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Anti-Inflammatory Properties of Nigela Sativa on Different Cytokines in Patients of Bronchial Asthma

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ABSTRACT

Introduction:-The worldwide incidence, morbidity and mortality of asthma are increasing dramatically. It is one of the most common disorders encountered in clinical medicine in both children and adults. Asthma is an inflammatory disorder of the airway. Cytokines play an important role in the co-ordination and persistence of inflammation in asthma. In our study we aimed to observe the Anti-inflammatory effect of Nagila sativa among asthmatic patients.

Material & Methods:- The present study was conducted at the Department of Tuberculosis & Respiratory Diseases, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Total 152 patients were enrolled in this study, in which 102 were male and 50 were female along with 20 healthy controls. The patients were randomized in two groups. Group I (76 patients) was given conventional treatment, while Group II (76 patients) received extract of Nigella Sativa (N.sativa). Measurements of all cytokines were performed by sandwich ELISA method and using commercially available ELISA kits.

Results:- We found that levels of cytokines (IL-4, IL-6, IL-8, IL-10 & IL-12) increases in asthmatic patients. But when these were treated with N. sativa levels of these cytokines decreased. The present study showed that the extract from N. sativa caused inhibitory effect on these cytokines.

Conclusions:- N. sativa drug may be useful for asthmatic patients by substituting the allopathic drug or by reducing the dose of the drugs. The results also suggest that N. sativa a preventive effect on asthma.

KEYWORDS

Introduction:-

Asthma is chronic respiratory allergic disease [1, 2]. It prevalence increases globally by 50% every decade in developed and developing countries [3]. Asthma is one of the few chronic diseases in the developed world that is increasing in prevalence, despite better understanding of its pathogenesis and improved treatments [4]. It is one of the most common disorders encountered in clinical medicine in both children and adults.

Asthma is a very complex and difficult term to define in simple manner, currently it is considered to be a group of different disorders characterized by three major features: i) intermittent and reversible airway obstruction leading to recurrent episodes of wheezing, breathlessness, chest tightness and cough ii) broncho-hyper responsiveness (BHR) which is defined as an in-creased sensitivity to bronchoconstrictors such as histamine and cholinergic agonists and iii) airway inflammation [5].

Cytokines play a critical role in orchestrating, perpetuating and amplifying the inflammatory response in asthma that is evident in the asthmatic airways [6]. It is considered as a novel media-tor of chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, inflammatory bowel disease and allergic inflammation [1].

IL-4 is a short chain α-helical bundle having molecular weight 18 KD. The major cellular sources of IL-4 are thymocytes, mature T-cells, mast cells, basophils and CD4+ Th2 cells. IL-4 is thought to be an upstream cytokine that regulates allergic inflammation by promoting Th2 cell differentiation (7). IL-4 was originally identified as a B-cell growth factor, which drives the optimal stimulation of B-cells by antigen [8]. IL-4 can be described as the main cytokine involved in the pathogenesis of allergic disorders [9]. IL-4 has long been considered as a potential target in allergies and asthma.

IL-6 is a small size glycoprotein (21 KDa) secreted by monocytes/ macrophages, T cells, B cells, fibroblasts, bone marrow stromal cells, keratinocytes, and endothelial cells [10]. The levels of IL-6 in serum have been found to be elevated in a number of inflammatory diseases. IL-6 has long been considered a general marker of inflammation. Increased levels of IL-6 in serum have been found in asthmatic patients [[11].

IL-8 takes role in the activation of neutrophils and is a potent chemoattractant of neutrophils during the airway inflammation [12]. IL-8 is a pro-neutrophilic chemokine that is secreted by various cell types. It is thought to play an important role in asthma, with levels correlated with the severity of disease [13,14, 15, 16].

IL-10 is a pleotropic cytokine that has the potential to down regulate both Th1 and Th2-driven inflammatory processes [17].

IL-12 plays an important role in Th1/Th2 differentiation during primary allergen presentation. IL-12 is secreted by antigen presenting cells including-lymphocytes, monocytes/ macro-phages, and dendritic cells [18].

Herbal medicines which are derived from plants and plant extracts had been traditionally used for health improvement and scientists in recent decades are interested in understanding their mechanism of action and identifying their main constituents. Many studies have been done to show the beneficial therapeutic effects of herbal medicines, including anti-oxidant, anti-inflammatory, anti-cancer, antimicrobial and immunomodulatory [19].

Nigela sativa (N.sativa) that belongs to the Ranunculacea family has a rich historical and religious background [20]. It is native to southwest Asia but it has been cultivated and used in different parts of the world as a spice, food additive, as well as herbal medicine for a wide range of illnesses, including bronchial asthma, headache, toothache, nasal congestion, infections, obesity, back pain, hypertension, diarrhea, gastrointestinal problems and many types of cancer [21,22].

The anti-inflammatory effects of *N. sativa* and its constituents have also been reported in several diseases like experimental allergic encephalomyelitis, colitis, arthritis, sensitized animals [23,24], asthmatic patients [25,26].

Cytokines play a major role in the promotion of immune responses against invading pathogens [27]. There are two distinct cytokine producing T cell TH1 and TH2. Type 1 lymphocytes are essential for the cell-mediated immune and defence against intracellular pathogen by producing interferon γ (IFNγ), interleukin 2 (IL-2) and tumour necrosis factor-α cytokines. Whereas, type 2 lymphocytes produce cytokines including IL-4, IL-5, IL-6, IL-10, and IL-13 and are responsible for defence against extracellular pathogens by the development of humoral immunity [28,29].

We aim to study the Anti-inflammatory effect of *N. sativa* on the immune system by evaluating the serum concentrations of IL-4, IL-6, IL-8, IL-10 and IL-12 among asthmatic patients.

Material & Method:- The present study was conducted at the Department of T.B. & Respiratory Diseases, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Total 152 patients were enrolled in this study, in which 102 were male and 50 were female along with 20 healthy controls. Subjects were undergoing 4-16 week run in period in order to ensure that subjects were symptomatic prior to randomisation. A step down approach was planed during the run in period as follows:-

Group I :- Subjects who were using ICS alone in past 4 weeks prior to screening. Subjects stable on BDP 400 mcg BID or other equivalent ICS dose continue on same dose for 4 weeks.

Group II :- Subjects who were using ICS + long acting bronchodilator combination

Complete clinical and treatment data were collected. Informed consent was obtained from all the subjects. The study was approved by Institution ethics committee.

Inclusion Criteria:-

- Diagnosed asthma by a physician and having two or more of the following symptoms: recurrent wheeze, recurrent cough, chest tightness at rest, wheeze, cough or tightness during night or early morning, wheeze or cough during exercise
- Having FEV1 less than 80% predicted value
- At least 15% increase in FEV1 due to 200µg salbutamol inhalation

Exclusion Criteria:-

- Patients with cold or other respiratory or heart diseases were excluded from the study.
- Evidence of alcohol, drug or chemical abuse
- Clinically significant abnormal laboratory values
- Current smokers or those who have smoked within the last six months or those who have significant smoking history
- Acute lower respiratory tract infections within 4 weeks of the screening visit.
- Women of child bearing age
- Pregnant or lactating women

Blood collection:- Blood samples (3-5 mL) were collected in plain vacutainers from the asthmatic patients before giving them *N. Sativa* and after the treatment of *N. Sativa.* The blood samples were centrifuged at 5,000 rpm for 10 min and then the serum samples were collected and stored at -20°C until they were assayed.

Preparation of the drug *(N. sativa):-*

Formation of the Tablets:- The seed of the plant *N.sativa* were collected from the open market.

1500 ml of Distilled water was poured into round bottom flask. 500 gm of the sample was placed in the thimble and was inserted in the centre of the extractor. The Soxhlet was heated at 70° C. When the solvent was boiling, the vapour rises through the vertical tube into the condenser at the top. The liquid condensate drips into the filter paper thimble in the centre which contains the solid sample to be extracted. The extract seeps through the pores of the thimble and fills the siphon tube where it flow back down in to the round bottom flask. This was allowed to continue for the time until the fluid in the siphon tube gets cleared.

The extract thus obtained was dried by evaporation and a semi solid, lusturious substances was obtained.

The extract after mixture of gum and marc needed well within aim of uniform distribution of drug constituents and the granule was prepared with an automatic granular. The tablets were prepared there after using tablet making machine.

Friability Test:- The friability test was carried out in an instrument called Friabilator. A friability test apparatus should stimulate the conditions that the product will be exposed to during the process of production. This test is used to determine the physical strength of uncoated tablets upon exposure to mechanical shock and attrition.

The friability is related to tablet hardness and is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping. We were used Roche Friabilator for friability test.

Tablet Disintegration:- The disintegration test is measure of the time required under a given set of conditions for a group of tablets to disintegrate into the particles which will pass through a 10 mesh screen. Generally, the test is useful as a quality assurance tool for conventional dosage forms.

Dissolution Test:- Dissolution is pharmaceutically defined as the rate of mass transfer from a solid surface into the dissolution medium or solvent under standardized conditions of liquid/solid interface temperature and solvent composition.

Cytokine assay:- Measurements of all cytokines were performed by sandwich ELISA method and using commercially available ELISA kits. ELISA test was performed following the supplier's instructions. The absorbance values were analyzed by ELISA reader at 450 nm (Thermo Electron Corporation, Vantaa, Finland).

Statistical Analysis:- All statistical analyses were performed using SPSS Statistics (version-20). Pair wise analysis using Chi square test was done to compare differences in cytokines levels between groups of patients. Analysis and results were con-

sidered significantly different at *p<0.05*.

Results:- Total 152 patients were enrolled in this study, in which 102 were male and 50 were female along with 20 healthy control. The patients were randomized in two groups. Group I (76 patients) was given conventional treatment, while Group II (76 patients) received extract of *Nigella Sativa*. These patients were divided in to five age groups (Table-1). It is observed that maximum number of patients belongs to age group of 20-30 years. During the study we observed that according to the occupation maximum number of the patients by profession was farmers (Table-2). On the bases of the clinical feature we divided patients into four groups, we observed that breathlessness and wheeze were present in all 152 cases (102 males and 50 females) (Table-3).In our study levels of IL-4 and IL-6 has been shown to be elevated in asthmatic patients. These cytokine plays an active role in pathogenesis of asthma. The present study showed that the extract from *N. sativa* caused inhibitory effect on IL-4 (Fig-1).

The levels of IL-6 in serum have been found to be elevated in a number of in Asthma, but after the treatment of *N. sativa* level of IL-6 has been decreased (Fig-2).In Asthmatic patients levels of IL-6 has been found increased compared to healthy control (P<0.005). As a result, IL-6 has been considered a general marker of inflammation.

The levels of IL-8, IL-10 and IL-12 have been elevated in asthmatic patients compared to healthy control and after the treatment of *N.sativa* the level was decreased *(P<0.005)*. These cytokines may be considered as anti-inflammatory and used as a bio-marker for asthmatic patients.

The levels of these cytokines have been decreased among those patients who were treated with *N. sativa* (Group 2) compared to those patients who were on conventional treatment (Group 1) (Fig3,4,5 Respectively) *(P<0.005)*.

Discussion:- The present study was aim to understand the anti-inflammatory properties of *N. Sativa* and to observe the expression of several circulating cytokines in asthmatic individuals. To assess the potential value of these cytokines as biomarker for asthma. We compared serum levels of these asthma associated mediators in three groups: 1) Patients of asthma 2) Asthmatic patients on conventional treatment with *N.sativa* 3) healthy control.

In our study we found the elevated levels of these cytokines (IL-4, IL-6, IL-8, IL-10 & IL-12) compared to healthy control (P<0.005). When we treated these asthmatic patients with N. Sativa the levels of these cytokines was found significantly decreased (P<0.005)

IL-4 can be described as the main cytokine involved in the pathogenesis of allergic disorders [9]. IL-4 has long been considered as a potential target in allergies and asthma.

IL-6 has long been considered a general marker of inflammation. However, it is becoming evident that IL-6 is not simply a pro-inflammatory marker, but an active factor that contributes to the pathogenesis of certain inflammatory diseases such as rheumatoid arthritis, and a successful target for some of these diseases [16]. Our studies shows that the presence of IL-6 in the lung airways correlates with an impaired lung function in different subsets of asthmatic patients, and suggests that IL-6 is likely to be directly involved in the pathogenesis of asthma and the progressive loss of lung function observed in patients who remain untreated. As such, IL-6 is likely a potential target for new treatments for this important disease.

N. sativa, a plant native to South East Asia is known for its relaxant effect on smooth muscle tissue [30, 31, 32]. In addition, Boskabady and colleagues have demonstrated that extracts and oils from *N. sativa* have inhibitory effects on histamine (H1) receptors [33], stimulatory effects on α-adrenergic receptors [34] and anti-tussive effects [35]. To translate these promising in vitro findings Boskabady and colleagues set out to investigate the effect of boiled *N. sativa* extract on lung function in asthmatic patients [36]. In this study they compared the bronchodilatory effects of *N. sativa* with those of theophylline in 15 asthmatics. They found that *N. sativa* did induce bronchodilation within 30 min of administration but the magnitude was significantly less than observed with theophylline and that both agents were significantly less effective than the beta-2 agonist salbutamol.

N. sativa can enhance immune responses in human. The majority of subjects who treated with N. sativa oil for 4 weeks showed a 55% increase in CD4 to CD8 T cells ratio, and a 30% increase in natural killer (NK) cell function. The results have been presented by A. E1-Kadi and O. Kandil to the 1st International Conference on Scientific Miracles of Quran and Sunnah, held in Islamabad, Pakistan [37].

A variety of experiments have shown that excessive or insufficient production of cytokines may significantly contribute to the pathophysiology of a range of disease responses and are thought to be decisive for pathological or physiological consequences [38]. After activation, CD4 T helper cells differentiate into either TH1-type cells, secreting IL-2, IL-12, IFN-g and TNF-a, or TH2- type cells secreting IL-4, IL-5, IL-10, and IL-13. Indeed, the balance between TH1 and TH2 cytokines is critical for the orientation of the inflammatory response toward cell-mediated or humoral-mediated responses. Thus, any factors that can interfere with TH1/TH2 axis might affect the outcome of the response [39]. Therefore, further studies are required to explore the specific cellular and molecular targets of *N. sativa.*

In our study for this purpose we evaluated the cytokine levels of different T cell sub-types. IL-8 IL-10 and IL-12 levels were higher in Asthmatic patients compared to healthy individuals and treated with *N. sativa.* As it was previously reported that *N.sativa* reduced these cytokines release [17, 18] In contrast, IL-8, IL-10 and IL-12 showed a statistically significant difference in patients before/after therapy, suggesting a value in monitoring circulating cytokine levels in severe persistent asthma patients receiving *N.sativa* therapy that also indicates that *N.sativa* therapy provides clinical benefits.

Conclusions:- In conclusion, the results of the present study indicated that *N. sativa* extract prevents pathological changes of the lung. Our study showed that levels of these cytokines decreases by the treatment of *N.sativa.*

Modern drugs which are extensively used for the treatment of Bronchial asthma has many side effects, *N. sativa* drug may be useful for asthmatic patients by substituting the allopathic drug or by reducing the dose of the drugs.

Future studies will be needed to address the clinical importance of these findings on these cytokines and *N. sativa* drug for the treatment of pulmonary diseases.

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Table:- 1 Distribution of Patients according to age and sex

Age Group	Males	Females	Total
10-20	18	5	23 (15%)
20-30	40	12	52 (34%)
30-40	24	7	31 (20%)
40-50	10	20	30 (19%)
50-<	10	8	18 (11%)
Total	102	52	152

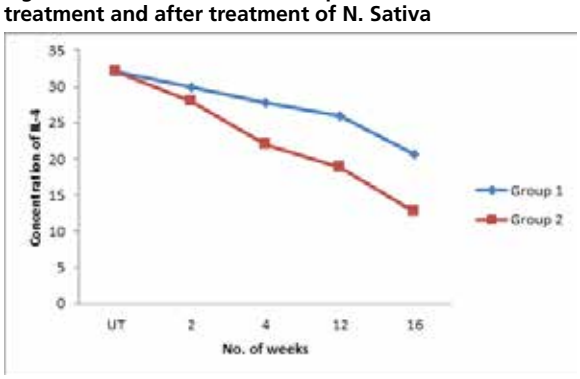
Table-2 Distribution of patients according to occupation

Occupation	No. of patients	Percentage
Labour	22	14%
Farming	59	38%
Business	18	11%
House wives	9	5%
Students	22	14%
Executives	22	24%

Table-3 Distribution of patients according to Clinical features

Symptoms	No. of patients		Total
	Male	Female	
Breathlessness	102	50	152 (100%)
Wheeze	102	50	152 (100%)
Cough	58	18	76 (50%)
Sputum	32	20	52 (34%)

Fig.1 Level of IL-4 in Asthmatic patients on conventional treatment and after treatment of N. Sativa

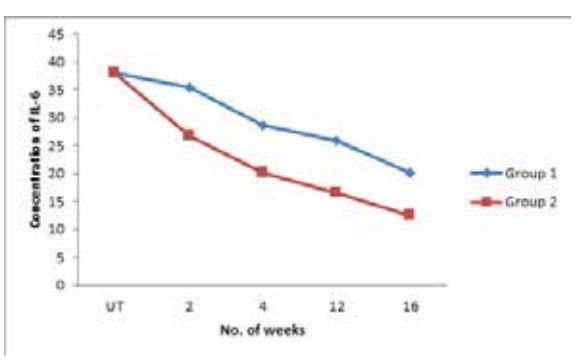


Group 1 :- Asthmatic Patient on Conventional treatment

Group 2 :- Asthmatic patients after treatment of *N. sativa*

UT:- Untreated

Fig.2 Level of IL-6 in Asthmatic patients on conventional treatment and after treatment of N. Sativa

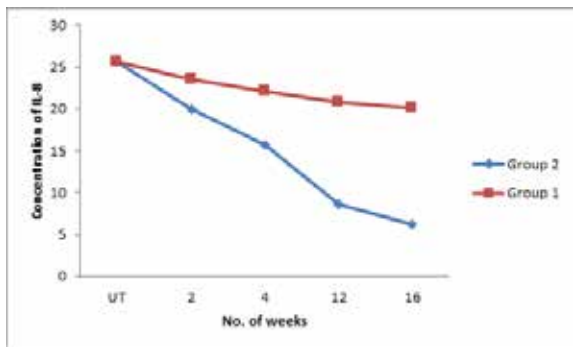


Group 1 :- Asthmatic Patient on Conventional treatment

Group 2 :- Asthmatic patients after treatment of *N. sativa*

UT:- Untreated

Fig.3 Level of IL-8 in Asthmatic patients on conventional treatment and after treatment of N. Sativa

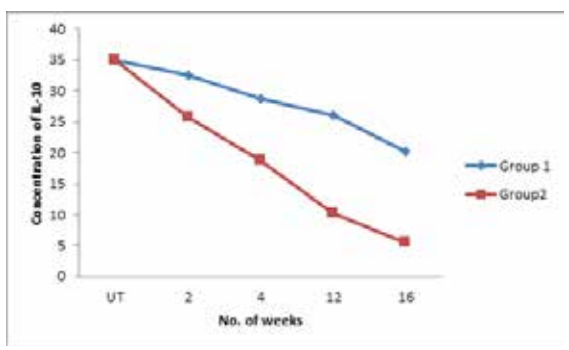


Group 1 :- Asthmatic Patient on Conventional treatment

Group 2 :- Asthmatic patients after treatment of *N. sativa*

UT:- Untreated

Fig.4 Level of IL-10 in Asthmatic patients on conventional treatment and after treatment of N. Sativa

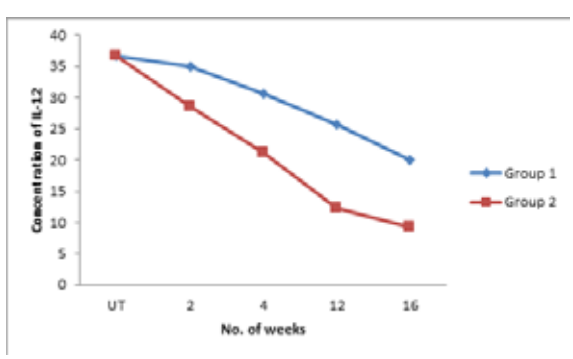


Group 1 :- Asthmatic Patient on Conventional treatment

Group 2 :- Asthmatic patients after treatment of *N. sativa*

UT:- Untreated

Fig.5 Level of IL-12 in Asthmatic patients on conventional treatment and after treatment of N. Sativa



Group 1 :- Asthmatic Patient on Conventional treatment

Group 2 :- Asthmatic patients after treatment of *N. sativa*

UT:- Untreated

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