dry. Leaves were simple, subsessile, exstipulate, opposite, decussate, wavy margin, slightly acuminate and pubescent due to the presence of thick coat of long simple hairs. Flowers were greenish white, numerous with small dense axillary heads or spikes. Bracts and bracteoles were persisting, ending in a spine. Seeds were sub-cylindric, truncate at the apex, round at the base, black and shining.

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

Powder study of *A. aspera* showed plenty of simple multicellular spiral or straight walled warty trichomes from leaf and stem, fragments of the leaf with wavy epidermal cells and anomocytic and few anisocytic stomata in surface view, longitudinally cut fragments of xylem showing lignified spiral, pitted, scalariform and annular thickened vessels and parianth in surface view showing parallelly running thin walled narrow elongated parenchymatous cells. All these microscopic characteristic details of *A. aspera* were identical to those reported earlier (Prasad and Bhattacharya, 1961).

*A. aspera* used in the study showed that it contained 1.1 % of foreign matter, 12.66 % of total ash, 2.53% of acid-insoluble ash, 8 % of ethanol soluble extractive, 19.2 % of water soluble extractive. All these values are found to be within pharmacopoeial limit. We have measured the amount of Sodium (Na) and Potassium (K) with the help of Flame Photometer in the ash of *A. aspera*. It has been found that the ash contains 6 % Sodium (Na) and 41% Potassium (K). It has been reported that *A. aspera* contains high amount of Potash (Kirtikar and Basu). Further salt which was prepared from the ash of *A. aspera* is reported to be efficacious in bronchial asthma (Saad et al 2003). It possesses anti-asthmatic effect by dilating the trachio-bronchial tree and expelling the phlegm. Estimation of these elements has not been reported earlier. Hence it can be taken as one of the tool for the standardization of the plant material of *A. aspera*. 
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In preliminary phytochemical screening, alcoholic extract of *A. aspera* showed presence of alkaloids, flavanoids and saponins. Kumar et al (1990) have reported the presence of alkaloids and saponins, while Inflorescence showed the presence of flavanoids and alkaloids (Singh and Dogra, 1985). All these tests further justified that the drug used under study, pass the quantitative limit test as prescribed by Pharmacopoeia.

Clinical efficacy of *A. aspera* in treatment of bronchial asthma was determined by performing three weeks open label noncomparative study on 20 patients of either sex, satisfying inclusion and exclusion criteria and measuring lung obstruction with the help of spirometer. The result of clinical study revealed that, out of 70% male asthmatic patients, the habit of smoking was found to be prevalent to extend of 43%. The family history of patients for asthma revealed that 60% had such background.

Further, it did not produce any change in general physical parameters like temperature, heart rate, and blood pressure after 3 weeks of treatment. Hematological parameters were also not altered by treatment with *A. aspera*.

*A. aspera* reduced the basic symptoms of bronchial asthma like dyspnoea, wheezing, chest tightness and cough. This suggests the effectiveness of *A. aspera* in ameliorating the symptoms of bronchial asthma.

Along with the improvement in symptoms, spirometry tests done before and after 3 weeks treatment with *A. aspera* revealed significant increase in FVC and FEV₁. FVC values were increased from $1.681 \pm 0.21$ lit to $2.131 \pm 0.24$ lit with a mean % increase was found to be $30.66 \pm 8.07\%$. FEV₁ values were increased from $1.164 \pm 0.112$ lit. to $1.442 \pm 0.127$ lit. with a mean % increase was found to be $27.89\% \pm 9.89\%$. Out of total 20 patients, 25% of patients were found to have predicted values of FVC above 80% before treatment with *A. aspera*. After treatment with *A. aspera*, 30% of patients were found to have predicted values of
FVC above 80%. 45% of patients were having predicted values of FVC in 20-40% before treatment with *A. aspera*. After treatment with *A. aspera*, 30% of patients were found to have predicted values of FVC below 40%. Out of total 20 patients, 50% of patients were having predicted values of FEV1 above 60% before treatment which was increased to 60% by treatment with *A. aspera*. However, no significant change was observed in ratio of FEV1 and FVC (FEV1 / FVC %) by treatment with *A. aspera*.

Further, *A. aspera* also significantly increased PEFR and MVV. PEFR values increased from 2.37±0.17 lit/sec to 2.93 ± 0.16 lit/sec with a mean % increase of 28.64 ± 6.92%. MVV values were increased from 29.87 ± 2.52 lit/min to 37.07 ± 2.47 lit/min with a mean % increase was found to be 32.35 ± 10.98%. Out of total 20 patients, 40% of patients were having PEFR in range of 20-40% of predicted values before treatment with *A. aspera*. After treatment, this was decreased to 20% of patients having PEFR less than 40 % of predicted values. Only 15% of patients were having PEFR above 80% of predicted values before treatment with *A. aspera*. After treatment, this was increased to 40% of patients having PEFR above 80% of predicted values. However, Forced Expiratory Flow between 25 and 75% (FEF25-75%) was not significantly increased by treatment with *A. aspera*. Statistical significant increase in lung volumes (FVC and FEV1) and lung flow rates (PEFR, MVV) suggests the usefulness of *A. aspera* in the treatment of bronchial asthma.

*The results from the clinical study on *A. aspera* suggest that, there was appreciable decrease in severity of symptoms of asthma and also simultaneously improvement in lung function parameters. Also none of the patients showed change in any general parameters or hematological profile or any adverse effect in dose used suggesting good tolerability to the drug. Moreover, considering the availability along with convenience and efficacy in oral administration, the drug offers a good future in treatment of asthma.*
Discussion

Since *A. aspera* was found to be effective in reducing the symptoms of bronchial asthma and improve the lung function parameters of asthmatic subjects, several experimental studies were done on the alcoholic extracts of *A. aspera* to reveal the possible mechanism of action of anti-asthmatic activity.

Bronchial asthma is characterized by increased airway reactivity to spasmogens. In present study, the bronchodilating effect was evaluated by observing the effect of alcoholic extract of *A. aspera* on acetylcholine and histamine aerosol induced bronchoconstriction in guinea pigs. Significant increase in preconvulsion time was observed due to pretreatment with *A. aspera*, when the guinea pigs were exposed to either Ach or histamine aerosol. The bronchodilating effect of *A. aspera* was comparable to ketotifen.

Spasmolytic effect of *A. aspera* was also evaluated by observing the effect of their alcoholic extract on histamine, Ach, 5-HT and BaCl<sub>2</sub> induced contractions of guinea pig ileum. *A. aspera* was found to be dose dependently inhibited ileal contractions induced by histamine and Ach.

These effects of *A. aspera* support the improvement in the symptoms and lung function parameters of asthmatic subjects. The possible mechanism of action may be blockade of H<sub>1</sub> and Ach receptors leading to inability of smooth muscle to respond to histamine and Ach induced spasm leading to inhibition of bronchoconstriction.

In addition to bronchodilators, a significant number of therapeutic approaches for bronchial asthma have been designed based on antagonising specific mediators released from mast cells. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Mast cells are found throughout the walls of the respiratory tract, and increased numbers of these cells have been described in the airways of asthmatic with an allergic component. Compound 48/80 is one of the most potent mast cell degranulators,
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which causes liberation of mediators of inflammation such as histamine, leukotrienes, platelet activating factors, chemotactic factors for eosinophils, neutrophils etc. from mast cells. A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by aqueous extract of *A. aspera* points towards its ability to interfere the release and/or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity.

Airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The degree of bronchial hyperresponsiveness and airway obstruction is closely linked to the extent of inflammation (Bousquet et al, 2000). Thus inflammatory mediators have been implicated in the pathogenesis of allergic and inflammatory disorders like bronchitis (West 1983). Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release of mediators and the effects of inflammatory mediators. The carageenan induced paw edema model in rats is known to be sensitive to cycloxygenase inhibitors.

Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial asthma and inflammation, the beneficial effect of alcoholic extract of *A. aspera* could be due to inhibition of their release possibly due to inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis.

In addition to these, antibacterial activity of water extract and alcoholic extract of *A. aspera* was tested against organisms like *E-coli, P.aeruginosa*, and *S.aureus*. Minimum inhibitory concentration (MIC) for water extract was found to be 10, 20 and 10 mg/ml respectively. Same way, MIC for alcoholic extract was found to be 15, 30 and 10 mg/ml respectively. Water extract was found to be more active as compared to alcoholic extract.
Results of the experimental studies of A. aspera suggest that anti-asthmatic activity could be due to its bronchodilator, mast cell stabilizing and anti-bacterial property. The possible mechanism of action may be blockade of H1 and Ach receptors leading to inability of smooth muscle to respond to histamine and Ach induced spasm leading to inhibition of bronchoconstriction.