

EVALUATION OF THE EFFECT OF *Vitex nigundo* LEAVES EXTRACT ON BRONCHOCONSTRICTION AND BRONCHIAL HYPERREACTIVITY IN EXPERIMENTAL ANIMALS

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Summary

The present investigation was undertaken to evaluate the bronchodilating and bronchial hyperreactivity of alcoholic extract of *Vitex nigundo* Linn. leaves in experimental animals. Effect of extract was studied on Toluene diisocyanate induced asthma. Bronchodilator activity of alcoholic extract of *V. nigundo* leaf studied on the histamine and acetylcholine aerosol induced bronchospasm in guinea pigs and bronchial hyperreactivity was studied on broncho-alveolar lavage fluid in the egg albumin sensitized guinea pigs. Effect on hypersensitivity reaction was studied by hetrologus passive cutaneous anaphylaxis model using rats and mice. *In vitro* mast cell stabilizing activity was studied using compound 48/80 (p-Methoxy-N-methylphenethylamine) as degranulating agent. Histopathological studies clearly showed protective effect of extract against toluene diisocyanate induced asthma. Treatment with alcoholic extract of *V. nigundo* leaves (200 and 400 mg/kg, p.o., for 7 days) showed significant protection against histamine and ach aerosol induced bronchospasm in guinea pigs. Significant decrease in the total leukocyte and differential leukocyte count in the broncho-alveolar lavage fluid of the egg albumin sensitized guinea pigs was observed by administration of alcoholic extract of *V. nigundo* leaves (200 and 400 mg/kg, p.o., for 15 days, p.o.). Three days treatment with extract of *V. nigundo* leaf also inhibits hypersensitivity reaction. Alcoholic extract of *V. nigundo* leaves dose dependently protected the mast cell disruption induced by compound 48/80. These results suggest that alcoholic extract of the *V. nigundo* leaves has not only bronchodilating activity but also decreases bronchial hyperreactivity and provide protection against asthma. This activity is may be due to flavonoids and phenolic compounds present in extract.

Key words: *Vitex negundo* Linn, Toluene diisocyanate, Mast cell stabilization, Hetrologus passive cetaneous anaphylaxis, Broncho-alveolar lavage

Introduction

Bronchial asthma is a chronic respiratory disorder affecting large population of the world. Most asthmatic patients are diagnosed by a triad of episodic symptoms: cough, wheezing and dyspnoea. Asthma no longer can be viewed as a result of combination of hyperreactivity and inflammatory response. Majority of drugs currently used in the treatment of asthma disease fall in to two broad therapeutic categories: Bronchodilator and anti-inflammatory drugs (1). None of the available modes and regimens in modern medicine can be considered ideal taking in to account their only symptomatic relief and loss of effectiveness on continued use(2). Hence we are still in search for newer and safer drugs for the treatment of not only asthma but in many other disorders related to that. Herbal medicines are recognized by WHO as essential building blocks for primary health care, especially in developing countries like India, but the herbal and other indigenous source have not adequately been explored for the presence of safe and effective anti asthmatic drugs. Though certain plants and their constituents have been reported to posses significant anti asthmatic potential during last three decades, the studies have not been carried forward to a logical conclusion for establishing their clinical usefulness as has been done for various modern drugs like β_2 agonist and steroids. *Vitex negundo* (Nagod) is highly reputed plant in ayurveda (Family: Verbenaceae). It is having various activities like in cough, asthma, rheumatic disorder, as tonic and in snake bite also (2)Anti asthmatic activity of Nagod is quoted in many books of ayurveda and herbal pharmacopoeia(2) *V. negundo* leaf extract have free radiacal scavenging activity (3). Mature leaves have anti-inflammatory activity (4).The bronchodilator and mast cell protection activity of *V. negundo* leaves was already studied using alcoholic extract (5). In the light of above reports the present investigation was undertaken to study the effect of alcoholic extract of *V. negundo* leaves for the anti-asthmatic potential.

Materials and Methods

Preparation of plant extract

The leaves of plant *V. negundo* collected and identified by morphological and microscopical studies (voucher specimen no. MAP/27/2008). The leaves were air dried under shade. The powdering was done after drying of a week by grinding. The powder was passed through 40 # sieve. The extract was prepared using soxhlet extractor using 70% ethanol as a solvent. The extract was concentrated under controlled temperature below 50° C in porcelain dish to get the syrupy mass (yield 5.2%). The extract was weighed and dissolved in sufficient amount of water to prepare required concentration. Alcoholic extract of leaves was subjected to various qualitative tests for the identification of phytoconstituents (6).

Experimental animals

Wister rats (175-200 g) and guinea pigs (400-600 g) of either sex housed in standard conditions of temperature ($22 \pm 2^\circ\text{C}$), relative humidity ($55 \pm 5\%$) and light (12 hrs light/dark cycles) were used. They were fed with standard pellet diet and water *ad libitum*. In addition to pellet diet guinea pigs were supplemented with Lucerne. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of CPCSEA. A minimum of six animals were used in each group. Throughout the experiments, animals were processed according to the suggested ethical guideline for the care of laboratory animals.

Acute toxicity test

Acute toxicity of the plant extract was determined using female albino mice. The animals were fasted for 3 hr prior to the experiment according to the recommended procedure (OECD guideline No. 425). Animals were observed for 48 hr for any mortality following oral administration of the different doses of the preparation.

Studies on histamine and acetylcholine induced bronchospasm in guinea pigs

Experimental bronchial asthma was induced in guinea pigs by exposing them to histamine and acetylcholine aerosol (7). Guinea pigs were selected and divided into four groups each containing six animals out of which groups I and group III were exposed to 0.1% w/v of histamine dihydrochloride aerosol and another group II and group IV were exposed to 0.5% w/v of acetylcholine bromide aerosol. The animals exposed to histamine and acetylcholine aerosol showed progressive dyspnoea. The end point preconvulsive dyspnoea (PCD) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from chamber and placed in fresh air. This time of PCD was taken as day 0 value. Guinea pigs of group-I and group III were treated with the alcoholic extract of *V. negundo* leaves 200mg/kg, p.o. and group II and group IV were treated with 400 mg/kg, p.o. once a day for 7 days. On the 7th day 2 h after the last dose, the time for the onset of PCD was recorded as on day 0. The percentage increased in time of PCD was calculated using following formula (8).

$$\text{Percentage increased in time of PCD} = \left(1 - \frac{T_1}{T_2}\right) \times 100$$

Where: T_1 = time for PCD onset on day 0, T_2 = time for PCD onset on day 7

Hetrologus passive cutaneous anaphylaxis model (9)

The present study was done using Hetrologus PCA model i.e from mice to rats for evaluation of alcoholic extract of *V. negundo* leaves on immediate hypersensitivity reaction. The rats were injected with 0.1ml, 1% egg albumin i.p and 0.1 ml of Bordetella Pertusis vaccine (BPT vaccine) on 1st, 3rd and 5th day. After 21 days of the immunization, blood was collected from tail vein. Serum was separated by centrifugation at 3000 rpm for 15 min. and stored below -20°C before use. Mice were selected randomly and divided in following Groups: control group, anti-ovalbumin sensitized group, Alcoholic extract of *V. negundo* leaves (200mg/kg) treated group, Alcoholic extract of *Vitex negundo* leaves (400mg/kg) treated group. The anti-ovalbumin serum was injected intradermally on the clipped dorsal skin of the mice. Extract was administered to mice according to their group for 3 consecutive days from the day of passive sensitization. After treatment, injection 1 ml of 0.5% Evans blue solution containing 20mg of egg albumin was injected intravenously through tail vein. Because of antigen-antibody reaction there was increased vascular permeability and dye will penetrate in that tissue where hypersensitivity reaction takes place. This area of skin was removed after rats were sacrificed. Transferred the skin portion to the solution of 70% acetone for 24 hrs. The dye was extract out in the acetone and Evans blue dye was measured colorimetrically at 620 nm. The amount of dye penetrates in the skin area reflect the severity of hypersensitivity reaction.

Studies on broncho-alveolar lavage fluid (BALF) in egg albumin sensitized guinea pigs (10-11)

Guinea pigs were selected and divided in four groups i.e. group I (control saline 10 ml/kg), group II (sensitized), group III (sensitized + Alcoholic extract of *Vitex negundo* leaves

200mg/kg, p.o.) and group IV (sensitized + Alcoholic extract of *Vitex negundo* leaves 400mg/kg, p.o.) each containing six animals. The guinea pigs of group II, group III and group IV were sensitized with egg albumin (1 ml, 10% w/v, i.p.) on the 1st day. The animals of group III and group IV were dosed once daily for fifteen days with alcoholic extract of leaves of *V. nigundo*. Two hour after the last dose of drug administration (on 15th day), all the animals of group II, group III and group IV were again challenged with egg albumin (0.5 ml, 2% w/v, i.v.) through saphenous vein. After 3 h of the challenged of the egg albumin or just prior to death of animals which ever was earlier, the trachea was immediately cannulated after anaesthetization and the airways lavaged with saline at 25°C (two aliquots of 1 mL/100 g body weight). Broncho-alveolar cells were collected in two successive lavages using saline and recovered through a tracheal cannula. The broncho-alveolar lavage fluid (BALF) was stored on ice and total WBC cell counts were performed using light microscope. Dilutions of lavage fluid (1 in 10) were made in saline, and differential WBC were counted by light microscopy stained with Leishman's stain. At least 200 cells were counted on each slide. Cells were differentiated using standard morphological criteria. All differential cell counts were performed blind and in randomized order at the end of the study. The result obtained were compared with controlled with sensitized group and sensitized treated groups.

Toluene diisocyanate induced asthma in rats (12)

The Wistar rats were randomly selected and divided in to 4 different groups. Group 1 (Control group) Group 2 (Toluene diisocyanate 5 μ l, 5%, intranasal), Group 3 (Toluene diisocyanate 5 μ l, 5%, intranasal + Alcoholic extract of *V. nigundo* leaf, 200mg/kg, p.o), Group 4 (Toluene diisocyanate 5 μ l, 5%, intranasal + Alcoholic extract of *V. nigundo* leaf, 400mg/kg, p.o). All the rats (except group 1) were sensitized by dropping 5 μ l of 10% toluene diisocyanate into each nostril for seven consecutive days. After a week of rest all the rats (except group 1) were resensitized for seven days. A week after the second course of sensitization the rats were provoked by intranasal administration of 5 μ l of 5% toluene diisocyanate. All the animals of group 3 and 4 were given the respective drug treatment for 15 days prior to the initiation of sensitization till the day of final challenge with 5% toluene diisocyanate. The last dose of drug treatment was given 2 hrs prior to challenge with 5% toluene diisocyanate. After the completion of exposure the animals were sacrificed and lungs were removed, fixed in 10% formaldehyde in saline for 5 min and dehydrated through a series of ethanol solutions, and embedded in paraffin. Sections were sliced and stained with hematoxylin-eosin for examination. The sections were observed for any pathological change in lungs.

In vitro mast cell degranulation by compound 48/80

Effect of alcoholic extract of *V. nigundo* leaves on compound 48/80 induced mast cell degranulation was studied according to methods described by Norton (1954) (13) and Kanemoto et al. (1993) (14). Normal saline containing 5 units/ml of heparin was injected in the peritoneal cavity of ale rats lightly anaesthetized with ether. After a gentle abdominal massage, the peritoneal fluid containing mast cells was collected in centrifuge tubes placed over ice. Peritoneal fluid of rats was collected and centrifuged at 2000 rpm for 5 min. Supernatant solution was discarded and the cells was washed twice with saline and resuspended in 1 ml of saline. All the solutions were prepared in normal saline. The peritoneal cell suspension divided in six parts viz. negative control, positive control, reference standard (Ketotifen 10 μ g/ml), Extract of *V. nigundo* of three concentration i.e. 500, 750, 1000 μ g/ml each containing 0.1 ml of cell suspension and incubated in a constant temperature in water bath at 37°C for 15 min.

Then 0.1 ml of compound 48/80 (phenethylamine p-methoxy n-methyl) (10 µg/ml) was added in all samples except in negative control and the suspensions were further incubated for 10 min at 37°C. The cells were then stained with 10% of Toluidine blue solution and observed under the high power of light microscope. The percentage granulated and percentage degranulated mast cell were counted. Positive control group in which compound 48/80 was added without addition of test agents i.e. kitotifen and *V. nigundo* and a negative control group in which neither compound 48/80 nor the test agents were added to correct for spontaneous degranulation of mast cells without any degranulating agent.

Statistical analysis

The results of various studies were expressed as mean ± SEM and analyzed statistically using one way ANOVA followed by Student's *t*-Test to find out the level of significance. Data were considered statistically significant at minimum level of $p < 0.05$.

Results

Acute toxicity test

In toxicity test, no mortality was observed and the extract was found to be safe up to a dose of 2000 mg/kg of body weight.

Phytochemical screening of alcoholic extract of *V. nigundo*

Qualitative screening of phytochemical showed presence of flavonoids, carbohydrates, phenolic compounds and alkaloids in alcoholic extract of *V. nigundo*

Studies on histamine and ach induced bronchospasm in guinea pigs

alcoholic extract of *V. nigundo* significantly and dose dependently increased PCD time following exposure to histamine ($p < 0.001$) and acetylcholine ($p < 0.01$) aerosols induced bronchospasm in guinea pigs (Table 1). Increased in the time of PCD was more against histamine aerosol as compared to acetylcholine aerosol following administration of *V. nigundo* leaves extract.

Heterologous passive cutaneous anaphylaxis model

Treatment with alcoholic extract of *V. nigundo* leaves decrease amount of dye leaked in area of hypersensitivity reaction. Decrease in amount of dye leaked indicate that extract treatment inhibit hypersensitivity reaction (Table 2).

Studies on broncho-alveolar lavage fluid (BALF) in egg albumin sensitized guinea pigs

After fifteen days guinea pigs were again challenged with egg albumin, in the broncho-alveolar lavage fluid significant increase in the total leukocyte count and differential leukocytes count were observed in sensitized i.e. group II ($p < 0.001$) as compared to the control i.e. group I. alcoholic extract of *V. nigundo* (200 and 400 mg/kg, p.o., for 15 days) significantly and dose dependently decreased in the total leukocyte count ($p < 0.05$) and differential leukocytes count ($p < 0.001$) was observed in group III and group IV as compared to group II (Table 3).

Toluene diisocyanate induced asthma in rats

Histological analysis of the lungs from non-sensitized i.e. group I showed normal lung histology (Figure 1A). Histological sections of lung tissue of toluene diisocyanate exposed group

(group 2) exhibited airway inflammation, infiltration of eosinophils, lymphocytes and sub mucosal edema of the lungs, bronchoconstriction shown as lumen plugging by mucus and cells (Figure 1B). Treatment with *V. nigundo* i.e. group III and group IV prevented the tissue edema, epithelial cell hypertrophy, infiltration of inflammatory cell, and airway lumen plugging thereby decreasing inflammation and bronchoconstriction which leads to normal lumen size (Figure 1C, 1D).

In vitro mast cell degranulation by compound 48/80

Alcoholic extract of *V. nigundo* and ketotifen was found to significantly ($p < 0.001$) inhibit *in vitro* rat peritoneal mast cell degranulation induced by compound 48/80 as compared to base line value i.e. positive control group (Table 4).

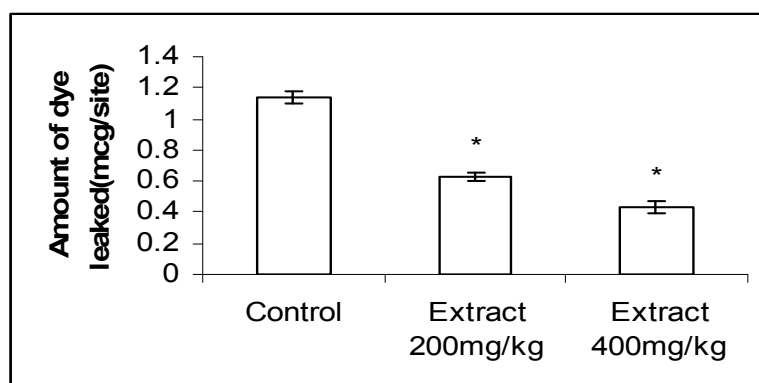
Table 1: Effect of alcoholic extract of *V. nigundo* leaves (p.o., for 7 days) on histamine and ach aerosol induced bronchospasm in guinea pigs.

Groups	Preconvulsion Dysepnoea Time (sec)		
	Before Treatm (control)	After Treatm	% increase in th econvulsion Dysej
Histamine Aerosol (0.1% w/v)			
I- <i>V. nigundo</i> (200 mg/kg)	125.8 ± 18.5	447.2 ± 25.34	72.82 ± 3.14 [†]
II- <i>V. nigundo</i> (400 mg/kg)	127.4 ± 20.3	632.7 ± 53.47	80.67 ± 6.23 [†]
Ach aerosol (0.5% w/v)			
III- <i>V. nigundo</i> (200 mg/kg)	149.27 ± 27.1	354.5 ± 45.09	58.73 ± 3.09 [†]
IV- <i>V. nigundo</i> (400 mg/kg)	137.4 ± 23.6	438.57 ± 43.71	67.98 ± 4.61 [†]

Values are expressed as mean ± SEM for 6 guinea pigs in each group,

* $p < 0.001$, # $p < 0.01$ when compared with control group

Table 2. Effect of alcoholic extract of *V. nigundo* leaves on hetrologus passive cutaneous anaphylaxis model.



• $p < 0.05$ when compared with control, $n = 6$ in each group.

Table 3. Effect of alcoholic extract of *V. nigundo* leaves (p.o., for 15 days) on Broncho-alveolar lavage fluid in egg albumin sensitized guinea pigs:

	Control	Sensitized	Sensitized + <i>V. nigundo</i> (200 mg/kg)	Sensitized + <i>V. nigundo</i> (400 mg/kg)
Total leucocyte count/cmm	8,842 ± 429	14,740 ± 670.9 *	11,680 ± 313.5 @	9,680 ± 248.32 \$
Neutrophil count/cmm	2,774 ± 304.2	4,100 ± 169.9 *	3,921 ± 287.25 #	3,081 ± 267.34 \$
Lymphocyte count/cmm	4,472 ± 384.8	9,130 ± 235.2 *	6,772 ± 343.2 \$	5,892 ± 327.82 \$
Eosinophil count/cmm	184.8 ± 20.82	506.2 ± 36.71 *	420.3 ± 19.45 \$	337.78 ± 13.26 \$
Monocyte count/cmm	109.3 ± 19.28	263.1 ± 44.52 *	200.5 ± 21.08 \$	153.6 ± 18.31 \$

Values are expressed as mean ± SEM for 6 guinea pigs in each group,

* $p < 0.001$ when compared with control group,

@ $p < 0.05$, # $p < 0.01$, \$ $p < 0.001$ when compared with sensitized group

Table 4. Effect of alcoholic extract of *V. nigundo* leaves on Compound 48/80 induced mast cell degranulation:

Treatment	Concentration (µg/ml)	Mast cells	
		% Granulated	% Degranulated
negative control (untreated mast cell)	-	91.83 ± 0.654	8.167 ± 0.654
Positive control (treated with compound 48/80)	-	26.5 ± 1.176	73.5 ± 1.176
Ketotifen	10	80.5 ± 0.9574*	19.5 ± 0.9574
<i>V. nigundo</i>	500	41.17 ± 0.9457*	58.83 ± 0.9457
<i>V. nigundo</i>	750	50.67 ± 0.666*	49.33 ± 0.666
<i>V. nigundo</i>	1000	63.17 ± 0.9457*	36.83 ± 0.9457

Values are expressed as mean ± SEM, n=6 in each groups,

- $p < 0.001$ when compared with base line value i.e. positivecontrol

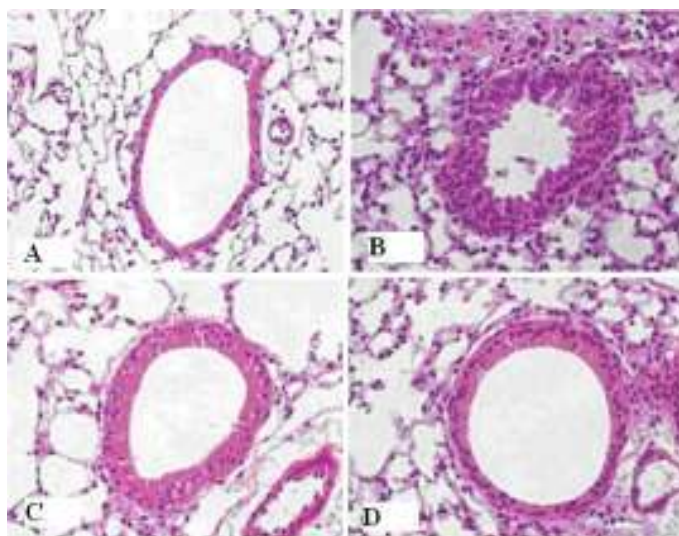


Figure 1: Effect of alcoholic extract of *V. nigundo* leaves on the histology of lungs of toluene diisocyanate induced asthmatic rats. (A) Control group (B) Toluene diisocyanate exposed group (C) Toluene diisocyanate + *V. nigundo* treated (200 mg/kg, p.o.) (D) Toluene diisocyanate + *V. nigundo* treated (400 mg/kg, p.o.)

Discussion

Bronchial asthma is commonly characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators like histamine triggered by exposure to allergens that directly cause acute bronchoconstriction (15-16). In the present study, histamine and acetylcholine were used as spasmogens in the form of aerosol to cause immediate bronchoconstriction in the form of PCD in guinea pigs. Bronchodilating effect of alcoholic extract of *V. nigundo* leaves was evaluated by observing its effects on the time of PCD. In our study we found that the time of occurrence of PCD was significantly increased that suggestive of bronchodilating activity following treatment with *V. nigundo* against spasmogens. Mediators like leukotriene, prostaglandins, PAF and cytokines are reported to be responsible for the immediate hypersensitivity reaction. Enhanced vascular permeability and leukocyte infiltration also observed in hypersensitivity reaction at the sites of allergen challenge. In Heterologous PCA model antiovalbumin serum obtained from sensitized rats was injected to the mice. The enhanced vascular permeability was estimated by Evans blue dye. The leakage of dye was significantly less in the mice treated with alcoholic extract of *V. nigundo* leaves than the control animals. This activity may be partly due to inhibition of leukotriene synthesis.

Increasing evidence suggests that the frequently observed association between activated T lymphocytes and eosinophils play a major role in the development of airway inflammation and in the accompanying bronchial hyperreactivity (17-18). Neutrophils and monocytes play a pivotal role in the disease process as they are source of variety of inflammatory mediators which are responsible for bronchial hyperresponsiveness and airway inflammation (19). In association with asthma, elevated numbers of these inflammatory cells like eosinophils, neutrophils, lymphocytes, monocytes have been identified in various tissue compartments like blood, biopsies of lung tissue, in broncho-alveolar lavage fluid and in sputum. In present study,

sensitization using egg albumin (1 ml, 10 % w/v, i.p.) and then second exposure to same antigen i.e. egg albumin (0.5 ml, 2 % w/v) through saphenous vein causes acute anaphylactic shock resembling the acute asthmatic attack resulting in the release of various mediators and cellular infiltration. Antigen challenge resulted in significant increase in the number of eosinophil in the broncho-alveolar lavage fluid. This was accompanied by intense eosinophil infiltration, accumulation and degranulation in the guinea pig lungs as evident of histopathology which is consistent with human asthmatic lungs. In our study we found that treatment with *V. nigrundo* in antigen challenged animal significantly inhibited antigen induced hyper reactivity by preventing increase in infiltration of total leukocyte count, eosinophils count. After antigen challenge airway hyperresponsiveness is supported by inflammatory pathology suggesting involvement of other mediators in pathogenesis of asthma. Neutrophil numbers have also been reported to increase in bronchial lavage fluid in asthmatics, but neutrophilia is generally shorter duration than eosinophilia (20-21). This was observed with our result that treatment with *V. nigrundo* resulted in significant inhibition of antigen induced bronchial hyperreactivity by decreasing neutrophil count. The participation of T lymphocytes in the pathogenesis of bronchial asthma and the accompanying bronchial hyperreactivity has been widely demonstrated (18). Indeed, activated CD4⁺ T lymphocytes are found in the blood and bronchial lumen from asthmatics (22). Recently, interest has been focused on the characterization of CD4⁺ T lymphocytes based on their repertoire of secreted cytokines and its possible role in the pathogenesis of allergic disorders. Thus, CD4⁺ T cells from asthmatics preferentially elaborate Th2-derived cytokines, such as IL-4 and IL-5, which have been shown to enhance IgE synthesis (23), and to act specifically on eosinophil survival, activation, and secretion of proinflammatory mediators (24). Large numbers of T lymphocytes, mainly of the CD4⁺ subset, have been identified in the bronchial mucosa of antigen challenged guinea pigs (25). In line of above the present finding show that treatment with *V. nigrundo* in sensitized animal produce significant decrease in lymphocyte count as compared to sensitized animals. The predominant cells in broncho-alveolar lavage fluid recovered from unchallenged guinea-pigs were those of the monocyte. The numbers of these cells were increased after antigen challenge (26). In the line of above context treatment with *V. nigrundo* significantly decreased monocyte as compared to sensitized guinea pigs. *V. nigrundo* inhibit infiltration of inflammatory cell, thereby decreasing the release of preformed inflammatory mediators. This decrease in release of inflammatory mediators prevents the direct damage to airway, which in turn prevent airway hyperresponsiveness.

Various processes involved in bronchial asthma such as inflammatory response can explain various histopathological alterations observed in biopsy of asthmatic patients. In asthma chronic inflammation is responsible for the bronchoconstriction which leads to airway narrowing and decrease in the lumen size of the bronchiole (27). This can be clearly seen by the histopathological studies of the lung tissue by observing the cross section of bronchi. In the present study, the sections of the lung tissues of animals exposed to TDI depicted marked bronchitis and severe bronchoconstriction. Treatment with *V. nigrundo* prevented the inflammation and bronchoconstriction which leads to normal lumen size and normal cellular structure compared to only TDI treated animals. These results suggest that *V. nigrundo* decreases bronchial hyperresponsiveness by decreasing the infiltration of inflammatory mediators like eosinophils, neutrophils. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens (28). Once binding of allergen to cell-bound IgE occurs, mediators such as histamine; eosinophil and neutrophil chemotactic factors; leukotrienes C4, D4, and E4; prostaglandins; platelet-activating factor; and others are released from mast cells which are responsible development of airway inflammation and bronchoconstriction. An attempt was made to find out whether alcoholic extract of *V. nigrundo* has any effect on the rate of

disruption of mast cells following exposure to compound 48/80, an agent which causes histamine release from mast cell (29). It has been assumed that the process leading to histamine secretion may be mediated by calcium release from an intracellular store of mast cells (30). In this study, *V. nigundo* offered significant protection against Compound 48/80 induced mast cell degranulation by stabilizing it, which is responsible for the decreasing airway inflammation by preventing release of various inflammatory mediators.

Conclusion

The alcoholic extract of *V. negundo* leaves is assumed to be effective in various animal models of asthma. This activity of extract is may be because of presence of flavonoids, phenolic compounds and terpens. Flavonoids of *V. nigundo* have anti-inflammatory, mast cell stabilizing and free radical scavenging activity (31-32). Phenolic compound also have free radical scavenging activity so they can inhibit inflammation (31). Further study is required to find out the major constituents responsible for this activity

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