Asthma is characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyperresponsiveness to the spasmogens such as histamine, acetylcholine and 5-hydroxytryptamine (5-HT) (330). The pathophysiological hallmark of asthma is the infiltration of inflammatory cells includes eosinophils (331), neutrophils, lymphocytes and macrophages (332). These cells release various inflammatory mediators such as histamine (333) and IL-4, 5, and 13 (334). Histamine released during the early and late phase allergic reactions (7); induces the secretion of mucus that cause extravasation of plasma proteins leading to airways mucosal oedema and contract the airway smooth muscle (99). Histamine may play a role in the immunomodulation of the immunoglobulin E (IgE) immune response (335), as well as in the regulation of the airway inflammation by the activation of epithelial cells and macrophages and possibly by inducing smooth muscle hyperplasia. Recent evidence indicates that acetylcholine is also released from non-neuronal origins such as the bronchial epithelium and inflammatory cells (336). Acetylcholine may play an essential regulatory role in airway remodelling, that are associated with chronic airway inflammation (337, 338). Acetylcholine is the dominant neurotransmitter involved in mucus secretion in the central airways (339). Acute airway inflammation can regulate mucus hypersecretion by augmenting acetylcholine release. In addition, cholinergic receptor stimulation interacts synergistically with epidermal growth factor (EGF) on mucus cell activation in airway submucosal glands (340).

Current asthma treatments are based on inhaled corticosteroids, long and short acting β2-adrenoreceptor agonists as well as leukotriene antagonists. Inhaled corticosteroid has become first-line treatment for most of the patients (341). However; long term use of corticosteroid is known to induce various side effects like hyperglycemia, hypertension, cardiometabolic abnormalities, psychiatric adverse effects, weight gain and osteoporosis (30). Moreover chronic use of corticosteroid induces vitamin D deficiency and low level of vitamin D contributes to increase in asthma severity (31).
Vitamin D is a steroidal hormone which not only plays an important role in the metabolisms of calcium, phosphorus and bone but is also involved in regulation of immune system. Vitamin D from the skin and diet is metabolized in the liver to 25-hydroxyvitamin D (25(OH) vitamin D) which is further metabolized in the kidneys to its active form, 1,25-dihydroxyvitamin D (1,25(OH)2D3). The biological responses to the 1,25(OH)2D3; are mediated by vitamin D receptor (VDR); a member of the superfamily of nuclear hormone receptors. VDR is expressed by many cells of the immune system, including activated B and T cells, monocytes, and dendritic cells (55, 342-344). Vitamin D inhibit Th1 (345), Th2 (235), Th17 (51) associated cytokines while some evidence shows that vitamin D may enhance (234) Th2 responses. Tregs are a subpopulation of T cells which modulate the immune system (346) which exert their effect by inhibiting transcription of the inflammatory cytokine IL-2, expressing IL-10, and potentially converting effector T cells to hyporesponsive or regulatory forms (55, 347). Vitamin D causes induction as well as proliferation of Tregs (55, 241) and increased IL-10-secretion and TLR-9 expression (56). Both in vivo and in vitro studies have shown that vitamin D enhances the production of anti-inflammatory cytokine IL-10 by human T cells (348, 349). Moreover, altered vitamin D homeostasis is associated with increased risk of developing glucose intolerance (350), metabolic syndrome (76), cardiovascular events (77), psychiatric effects (78), obesity (79) and hypertension (80).

In the present study, vitamin D was screened for efficacy studies with the help of various animal models such as histamine and acetylcholine induced bronchoconstriction in guinea pigs, clonidine induced mast cell degranulation and haloperidol induced catalepsy in mice.

In the acute phase of asthmatic episode, exposure to allergens and irritants like pollens, molds, house dust mites, animals’ dander, occupational chemicals cause activation of inflammatory cells. These cells can synthesize and secrete a vast numbers of mediators like histamine, tryptase, leukotrienes and prostaglandins that directly cause bronchoconstriction, submucosal gland secretion and vasodilation resulting in the early phase asthmatic response (351). In the present study histamine hydrochloride and acetylcholine were used as spasmogens in the form of aerosols (0.25%) to cause immediate bronchospasm in guinea pigs. Bronchodilatory activity was evaluated by observing effects of vitamin D on pre-convulsion dyspnea (PCD) time.
Histamine induced bronchoconstriction is the traditional immunological model of airway obstruction. Guinea pigs are extremely sensitive to primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins; also guinea pig airways react to spasmogens such as histamine, acetylcholine, leukotrienes, and other bronchoconstrictors in a manner similar to that seen in humans (352). Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to β-adrenergic antagonists (353). Thus, the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release. When histamine exposed to guinea pigs; it causes hypoxia and leads to convulsion. Histamine also causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in cardiovascular system of guinea pigs. A prominent effect caused by histamine leads to severe bronchoconstriction in the guinea pigs that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms (7).

Histamine contributes to the progression of allergic inflammatory responses by enhancement of the secretion of proinflammatory cytokines like IL-1α, IL-1β, IL-6 and IL-8 in local tissues (335, 354, 355) by regulating the expression of its own receptors on endothelial cells and influences the overall inflammatory reaction (356). Histamine also regulates granulocyte accumulation to tissues in distinct ways. Allergen-induced accumulation of eosinophils in the skin, nose and airways is potently inhibited by H1-antihistamines (357). The second-generation H1-antihistamines ebastine, cetirizine and loratadine has shown efficacy in guinea-pig model of bronchoconstriction elicited by histamine. Moreover, effects of ebastine, loratadine and cetirizine lasted 21, 19 and 15 hrs, respectively (358). In similar line our study demonstrated that pretreatment with vitamin D at all doses level (50, 100 and 200 IU/kg) were found to significantly increase the PCD time against histamine aerosol I guinea pig as compared to control and effects were lasted for 24 hrs. This effects produced by vitamin D were comparable to chlorpheniramine maleate suggesting their protective effect might be due to their bronchodilatory activity.

Acetylcholine-aerosol provoked bronchoconstriction in all animals of control group. Vitamin D significantly protected animals from acetylcholine-induced bronchoconstriction. In the early stage of asthma, release of inflammatory mediators like histamine, acetylcholine, leukotrienes, and prostaglandins directly cause acute bronchoconstriction
(332). Spasmolytic drugs like β-adrenergic agonists, xanthine derivatives and anticholinergic drugs are used as quick relief medications in such acute asthmatic attacks (359). Vitamin D is has anti-inflammatory activity and study have shown that vitamin D supplementation potentiate the anti-inflammatory function of corticosteroids in asthmatic patients (31). In the present study, animals treated with vitamin D at all doses level (50, 100 and 200 IU/kg) were found to significantly increase the PCD time against acetylcholine aerosol as compared to control. This bronchodilatory effect of vitamin D was found comparable to the protection offered by atropine sulphate.

Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. 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 (360). Uvans has studied mast cell degranulation and its correlation with the release of histamine after administration of a mast cell degranulating agent (361). In similar line Lakdawala et al. have shown that clonidine releases histamine from mast cells in a similar manner to a compound 48/80 (362). Histamine secretion is mediated by calcium release from intracellular store of mast cell (363). In present study vitamin D significantly inhibited mast cell degranulations similar to that of ketotifen fumarate and supports the mast cell stabilizing properties of vitamin D. Previous reports showed that vitamin D regulates the maturation of mast cells via inducing apoptosis in mast cell precursors and inhibiting mast cell differentiation in various stages though triggering vitamin D receptor (VDR) in mast cells (364). In vivo studies in mice showed absence of VDR signaling, resulted in accelerated maturation of mast cells and an increase in the number of mast cells (365). Vitamin D inhibits mast cell activity by means of down-regulating mast cell development, differentiation, and eventually function (366). Vitamin D also inhibits calcium ionophore-induced histamine release from peritoneal mast cell (367). Thus our study also supports that vitamin D helps to stabilize mast cell by antagonizing histamine release from mast cell.

Several drugs are known to induce catalepsy in animals and different stages of catalepsy appear to be directly correlated with brain histamine content (368). Haloperidol induces
catalepsy by inhibiting dopamine D2 receptors and inhibits dopamine secretion. Dopamine is agonist for adrenaline. Adrenaline is physiological antagonist of histamine. So as there decrease in dopamine there is imbalance in neurotransmitters means high level of histamine (351). Puchacz et al (369) has shown that vitamin D increases availability of dopamine, noradrenaline and adrenaline in rat’s brain. Similarly in our study we found that vitamin D (at all doses level; 50, 100 and 200 IU/kg) treated animals showed significant protection against haloperidol-induced catalepsy and comparable to standard drug chlorpheniramine maleate; which support vitamin D may increase dopamine level and causes decrease in histamine level; ultimately protects animal from bronchoconstriction.

Thus, our study demonstrated that all three doses of vitamin D 50, 100 and 200 IU/kg have significant bronchoprotective and antihistaminergic activity and comparable to standard drugs.

On the basis of the above pharmacological results; we have made an attempt to investigate the mechanism of action for antiasthmatic activity of vitamin D in ovalbumin induced airway inflammation in Wistar rats. In this study we have selected two different dosage schedule of vitamin D as 50 IU/kg given daily and bolus dose; 60,000 IU given only single dose. The reason behind to this dosage is that there is disparity of vitamin D dosage which can be given as daily, weekly, monthly or annually (370). So to confirm this we have chosen this daily and single bolus dose. Further we have studied comparative study of vitamin D, dexamethasone and combinations of dexamethasone and vitamin D.

We used ovalbumin (OVA) induced asthma model in rats to find out the mechanism of action as it shows similar effects like human allergic asthma (371). Rats are widely used by various investigators for induction of allergic asthma. Another reason to used rats is that; our aim was to assess the efficacy, and side effects such as steroid induced metabolic, cardiovascular, and behavioral modulation by the monotherapy, and combination therapy rather than to concentrate only on asthmatic parameters such as IL-5, IgE, and differential WBC count. For this purpose, we have selected rats and not any other animals. Ovalbumin challenge evoked anaphylactic reactions in sensitized animals which result from the action of various mediators; histamine, leukotrienes, prostaglandins, thromboxane A2 and platelet activating factor from inflammatory cells (371).
In the present study, all the OVA-sensitized animals did not show any significant change in body weight. OVA-sensitized animals showed significant increase in eosinophils, neutrophils, lymphocytes, monocytes and total cell. However, pretreatment of animals with vitamin D, combination of vitamin D and dexamethasone in this asthma model decreased the production of the number of inflammatory cells such as eosinophils, neutrophils, lymphocytes, monocytes and total cell.

The recruitment of eosinophils into bronchial mucosa is critical contributor to the late asthmatic reaction of congestion and mucus hypersecretion (372). When these cells arrive they degranulate and perpetuate underlying airway inflammation. Eosinophils are a rich source of cytotoxic proteins, lipid mediators, oxygen free radicals and cytokines (373). In asthmatic patients, after transendothelial migration, eosinophils transmigrate and adhere to bronchial epithelium where they degranulate and release substances eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO) and superoxide which are toxic for epithelial cells (374). Damage and desquamation of cells, cilliostasis, and epithelial secretion manifest the toxicity to airway epithelium. MBP is a selective, allosteric antagonist for M2 muscarinic receptors (autoreceptors) (375). The loss of M2 muscarinic receptor function results in increased airway tone due to increased release of acetylcholine and potentiation of vagally mediated reflex bronchoconstriction and bronchial hyperresponsiveness (376). MBP also stimulates histamine release from basophils and mast cells. Lipid bodies (intracellular lipid rich domains) are induced to be developed in the activated eosinophils, and are the sites for enhanced synthesis of both lypoxygenase and cyclooxygenase-derived eicosanoids (377). Eosinophils are capable of producing significant quantities of cysteiny1 leukotrienes (especially LTC-4). Cysteinyl leukotrienes contract airway smooth muscle (100-1000 fold more potent bronchoconstrictors than histamine), increase vascular permeability, stimulate mucus secretion, decrease mucociliary clearance, stimulate eosinophil and neutrophil recruitment into the airways (100), stimulate smooth airway muscle proliferation and cause neuronal dysfunction (378). Eosinophils have the potential to synthetize and release a number of cytokines and chemokines. Cytokines produced by eosinophils include the autocrine-eosinophil active growth factors (IL-3, IL-5, GM-CSF), immunoregulatory cytokines (IL-2, IL-4, IL-1, TGF-β, IFN-γ), proinflammatory cytokines (IL-1, IL-6, TNF-αIL-16) and chemokines (IL-8, MIP-1α, RANTES) (379). Transforming growth factor-β (TGF-β) is an immunoregulatory factor with a direct effect on growth of some cell types (stimulation on
fibroblast growth and inhibition of epithelial cell growth) and upregulation of the synthesis of ECM proteins, inflammatory mediators and cytokines, making it an important factor in the remodeling process (380).

Inhaled GC are a first-line therapy for asthma due to their potent anti-inflammatory properties that primarily result in reduced numbers of airway inflammatory cells and their associated mediators. In addition, GC induce apoptosis in peripheral blood eosinophils, (381) as well as in tissue eosinophils resident in nasal polyp tissue sections, (382) suggesting that eosinophil apoptosis induction by GC might be relevant to their anti-inflammatory effects in asthma. The intracellular signaling mechanisms by which GC induce apoptosis in human eosinophils include the involvement of caspases and release of mitochondrial cytochrome C (383). Studies with eosinophils derived from both healthy and asymptomatic allergic individuals have demonstrated involvement of caspase-3 and -8 in glucocorticoid-induced apoptosis (384).

The data presented in this study show that pretreatment with vitamin D, combination of vitamin D and dexamethasone in this asthma model decreased the production of the inflammatory cells; eosinophils. Vitamin D is known for its beneficial effects in diseases with strong Th1 responses, perhaps by altering Th1/Th2 balance in vivo (235) Matheu et al (385) reported that 1,25(OH)2D3 treatments reduced airway eosinophilia using a mice model of allergic asthma while Topilski et al (235) reported that 1,25(OH)2D3 suppressed eosinophilia and lung inflammation scores in a model of allergic asthma induced in either BALB/c or C57BL/6 mice. They also observed that 1,25(OH)2D3 treatment inhibited chemokine-induced migration of T cells. In similar line a study conducted by Ma et al (386) has shown that mice treated with 1,25(OH)(2)D(3) could significantly inhibited the infiltration of eosinophils into lung tissues and BAL fluid. These results indicated that 1,25(OH)(2)D(3) pretreatment enhanced the inhibitory effects of immunotherapy on allergic airway inflammation and pretreatment of vitamin D may be beneficial for improving the efficacy of immunotherapy. Wittke (238) reported that VDR is believed to be involved in the allergic asthma response in mice, because in their experiments, VDR knockout mice showed mild symptoms of airway inflammation and failed to develop experimental asthma.
Neutrophil infiltration is prominent mostly during the late phase response, as well as in more severe, persistent asthma (387), making its role less clear in milder forms of asthma. Recently, higher levels of TGFβ expression and release from asthmatic neutrophils indicate that neutrophils may be involved in the airway remodeling process of asthmatic subjects. In inflammation, the potential for neutrophils to cause tissue damage via the release of toxic reactive oxygen species and granule enzymes such as proteases is very high. For example, it has been reported in the airways that secondary necrosis of apoptotic neutrophils leads to release of cytotoxic granules, causing harm to resident structural cells (387). It is known that neutrophilic asthma represents a fairly large proportion of the disease overall, up to 50% by some reports (388). Although glucocorticoids are generally considered to be the treatment of choice but glucocorticoid resistance in asthma (389) associated with neutrophilic inflammation. Although glucocorticoids lead to marked reduction of eosinophils, mast cells, T lymphocytes and macrophages in sputum, bronchoalveolar lavage and bronchial wall (388), changes in the neutrophilic components of asthma are often the opposite, with reports of increase in neutrophils after glucocorticoid therapy (390). Therefore, resolution of the neutrophilic inflammatory response is an active process involving down regulation of proinflammatory cytokines, upregulation of anti-inflammatory eicosanoids, decreased generation of reactive oxygen intermediates, and removal of the cells (391).

Several lines of evidence indicating that neutrophils express functional vitamin D receptors. 1,25-vit D3 decreases neutrophil activity (392) and has a potential to affect the inflammatory process by modulating the expression of neutrophil genes particularly, trappin-2/elafin/SKALP (393). Takano et al., showed that 1α,25(OH)(2)D(3) inhibited neutrophil recruitment in the lung by approximately 40% without increasing plasma calcium concentration (394). Therefore, our data presented in this study showed that pretreatment with vitamin D, combination of vitamin D and dexamethasone in this asthma model decreased the production of neutrophils.

Mucosal-biopsy specimens obtained from patients during an episode of asthma after the inhalation of allergen contain lymphocytes, many of which express surface markers of activation (395). T-lymphocytes play a very important role in coordinating the inflammatory response in asthma through the release of specific patterns of cytokines, resulting in the recruitment and survival of eosinophils and in the maintenance of mast
cells in the airway (13). There are two types of T-lymphocytes; helper CD4+ T cells and killer CD8+ T cells. There are four distinct subsets of CD4 lymphocytes, Th1, Th2, regulatory T cells (Treg), and T17 cells can differentiate from precursor T cells at the time of antigen presentation and influence cytokine production. The differentiated Th cells are characterized by the specific sets of cytokines they release when stimulated. Th1 cells produce IL-2 and interferon γ (INF-γ), both essential for cellular defense mechanism. Th2 cells produce cytokines (IL-4, IL-5, IL-6, IL-9 and IL-13) that mediate allergic inflammation. Furthermore, there is reciprocal inhibition, in that Th1-type cytokines inhibit the production of Th2-type cytokines and vice versa. It is hypothesized that allergic asthmatic inflammation results from a Th2-mediated mechanism (14). Increased numbers of activated T-cells as well as Th2-cytokines are found in bronchoalveolar lavage fluid, blood and bronchial submucosa from asthmatic patients (10).

Vitamin D acts on and VDRs are present on most types of immune cells and in highest density probably on monocytes, DCs and T lymphocytes. The recent data has shown that 1α,25(OH)2D3 has direct effects on activated helper T cells, regulatory T cells, activated B cells and dendritic cells (DC) (44). Baeke et al., shown that T cells are direct targets for 1,25-dihydroxyvitamin D3. (233). 1,25(OH)2D3 reduce the production and expression of the Th1 associated cytokines IL2, TNF-α, and IFNγ in T cells (236). These cytokines are characteristic of Th1 cell responses, and associated with the progression of several autoimmune diseases. More recently, 1,25(OH)2D3 inhibit Th17 associated cytokines have been shown both In vitro and in vivo in experimental which may be important in steroid refractory airway disease (396). The Th17 produced more inflammatory cytokines such as IL-6, IL-17 and IL-1β (53). Vitamin D regulates the differentiation and activity of CD4+ T cells both directly and indirectly by inhibiting Th1 and Th2 function to suppress autoimmune disease pathology (397). However there are conflicting evidences, both enhancement and inhibition for effects of vitamin D on Th2 responses (55, 234, 398). Treatment with vitamin D showed reduced level of IL-4 concentrations in bronchoalveolar lavage fluid, and an attenuated inflammatory response in vivo in a Th2 dependent murine model of allergic airways disease (235). Taher et al., established a beneficial effect of vitamin D administration to post allergic sensitization mice (237). However, Wittke et al., shown that 1,25[OH]D3 administration to mice had no effect on the severity of allergic airway disease induced by OVA (238). Also VDR gene knockout animals had reduced level of Th1 and Th2 (238, 399). Subsequent human studies suggest that vitamin D
deficiency causes impaired lung function (239). In asthma, reduced vitamin D levels are associated with impaired lung function, increased airway hyperresponsiveness, and reduced glucocorticoid response (49, 50). However, there are some reports shown non-linear response to vitamin D in that in that both high and low levels have been associated with increased Th2 activity. 1,25(OH)D3 inhibited both Th1 and Th2 cytokines production at low concentration but failed to inhibit at high concentrations (55, 240, 398). Our study also established that pretreatment with vitamin D, combination of vitamin D and dexamethasone in this asthma model decreased the production and expression of lymphocytes and thereby associated cytokines.

Monocytes which are important in the initiation of the adaptive immune response. Monocytes can be either immunogenic or tolerogenic and hereby modulate T-cell responses (400). Tolerogenic APCs are characterized by a reduced expression of costimulatory molecules and a cytokine production favoring regulatory T-cell (Treg) induction (401). Vitamin D has been shown to manipulate monocytes at different levels, enabling them to exert tolerogenic activities, which could be exploited to better control autoimmune diseases (402). Monocytes cultured with 1,25(OH)2D display a VDR-dependent loss of major histocompatibility complex-II (403). Monocytes pretreated with 1,25(OH)2D were less effective in inducing proliferation of T cells upon stimulation with tetanus toxoid (404). Additionally, 1,25(OH)2D inhibits the production of interleukin-1a (IL-1a), IL-6, IL-12 and tumour necrosis factor-a (TNF-a) by monocytes cultured in the presence of proinflammatory stimuli such as CD40L and LPS (404, 405). On the other hand, transcription levels of IL-10 mRNA are significantly upregulated by LPS-stimulated monocytes in the presence of 1,25(OH)2D (406). Also in vivo, 1,25(OH)2D treatment (1 μg twice daily for 7 days) in healthy volunteers showed a significant reduction in IL-6, but not IL-1a or TNF-a production by peripheral blood mononuclear cells (407). The cytokines affected by 1,25(OH)2D are typically involved in the differentiation of naïve T cells in distinct effector Th cell subsets. Monocytes activated in the presence of 1,25(OH)2D and co-cultured with anti-CD3-stimulated purified CD4+ T cells show a decreased interferon-g (IFN-g) and increased IL-10 production by CD4+ T cells. Furthermore, IL-6 has been described to prevent the development of TGF-β-induced Tregs and together with TGF-β to induce Th17 cell differentiation (408). Thus, by decreasing IL-6 production and by increasing IL-10 production in monocytes, vitamin D could modulate the T-cell response in a more anti-inflammatory and regulatory direction. Thus our study suggests that
treatment with vitamin D along with dexamethasone in asthma; decreases the production and expression of monocytes and associated cytokines.

The central component of allergic asthma is the development of an immunoglobulin E (IgE) antibody-mediated response to allergens that requires the interaction of a number of leukocytes. IgE is secreted from B cells into circulation, where it binds to high-affinity FcεR1 receptors on the surfaces of mast cells and basophil. Upon the next encounter with allergen, the antigen binds to membrane bound IgE, stimulating release of such mediators as histamine, leukotrienes, and cytokines that initiate acute bronchospasm and also to release pro-inflammatory cytokines to perpetuate underlying airway inflammation (126). Elevated level of IgE in both the serum and bronchoalveolar lavage (BAL) fluid is associated with bronchial asthma (409). IgE also may play a role in modulating the severity of asthma, because previous studies have found associations between high IgE levels and greater asthma severity (410), airway hyperresponsiveness (411) and lower baseline lung function (412). The development of monoclonal antibodies against IgE has shown that the reduction of IgE is effective in asthma treatment (413). These clinical observations further support the importance of IgE to asthma (414).

Vitamin D known as has beneficial roles in IgE synthesis and regulation. Due to the conflicting observations regarding vitamin D’s impact on allergy, in vitro human and in vivo murine studies have been performed to assess whether vitamin D directly affects serum levels of IgE. In one study, previously stimulated B cells showed markedly decreased production of IgE following the administration of vitamin D (415). In a second study, calcitriol and VDR agonists led to suppressed IgE production by cultured human B cells (416). Furthermore, calcitriol and VDR agonists also reduced IgE production in an allergy mouse model (416). Other researchers in human study of Cost Rica demonstrated that low vitamin D levels were associated with increased airway responsiveness, higher eosinophilic counts and total IgE levels, and increased risk of severe asthma exacerbations (73, 250).

Interleukin (IL)-5 has been recognized as the most specific cytokine in the eosinophil lineage (88, 417) and has been identified as the key common denominator in inflammatory pathways in asthma (418). IL-5 plays a key role in eosinophil proliferation, differentiation, maturation, migration to tissue sites and survival, as well as prevention of eosinophil
apoptosis (11, 419). IL-5 comprises of a functional site for binding to the specific receptor subunit, IL-5Rα, and a separate motif for binding to the signaling subunit, β-chain (420). The IL-5Rα subunit is specific only to IL-5 binding; the β-chain also binds the cytokines IL-3 and granulocyte-macrophage colony-stimulating factor via a shared extracellular domain (421). There is considerable emerging evidence that differentiation of eosinophils also occurs within tissues undergoing an allergic response, such as the airways in atopic asthma, and not only in bone marrow (11). Thus, in order to obtain the most clinical response in eosinophilic asthma, a systemic approach to anti-IL-5 therapy may be important.

In the lung, T-lymphocytes are the main source of IL-5 (422), but they do not express IL-5R. Eosinophils and basophils express IL-5R on their surface and also produce IL-5, contributing to the levels of this cytokine. Several lines of experimental evidence support the role of IL-5 in the pathogenesis of asthma. IL-5 mRNA is identified in increased quantities in bronchial biopsies taken from asthmatics compared with non-asthmatic controls (423). IL-5 mRNA expression in the airways has been shown to correlate with the clinical severity of asthma (424). Inhaled bronchial provocation with allergen gives rise to an increase in IL-5 mRNA in bronchoalveolar lavage from asthmatics patients (425). Finally, inhalation of recombinant human IL-5 leads to an increase in eosinophilia in induced sputum and in AHR (426). These data confirm that IL-5 is present in patients with asthma, making it a possible target for intervention.

In an experimental mice model of pulmonary eosinophilic inflammation, subcutaneous 1,25(OH)D injections abrogated IL-5 production in bronchoalveolar lavage fluid (385). Furthermore, of 1,25(OH)D decreased IL-5 production Th0 cells and remained unchanged in Th1 and Th2 cells (427). Regarding no change in IL-5, Yusupov et al. (428) demonstrated that vitamin D (cholecalciferol) supplementation at 2000 IU/d for 3 months was ineffective at altering circulating IL-5 concentrations in young, healthy adults. While Berker et al., 2012 reported low-daily dose of supplemental vitamin D IL-5 decreases concentration (429). Collectively, these findings highlight that the cytokine-modulating property of vitamin D could be physiologically dependent on the supplemental dose (and/or environmental factors and dietary intake), the immunological challenge (or lack thereof), experimental model (i.e., humans, rodents, or cell), duration of vitamin D treatment, the timing of data collection, and importantly, the form of vitamin D studied.
However, in our study treatment with vitamin D or vitamin D combined with dexamethasone significantly reduced the levels of IL-5 and IgE in serum and BALF. These results suggest that vitamin D has protective effects in allergic asthma by reducing in the levels of Th2 cytokines, and that it acts synergistically with GCs.

NO plays a key role in physiological regulation of airway functions and is implicated in airway diseases, including asthma (430-432). NO itself is a potent vasodilator and this may increase plasma exudation in the airways; it may also amplify the Th2-lymphocyte mediated response (432). Nitric oxide (NO) is produced by several cells in the airway by NO synthases (NOS) (431, 432). An inducible form of the enzyme (iNOS) is expressed in epithelial cells of asthmatic patients (433) and can be induced by proinflammatory cytokines in airway epithelial cells (434). This may account for the increased concentration of NO in exhaled air of asthmatic patients (435) and recent investigations measuring exhaled NO concentration have suggested that it may be a useful measure of ongoing lower airways inflammation in patient with asthma as well as for measuring effectiveness of therapy (436).

Chang et al. reported that 1,25(OH)2D3 inhibited iNOS expression and reduced NO production by lipopolysaccharides-stimulated macrophages in the range of physiological doses (437). Inhibition of the NO surge was coupled with a reduction in OONO- and lactate dehydrogenase production. They proposed that 1,25(OH)2D3 production by macrophages might protect themselves against oxidative injuries caused by the NO burst. Garcia et al. reported that 1,25(OH)2D3 or its analogues could have a therapeutic value in the management of iNOS-associated diseases of the central nervous system (438, 439). Zhou et al (439) reported vitamin D pretreatment significantly reduced the OVA-triggered up-regulation of iNOS and NO level. In similar way; our study also demonstrated that vitamin D, combination of vitamin D and dexamethasone significantly reduced the NO production and help to control reactive nitrogen species induced inflammation in asthma.

The role of platelets in inflammatory cell recruitment to the lungs is a relatively recent subject of interest. Over the past few decades, as it has become increasingly clear that asthma is more than bronchoconstriction and is, in fact, a chronic inflammatory disease of the airways, the mechanisms controlling this airway inflammation have been intensely investigated. It is of particular interest that platelet depletion, as well as administration of
prostacyclin (PGI2) and PAF antagonists in animal models of asthma, significantly inhibits eosinophil infiltration into the lungs (440). In a model of OVA-induced mouse allergic lung inflammation, it was recently shown that eosinophil recruitment is inhibited in thrombocytopenic animals (441), thus lending further support to the suggestion that platelets are essential for leukocyte recruitment in allergic models of asthma. Platelets secrete various mediators, including free radicals and cationic proteins that have been suggested to contribute to the increase in vascular permeability of the airway epithelium and the stimulation of mucus secretion. Studies by Pretolani and colleagues (442) have consistently shown that PAF administration to actively sensitized guinea-pigs induces significant hyperresponsiveness in both in vitro and in vivo animal models, a feature which they suggest may be due to an increased reactivity of resident bronchopulmonary cells or, potentially, to the infiltration of inflammatory cells, such as histamine-releasing mast cells, basophils and platelets, in the lungs (443). In a different model, PAF-treated rabbits were found to exhibit increased airway responsiveness to inhaled histamine. The response was, however, prevented in thrombocytopenic animals (444).

In experiments, vitamin D attenuates platelet activation while reducing the expression of two CAMs, VCAM-1 and MT1-MMP (445). Researchers in Israel also identified 50 patients on a regimen of 4,000 IU of vitamin D daily for 5 days. The vitamin D group showed a decrease in VCAM-1 as well as another inflammation marker, IL-6. The patients who did not receive vitamin D showed clear increases in both inflammation markers. Damera et al. show that calcitriol, inhibited thrombin and platelet-derived growth factor-induced airway smooth muscle cell proliferation (446). Both studies indicate that vitamin D has actions that reduce platelets activation. In the present study we found that animals treated with vitamin D, dexamethasone and combinations of vitamin D and dexamethasone showed significant reduction in platelets count.

Vitamin D is a steroid hormone that can act on the cellular differentiation and growth in the bone marrow either directly or through hyperparathyroidism. Both systemic or locally produced 1,25 (OH)(2)D(3) may play a role in modulating cell development processes such as hematopoiesis and lymphocyte differentiation (447-449). The Blood is an important for pulmonary and tissue respiration and serve as a medium for neurohumoral transmissions, biotransformation, metabolic excretion, nutritional and immunological processes, as well as homeostatic responses. The laboratory determination of blood
components is important for the treatment purpose and also help to determine deleterious effect of drugs on the blood and it’s relating functions (450). Vitamin D may have an effect on the RBC erythropoiesis in the bone marrow and partially explain the high incidence recurrent infections in low level of vitamin D in asthmatic patients. In the study of Ashraf et al., authors did not find any significant effects of vitamin D on RBC count and indices; that effects may be due to their study involved vitamin D deficient (vitamin D blood level < 15 ng/ml) patients and not suffers from asthma or any other health effects (451). In our study we found that animal treated with vitamin D showed significant increase in RBC count, hemoglobin, PVC while decrease in MCV, MCHC and no effects on MCH indices. Therefore, vitamin D may enhance erythropoiesis in the bone marrow and help to fight against infections. In our study this effects may be due to our study involved used of animals and also they were OVA sensitized and might not be vitamin D deficient.

Glucocorticoids are the most common cause of drug-induced diabetes. The 52.9% were developed diabetes in the first 3 months and 14.5% after 1 year after initiation of systemic glucocorticoids therapy in patients with respiratory disease (161). Glucocorticoid induced diabetes is similar to type 2 diabetes because glucocorticoids impair glucose metabolism mainly through increasing insulin resistance, which occurs in the liver with increased basal glucose production, and in the adipose and skeletal tissues with impaired glucose utilization. Two mechanisms are predominantly responsible for hyperglycemia secondary to GC: a decrease in insulin secretion and insulin sensitivity (452). Glucocorticoids causes β-cell dysfunction, reduced insulin sensitivity and impair β-cell function (453), by acting through glucocorticoid receptors which are also expressed on pancreatic β-cells. Glucocorticoids may also impair the uptake and the metabolism of glucose in β-cells through genomic actions (i.e., modulation of gene expression by nuclear glucocorticoid receptor) which lead to a decrease in the efficacy of cytoplasmic Ca²⁺ on the exocytotic process of insulin secretory vehicles (454). It was also reported that short-term exposure to glucocorticoids reduced the insulinotropic effects of GLP-1 (455). The most important metabolic consequences of glucocorticoid excess occur during the postprandial period when these hormones exert anti-insulin effects in liver, skeletal muscle, and adipose tissue. Insulin favours the uptake and storage of glucose as glycogen in muscle and fat and reduces lipolysis by the inhibition of fatty acid release into the blood; insulin also inhibits hepatic gluconeogenesis and glycogenolysis. Glucocorticoid excess may affect all these biological activities leading to the development of insulin resistance. Glucocorticoids also
inhibit insulin signaling network through several different mechanisms. Insulin acts by binding to its receptor (IR), leading to increased kinase activity and tyrosine phosphorylation of several downstream signaling molecules, including IRS-1 through IRS-4. These proteins then activate the PI3K and MAPK pathways, leading to a range of downstream effects (456). In skeletal muscle, glucocorticoids cause insulin resistance by decreasing transcription of IRS-1, while increasing transcription of two proteins that counter insulin action, protein tyrosine phosphatase type 1B (PTP1B) and p38MAPK (457). A similar increase in transcription of p38MAPK is observed in liver (458). Glucocorticoids also decrease IRS-1 and IRS-2 levels in fat (459), while there is a decrease in IR and IRS-1 phosphorylation in response to glucocorticoids in liver (459). Adiponectin which is secreted by adipose tissue, promotes insulin sensitivity in tissues and is suppressed by glucocorticoid treatment, serving as yet another component in the insulin resistance seen with glucocorticoid therapy (460). Glucocorticoids also promote proteolysis, lipolysis, free fatty acid production, and fat accumulation in the liver, which can contribute to insulin resistance (460). In addition, glucocorticoids directly promote hepatic gluconeogenesis, increasing hyperglycemia. Pancreatic β cell dysfunction allows further hyperglycemia to develop, likely contributing to the progression to frank diabetes sometimes seen with chronic glucocorticoid therapy (460).

In our study we found that dexamethasone induced hyperglycemia is attenuated by vitamin D. The β-cell in the pancreas that secretes insulin has been shown to contain VDRs as well as the 1 alpha hydroxylase enzyme (260). Evidence indicates that vitamin D treatment improves glucose tolerance and insulin resistance (461). Supplementation with vitamin D has been shown to restore insulin secretion in animals (262). Mechanisms associated with vitamin D and diabetes include improving insulin action by stimulating expression of the insulin receptor, enhancing insulin responsiveness for glucose transport, having an indirect effect on insulin action potentially via a calcium effect on insulin secretion, and improving systemic inflammation by a direct effect on cytokines (462). In studies of nonobese diabetic mice, high doses of 1 alpha 25-dihydroxyvitamin D3 (active form of vitamin D) have been shown to delay the onset of diabetes by means of immune modulation (263). This active form has been shown to protect beta cell function caused by inflammatory cytokines (IL-6 and TNF-alpha) (463). There is strong evidence that activation of inflammatory pathways interferes with normal metabolism and disrupts proper insulin signaling (266). Proinflammatory cytokines, such as IL-1β and TNF-α (266) are
exacerbating insulin resistance. Insulin resistance can also be triggered by the presence of metabolic stressors, such as high blood non-esterified fatty acids (NEFA) levels, which compromises insulin sensitivity by reducing the action of this hormone in peripheral tissues, such as the liver, skeletal muscle and adipose tissue. It is recognized that VDR, the receptor of the steroid hormone 1,25(OH)2D3, is widely distributed in more than 38 tissues, where it clearly controls vital genes related to bone metabolism, oxidative damage, chronic diseases and inflammation (464). VDR is constitutively expressed by macrophages and dendritic cells, which suggests that vitamin D plays an important role in the modulation of inflammatory response. Incubation of isolated monocytes with 1,25(OH)2D3 attenuates the expression of proinflammatory cytokines involved in insulin resistance such as IL-1, IL-6 and TNF-alpha in type 2 diabetic patients (463). A study of >10,000 Finnish children who were given 2,000 IU vitamin D3 per day during the first year of life demonstrated a 78% reduced risk of type 1 diabetes over a 30-year follow-up (269). Subsequently, this finding was confirmed by a meta-analysis of 5 observational studies in England (270). One study reported that a daily intake of >800 IU of vitamin D compared with <400 IU of vitamin D reduced the risk of T2DM by nearly one-third (271). In a 6-month study of 81 South Asian women with insulin resistance, 1,000 IU daily of vitamin D reduced insulin resistance and improved insulin sensitivity, reducing fasting insulin levels without changing insulin secretion (261). Vitamin D also regulates nuclear peroxisome proliferative activated receptor that has an important role in the insulin sensitivity (465). Thus combination of dexamethasone with vitamin D helps to prevent hyperglycemic side effects induced by dexamethasone.

Besides insulin resistance, GC treatment is also associated with dyslipidemia (466). Lipoproteins function in the body as transporters for lipids and are classified based on their density. High dose GC treatment has been associated with increased triglycerides, total and LDL-cholesterol levels 79 and leads to increasing the risk for cardiovascular diseases. These lipid changes can occur within 48 h of the initiation of (467) of GC therapy. Corticosteroids have multiple potential deleterious effects on cholesterol metabolism, including an increase in the activity of acetyl-coenzyme A carboxylase and free fatty acid synthetase, down regulation of LDL receptor activity, increase in the activity of HMG-CoA reductase and inhibition of lipoprotein lipase (LPL) (35). LPL is essential for the release of fatty acids from triglycerides in VLDL particles, in order to be taken up in the adipose tissue and GCs have been shown to increase LPL activity by both transcription as
well as posttranslational modifications (168). A high cumulative dose of corticosteroids is associated with increased levels of very low-density lipoproteins (VLDL), total cholesterol (TC), and triglycerides (TG), as well as a decrease in high-density lipoprotein (HDL) levels. This has been confirmed in early corticosteroid withdrawal analyses that show beneficial effects in reducing dyslipidemia (36). Glucocorticoids’ effects on lipid metabolism in adipose tissue are controversial and may involve stimulation of both lipolysis and lipogenesis (468). Recently, it has been demonstrated that glucocorticoids directly stimulate lipolysis in rat primary adipocytes in a dose- and time-dependent manner (469). Dexamethasone stimulated the release of free fatty acids (FFA) and glycerol after 24 h of incubation. This action occurred even at a low Dex concentration; it was rapid starting at 4–8 h and increased continually to 32 h. The findings that Dex increased intracellular cAMP levels and protein kinase A (PKA) activity and down-regulated cyclic-nucleotide phosphodiesterase 3B, the major enzyme responsible for cAMP hydrolysis, suggest that cAMP/PKA system is functionally involved in the mechanism by which glucocorticoids stimulate lipolysis. Furthermore, incubation with Dex caused phosphorylation and downregulation of perilipin, a phosphoprotein that coats lipid droplets in adipocytes which regulates lipolysis. Phosphorylated perilipin changes conformation and, exposing the stored lipids, facilitates lipolysis (469). Glucocorticoids may enhance lipolysis and modulate FFA mobilization through multiple mechanisms including a permissive effect, which has long been thought to be the principal one. In particular, glucocorticoids may modulate the dynamic responsiveness to other hormones such as catecholamines and GH, thereby increasing their lipolytic action (466).

Previous studies have examined the relationship between 25(OH) vitamin D and HDL-C, LDL-c, total cholesterol and triglyceride concentration (76, 470) and reported overall a positive correlation between 25(OH) vitamin D and HDL-C and an inverse correlation between 25(OH) vitamin D and LDL-c, total cholesterol and triglyceride levels. Activation of VDR results in decreased accumulation of neutral lipids (triglycerides and cholesterol). A decrease in triglycerides is mediated by vitamin D-induced 1) decrease in fatty acid synthesis as a result of decreases in sterol regulatory element binding proteins (SREBP)-1-dependent ACL, ACC, and FAS, which are the major mediators of fatty acid and triglyceride synthesis 2) increase in fatty acid oxidation as a result of increased PPAR-α, and 3) decrease in fatty acid uptake as a result of decrease in CD36. The decrease in cholesterol is mediated by vitamin D-induced 1) decrease in cholesterol synthesis as a
result of decreases in SREBP-2 and HMG CoA reductase and 2) decrease in cholesterol uptake as a result of a decrease in LDL receptor (273). The other possible indirect mechanism may involve is PTH. There is a reciprocal relation between serum 25(OH)D and PTH levels, and supplementation with vitamin D suppresses serum PTH as PTH has been reported to reduce lipolysis at least in vitro. In addition, as suggested by Zittermann et al., vitamin D could affect the serum lipids through an increased calcium level which may reduce hepatic TG formation and/or secretion (471). Furthermore, vitamin D may have an effect both on insulin secretion and insulin sensitivity and thereby indirectly influence lipid metabolism (472). Thus in our study dexamethasone induced dyslipidemia is attenuated by vitamin D; this effect may be due to vitamin D may affect serum lipids level both directly and indirectly.

Urea and creatinine is key marker for liver and kidney function test. The test is most important to check the function of these organs during drug treatment to assess any adverse effects related to these drugs. In the present study we did not find any significant changes in any parameters during treatments schedule of vitamin D, dexamethasone and combinations of both drugs. Thus drug found to safe in given dosage schedules.

Psychiatric disorder including anxiety, depression, mania, euphoria, irritability and hyperactivity were reported to be 52.8% of the observation cohort on long term (more than 3 months) or higher dose (more than 20 mg/day) in patients on glucocorticoids therapy (473). Studies have revealed incidence of psychiatric adverse events following glucocorticoid use to be anywhere ranging from 1.8% to 57% with severe psychiatric disturbance being 5.7% (169). Sirois et al reviewed studies from 1978 to 1983 and found depression was 28%, mania and depression 12%, psychosis 11% and delirium 13% (474). In a meta-analysis of 11 uncontrolled studies involving 935 adult patients, Lewis and Smith (170) found incidences of psychiatric reactions ranging from 13% to 62% with a weighted-average incidence of 27.6%. There are several case reports that suggest psychiatric disturbances may occasionally occur with steroid inhalers. A 5-year-old child with asthma developed symptoms of mania including agitation, irritability, and insomnia 2 days following the addition of inhaled budesonide at 200 g/day (170). Phelan (171) found that a 69-year-old man who had previously developed protracted manic symptoms with oral prednisolone became euphoric with pressured speech and visual hallucinations after receiving 400 g/day of inhaled beclomethasone for 3 weeks.
Chapter 5
Discussion

The biologic mechanisms by which corticosteroids may affect behavior are also not fully understood but evidences suggest that corticosteroids have direct and indirect effects on the central nervous system in animals and in humans (475-477). Corticosteroids freely cross neuronal cell membranes and, in neurons containing specific cytoplasmic steroid receptors, translocate as a steroid-receptor complex to the cell nucleus (475). Corticosteroid receptors are located in the hippocampus, septum, and amygdale (475, 478) areas of the brain which believed to be involved in behavior, mood, and memory. Many central nervous system effects of corticosteroids are mediated via genomically related events. Corticosteroids altered transcription of specific genes that code for tyrosine hydroxylase (475, 479), monoamine oxidase (475, 479) and dopamine-/3-hydroxylase (475, 479), enzymes which control the activity of catecholamines and other neurotransmitters. Also chronic exposure to corticosteroids may induce morphologic changes and even cell death in hippocampal CA3 neurons (480) which adversely affects its ability to provide negative feedback on glucocorticoid levels to the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased levels and cause more damage. This damage may be mediated by increases in excitatory amino acids and serotonin (38). Some data suggest that glucocorticoids act within 24 hours to significantly increase the rate of apoptosis in the hippocampus (478) probably via inhibition of glucose uptake in neurons (481) leading to decreased hippocampal volume and performance (482).

The VDR and its metabolizing enzymes are expressed in the midbrain, cerebellum, thalamus, hypothalamus, basal ganglia, hippocampus, olfactory system and the temporal, orbital and cingulate cortices (483). A calcitriol interacts with the synthesis and degradation of some neurotransmitters and activates the gene expression of the enzyme tyrosine hydroxylase (369) thereby increasing the availability of dopamine, noradrenaline and adrenaline. Also, calcitriol may enhance cholinergic function, both by increasing the activity of choline acetyltransferase (484) and by decreasing the activity of acetylcholine esterase (485). Dopamine, noradrenaline and acetylcholine are well-known actors in the pathophysiology of mood disorders (486, 487). Kalueff et al., show that the mice deficient in vitamin D receptor gene demonstrate increased anxiety-like behaviours when subjected to a battery of behavioural tests (488). Several studies have shown that increase in vitamin D level significantly reduced depression like behaviors (291, 296, 489). In our study we found that vitamin D and its combination with dexamethasone significantly reduced anxiety and depression like behaviors either by involving genomic or non-genomic
mechanism by increasing level of various neurotransmitter which involved in the pathophysiology of mood disorders.

The previous human studies showed that daily doses of vitamin D have better therapeutic effect than bolus doses of vitamin D (370) but in our study we did not find any statistically significance difference between daily dose and bolus dose of vitamin D in antiasthmatic activity as well as associated side effects with corticosteroids except in psychiatric side effect study. The bolus dose of vitamin D was more efficacious than daily dose in reversing anxiety and depression like behaviors. This difference may be due our study is on animals and may be difference in study duration. Therefore, in asthmatic patients vitamin D can be given as daily dose or bolus dose; there is no difference in efficacy. Bolus dose either weekly or monthly may enhance the compliance of patients.

The histological examination of lung tissue from the model control rats showed a typical damaged lung tissue with total and differential leukocyte infiltration, reduced lumen size, epithelial desquamation and angiogenesis while animals treatment with dexamethasone, vitamin D and combinations of vitamin D and dexamethasone showed a protective effect, as evidenced by the presence of milder or less pathological features.

Our data from above mentioned study suggest that, Vitamin D enhance the efficacy corticosteroids and showed significant protection in animals and that might be due to the inhibition of infiltration of inflammatory cells, release/synthesis of T-cell derived cytokine and immunoglobulin. Further, vitamin D reduces corticosteroid induced side effects like hyperglycemia, dyslipidemia and psychiatric adverse effects. There is no significant difference in efficacy; either received daily dose or bolus dose of vitamin D. The bolus dose can be used to improve compliance.

The mainstay treatment of asthma is inhaled corticosteroids and bronchodilators that are currently the most effective controller medications for the treatment of persistent asthma and are also recommended by the Global Initiative for Asthma (GINA) guidelines (24). The efficacies of glucocorticoids include reducing asthma symptoms, reducing exacerbation frequency, improving quality of life, improving lung function, decreasing airway hyperresponsiveness, controlling airway inflammation, and reducing mortality.
However; approximately 5-10% are severe refractory asthmatics who respond poorly to asthmatic drugs; associated with more drug medications and more hospital visits and admissions than mild to moderate asthma (5, 490). Also medical cost to treat severe asthma is more than 50% of the total medical cost for treating asthmatic patients (5). Severe refractory asthmatics have persistent symptoms, frequent symptom exacerbation, and severe airway obstruction even when taking high-dose inhaled steroids. Also the treatment responses to corticosteroids varies in these asthmatic patients and even respond poorly to systemic glucocorticoid (490). Therefore; asthmatic patients who receive high doses of systemic corticosteroids or those with corticosteroid-resistant asthma can benefit from medicines that involve novel mechanism of action, reversing the resistance of drugs or may act synergistically with existing drugs.

In further study we aim to study the effect of vitamin D with corticosteroids antagonist; mifepristone; whether vitamin D can suppress inflammatory cell in absence of corticosteroid receptor or involved receptor other than corticosteroid for mechanism of action.

Mifepristone is synthetic steroid with antiglucocorticoid activity. Mifepristone bind strongly to the glucocorticoid receptors, and to a lesser extent to the androgen receptor. Its relative binding affinity for glucocorticoid receptors is approximately three times greater than dexamethasone (327). We found animals pretreatment with vitamin D, combination of vitamin D and dexamethasone decreased the production of the inflammatory cells; eosinophils and neutrophils. However, animals pretreated with mifepristone and received dexamethasone are unable to suppress inflammatory cells like eosinophils and neutrophils but those animal who were pretreated with mifepristone and received vitamin D are able to suppress inflammatory cells like eosinophils and neutrophils. It is clear that vitamin D may act by other mechanism and does not involved glucocorticoids receptor. Therefore; vitamin D may be useful in that case who are steroid insensitive. In this experiment we did not find any effects of treatments on inflammatory cells like lymphocytes and monocytes. This may be due to short study period of experiments or may be biological or environmental variations in animals.

From the above experiments it is clear that vitamin D can suppress the inflammatory cell in corticosteroid insensitive. So in the light of above experiments we aim to study the effect
of vitamin D with PI3K inhibitors; wortmannin and demonstrated that vitamin D acts as synergistically with wortmannin.

PI3Ks represent important mediators in the signaling cascade leading to the initiation of the inflammatory response. Recently, several studies have demonstrated the importance of PI3K in cellular migration in vitro and in vivo (142, 491). Early evidence for the role of PI3K in chemotaxis was the demonstration that human T cell migration induced by regulated on activation, normal T expressed and secreted was PI3K activation-dependent (492). In the immune system, PI3K may also be activated by antigen receptors, such as the B cell, the T cell, and Fc receptors (FcRs)–receptors for mitogenic and inflammatory cytokines–and chemokine receptors (493).

In the present study we demonstrated vitamin D play an important role in modulating PI3K signaling in the pathogenesis of airway inflammation and targeting PI3Ks appears to have therapeutic benefit for the treatment of asthma. We found that OVA-sensitized animals showed significant increase in eosinophils and neutrophils. Pretreatment of animals with vitamin D, dexamethasone, wortmannin and combinations of vitamin D with dexamethasone and wortmannin significantly inhibited eosinophils and neutrophils in OVA model of asthma. However, vitamin D, acts as synergistically with wortmannin.

PI3K signaling is integral to both mast cell and eosinophil function (20, 494). Inhibition of PI3K signaling with the nonselective inhibitor LY294002 in an ovalbumin (OVA)-challenged murine model of asthma reduced inflammatory cell influx into the lung as well as reduction of IL-5, IL-13 and CCL11 (eotaxin) (21). In addition, tissue eosinophilia, airway mucus production and airway hyper-responsiveness to inhaled metacholine were all suppressed. These results are supported by other studies where pharmacological inhibitors of PI3K or adenoviral-mediated PTEN overexpression all reduced eosinophilia and AHR in models of allergen challenge (495). Collectively, these studies clearly demonstrate that direct targeting of PI3K in the lung reduces allergic inflammation.

PI3K regulates neutrophil migration, as evidenced by the ability of the PI3Kd-specific inhibitor IC87114 to reduce directional movement in response to chemotactic agents (496). In agreement with this finding, wild-type (WT) mice treated with the PI3Kd-specific inhibitor IC87114 show reduced infiltrating cells in a model of pulmonary inflammation
At the site of infection, neutrophils and macrophages exert their antimicrobial function by producing and secreting reactive oxygen species (ROS), an event known as respiratory burst. This event consists in a biphasic process, with an initial PI3Kg-dependent phase of PI(3,4,5)P3 production and subsequent amplification of the signal mediated by PI3Kd (496). Indeed, genetic ablation of PI3K impaired ROS production by neutrophils (142). Similarly, PI3Kg inhibition completely abrogates neutrophil respiratory burst, thus supporting a model where the class IB-dependent first phase is necessary for the class IA-mediated second phase (497).

Thus vitamin D is an imperative player in modulating PI3K signaling in the asthma and acts as synergistically with wortmannin. It suggests that vitamin D should be added to corticosteroid insensitive patients to improve the adherence of treatments in asthma.

In brief, our studies conclude that vitamin D has significant place in asthma. Vitamin D enhances the efficacy of corticosteroids by inhibiting inflammatory cells and cytokines. It also helps to reduce the corticosteroid induced side effects like hyperglycemia, dyslipidemia and psychiatric adverse effects. The vitamin D can be use as daily dose or bolus dose as per need and compliance. Further, vitamin D acts as synergistically with PI3K inhibitors that can play a key role in corticosteroid insensitive patients.