

# **Translational Research and Herbal Drug Development : An Experience with Bronchial Asthma**

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Global Summit on Herbals and Natural Remedies, Oct. 26-27, 2015

# Herbal Drug Research

- Herbal Drug Research is of global interest and emerging as a leading area for new drug discovery and development
- Demonstrated efficacy against a wide variety of diseases with reportedly, minimal adverse effects
- Need to explore and generate scientific information and develop skills in this relevant area

# Herbal drugs...

- Newer scientific techniques are being used for the validation of their demonstrated effects and initial results suggest a scientific basis for their actions
- This could revolutionize drug treatment in several pathophysiological states
- Crucial pharmacoeconomic implications

# Approaches.....

- Interactions between traditional and modern medicines : an emerging concept
- Two common approaches for herbal drug research:
  - Translational research (lab to clinic)
  - Reverse pharmacology (clinic to lab)
- Several effects of drugs from traditional systems of medicine explained by modern biological mechanisms (Concepts of cellular/molecular biology are being incorporated)

# Respiratory disease

- Respiratory diseases : a major cause of hospital admissions
- Obstructive airway disease (Bronchial Asthma and COPD) affect 5-7% population in industrialized countries
- Several factors (emotional and environmental) contribute to their genesis
- Optimization and rationalization of drug therapy : key to effective management

# Bronchial asthma..

- Bronchial asthma is a chronic inflammatory disorder associated with airway hyperresponsiveness leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing
- Many cells and cellular elements play a role in airway inflammation and contributes to hyperresponsiveness, airflow limitation, symptoms & chronicity

# Bronchial asthma..

- The aim/strategies of in asthma treatment is:
  - reduction of inflammation & bronchial hyper reactivity
  - bronchodilation
  - blockade of IgE antibody
  - prevention of release of inflammatory mediators and antagonism of released mediators
  - oxidative stress has also been implicated in this cascade

# Drugs in asthma...

- Bronchodilators and corticosteroids are the mainstay in the treatment of these conditions
- Safety concerns have prompted the search for viable alternatives
- Herbal drugs have been effectively used in traditional and modern medicine and may have beneficial/synergistic effects



# UNIM-352...

- A polyherbal Unani preparation, known for its use in bronchial asthma (Standardized and authenticated by CRIUM, Hyderabad and CCRUM, AYUSH, New Delhi)

- Composition :

*Linum usitattissimum linn* (Alsi)

*Trigonella foenumgraecum* (Methi)

*Allium sativum linn* (Seer)

*Apis mellifera linn* (Chillbenj)

*Caesalpineia bondumelo fleming* (Magz-e-Karanjwa)

*Pongomia glabra vent* (Magz-e-karanj)

Honey qs

# Aims of the study

- To clinically evaluate the efficacy and safety of UNIM-352 in patients of bronchial asthma
- To investigate the possible mechanism of anti-asthma action of UNIM-352 in experimental animal models

# The Clinical Study

- Single blind, randomized, placebo controlled, parallel design, prospective study
- Ethical clearance obtained and GCP guidelines followed
- Standard inclusion/exclusion criteria
- Subjects : OPD patients of bronchial asthma, Diagnosed by clinical features and PFT findings
- Written informed consent taken

# Inclusion/exclusion criteria

- Age : 18 – 60 years (either sex)
- Moderate, persistent asthma diagnosed by PFT and bronchodilator reversibility (GINA guidelines)
- No co-morbid conditions (DM, HT, HIV, TB, liver disease, kidney disease, alcohol intake and smokers, pregnancy etc.)
- No medication for diseases other than asthma

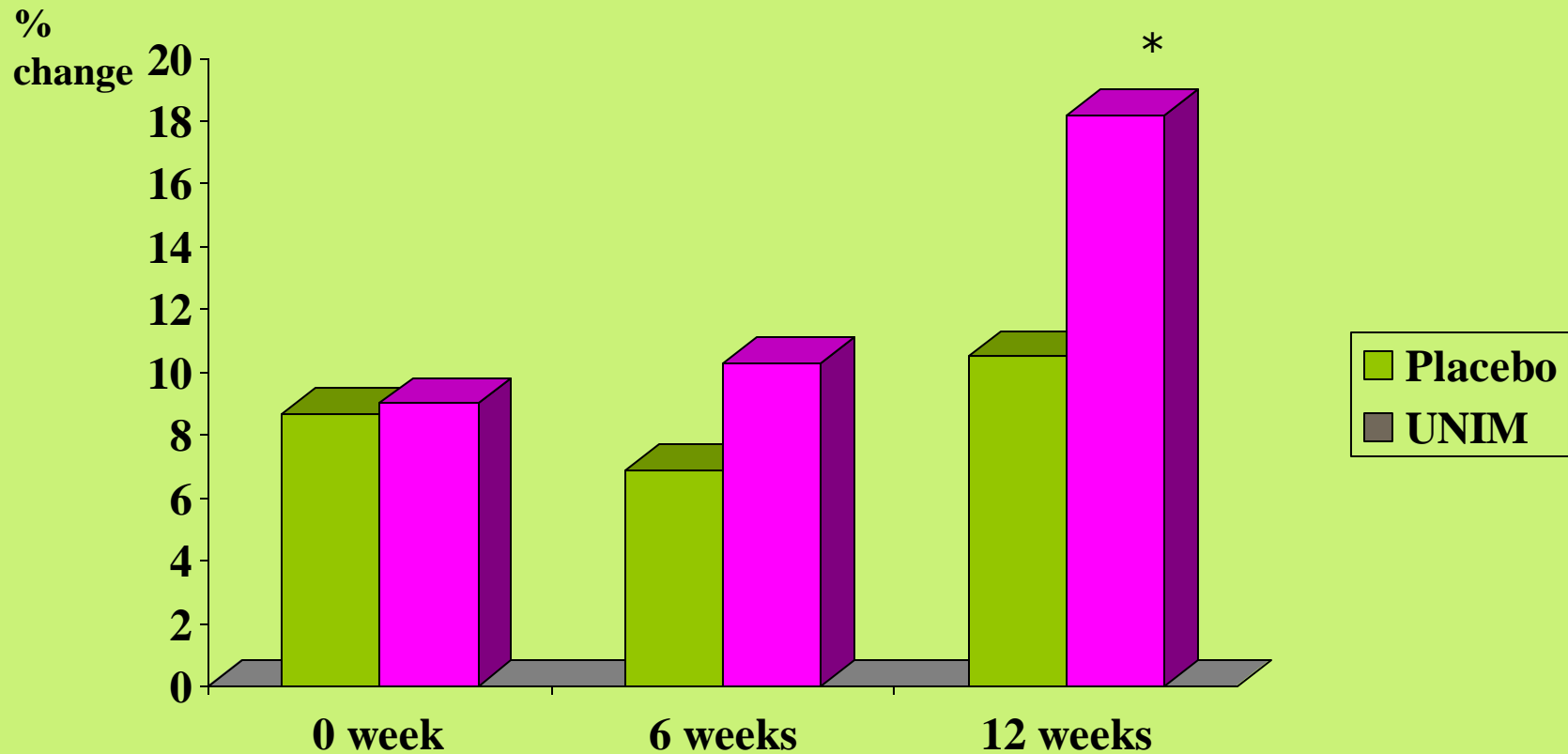
# Experimental design

- **Group I :**  
Formoterol (6 mcg) + Budesonide (200 mcg) + Placebo  
majoon (10 g) – BD
- **Group II :**  
Formoterol (6 mcg) + Budesonide (200 mcg) + UNIM-352  
majoon (10 g) – BD
- SOS Emergency Levosalbutamol inhaler (100 mcg) in both  
groups
- Treatment period : 3 months (12 weeks)

# Study markers and data analysis

- FEV1
- FEV1 / FVC ratio
- Incidence of asthma aggravation
- Frequency of emergency bronchodilator use
- Clinical symptoms (dyspnoea, wheezing, cough, etc.)
- These parameters were compared between placebo and UNIM-352 groups
- Data was analysed by Chi-Square test and Wilcoxon`s test (paired)

# Comparison of placebo and UNIM-352 treatments on FEV1 in asthma patients

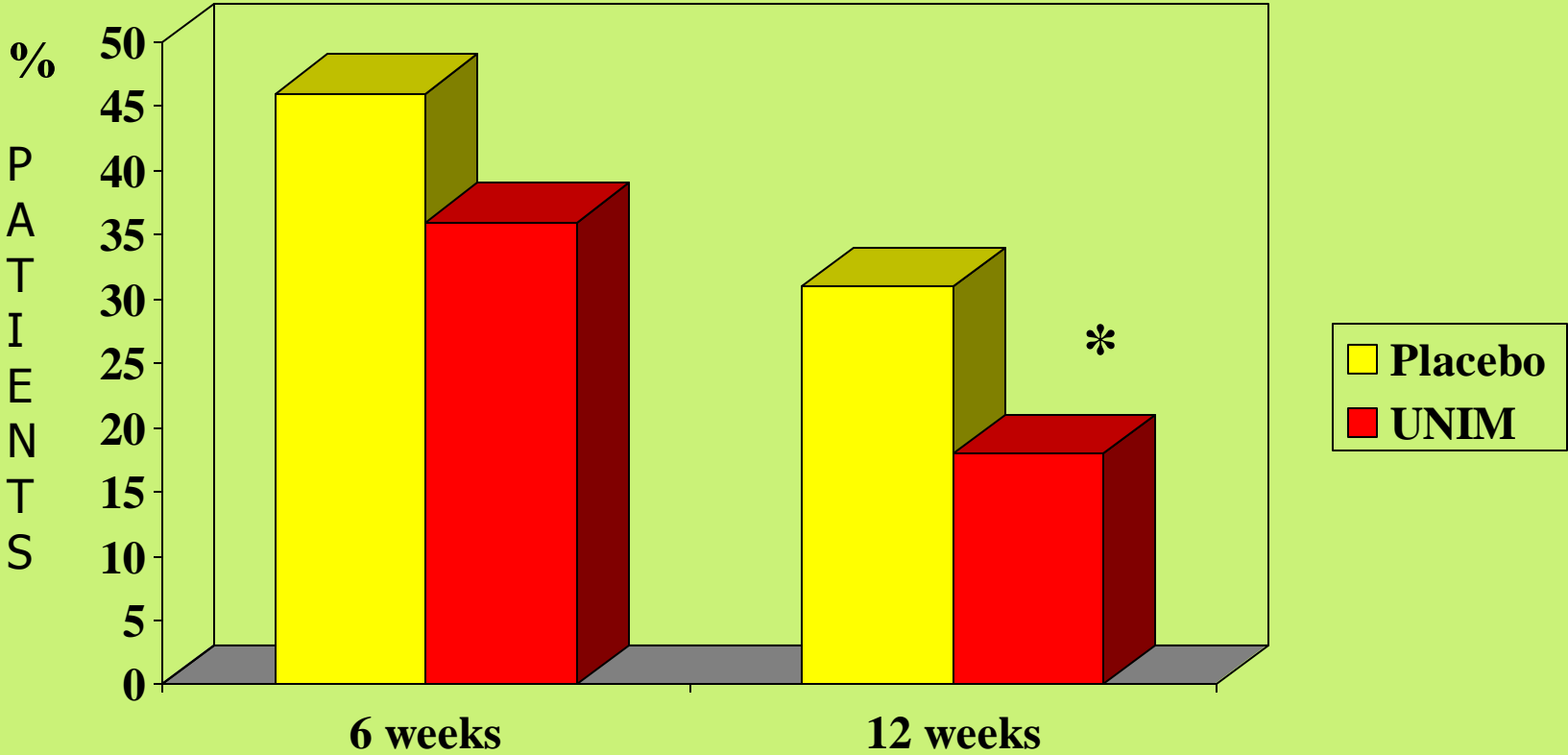


# Comparison of placebo and UNIM-352 treatments on PFT parameters in asthma patients

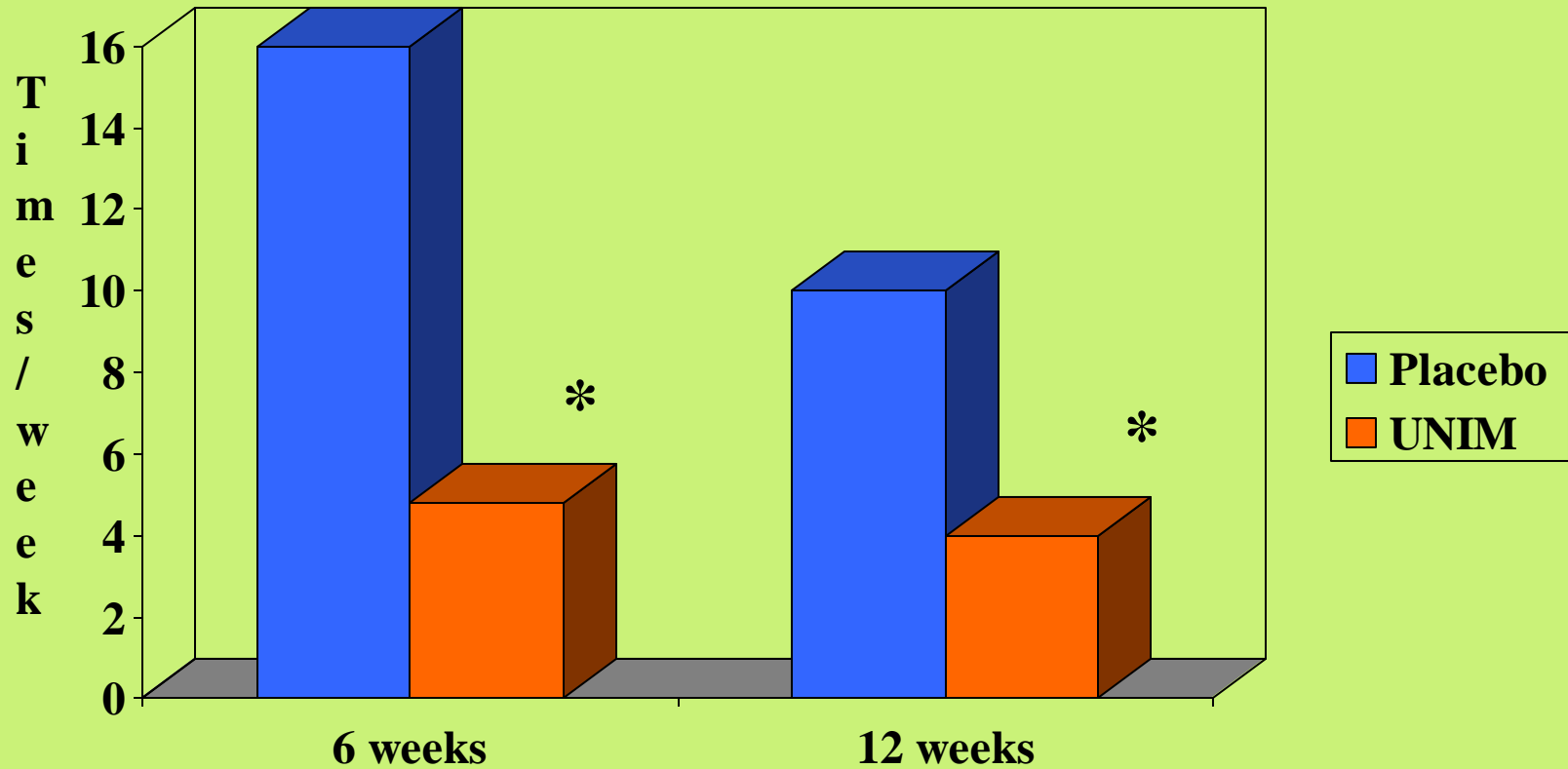
PFT Parameters	At 0 week	At 6 weeks	At 12 weeks
<b>Change in FEV1 (%)</b>			
<b>Placebo</b>	<b>8.7 ± 2.4</b>	<b>6.9 ± 1.1</b>	<b>10.5 ± 1.8</b>
<b>UNIM-352</b>	<b>9.0 ± 1.7</b>	<b>10.3 ± 2.6</b>	<b>18.2 ± 3.4 *</b>
<b>Basal FEV1 (%pred)</b>			
<b>Placebo</b>	<b>71.3 ± 4.8</b>	<b>74.7 ± 3.0</b>	<b>79.4 ± 2.9</b>
<b>UNIM-352</b>	<b>75.9 ± 4.9</b>	<b>83.5 ± 4.4 *</b>	<b>87.0 ± 4.2 *</b>
<b>FEV1/FVC ratio</b>			
<b>Placebo</b>	<b>5.7 ± 2.0</b>	<b>4.5 ± 1.1</b>	<b>6.6 ± 1.1</b>
<b>UNIM-352</b>	<b>5.3 ± 1.0</b>	<b>5.9 ± 2.5</b>	<b>8.4 ± 2.6 *</b>



# Incidence of exacerbation of bronchial asthma after placebo or UNIM-352



# Mean frequency of emergency bronchodilator use after placebo or UNIM-352 treatments



# Summary

- A total of 40 patients were enrolled (05 drop outs)
- Out of the 35 patients completing the study, 16 were in placebo group and 19 were UNIM-352 group
- Analysis of data showed that UNIM-352 had significant beneficial effects in bronchial asthma patients as compared to the placebo treated group, in the parameters studied
- UNIM-352 was generally well tolerated and adverse effects reported were rare

# Reverse Pharmacology

- A study was conducted to evaluate the cellular and molecular mechanism involved in the anti-asthmatic effects of UNIM-352
- Approach of reverse pharmacology – defined as validation of the effects of known drugs through experimental studies, after its clinical efficacy has been established
- A technique frequently used these days to experimentally evaluate disease ameliorating effects of investigational drugs

# The pre-clinical study

- To evaluate the effects of UNIM-352 on standard markers of inflammation and immunity in exptl. Model of asthma
  - Assay of pro- and anti-inflammatory cytokines (TNF $\alpha$  and IL-4, GM-CSF), HDAC in blood and BAL
- To evaluate the effects of UNIM-352 on experimentally induced airway remodelling
  - TGF- $\beta$  and hydroxyproline (markers of collagen formation, fibrosis) in lung tissue

- **Histopathology:** By the accredited laboratory, North Delhi Pathology Clinic, Delhi
- **Cytology of BAL fluid and blood :** Eosinophil and neutrophil counts in blood and BAL fluid were carried out using Leishman's stain
- **Oxidative stress markers** To study the effects of UNIM-352 on :
  - Lipid peroxidation (MDA levels)
  - Anti-oxidant defense profile (SOD, GSH)

# Methods..

- Experimental animals: Inbred Wistar rats (180-250g, either sex) and animal care was as per INSA guidelines and protocol was approved by the IAEC
- Rats were sensitized with ovalbumin (10 mg/rat, ip) adsorbed to 100 $\mu$ g of Al(OH)<sub>2</sub> and challenged with ovalbumin (1 mg, ip) on 14<sup>th</sup> day
- After 24 h of challenge, rats sacrificed and blood and BAL fluid( by lavaging the lung) were collected and assayed for various parameters

# Experimental procedure....

## For airway remodelling:

- Immunized with 100mg OVA with 200mg Al(OH)<sub>3</sub>, ip
- 15-21 days nebulized with 2% OVA daily for 30 min
- Sacrificed on 22<sup>nd</sup> day and lungs , blood, BALF collected

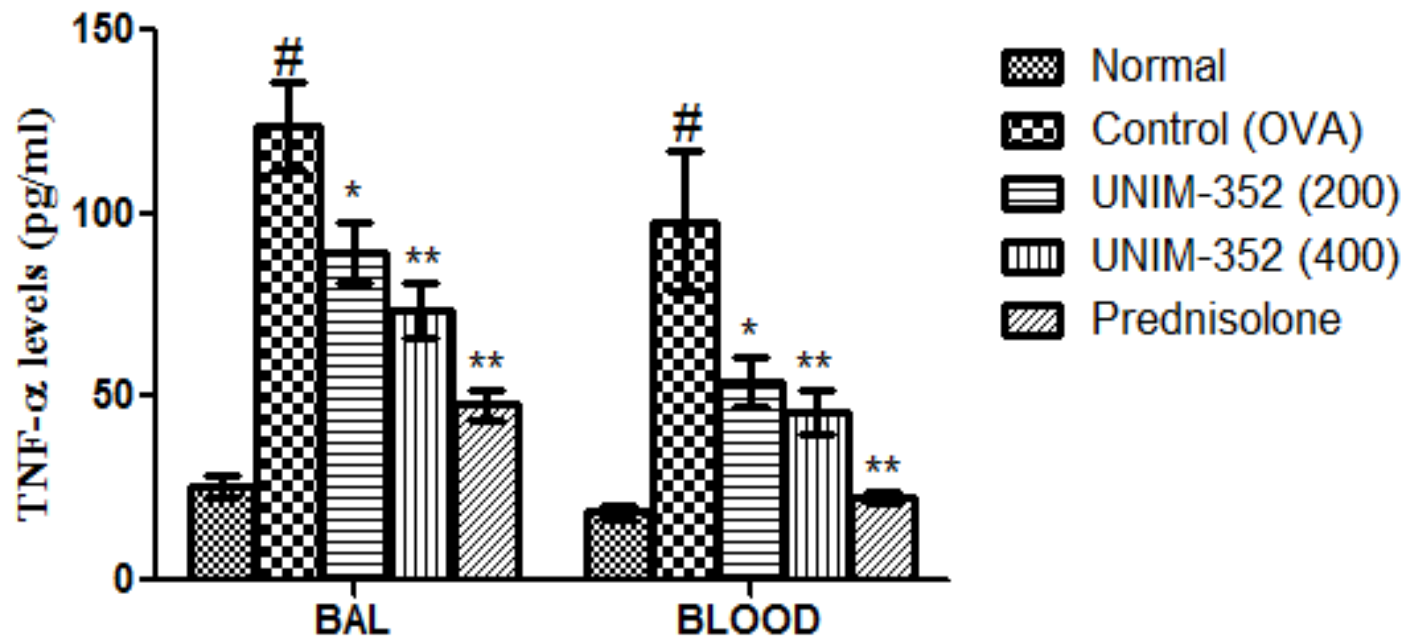


# Results...

- UNIM-352 was used at dose levels of 200 and 400 mg/kg, orally
- The animals behaved normally and did not show any overt signs of toxicity
- There was no loss of weight or reduction in food and water intake after the drug

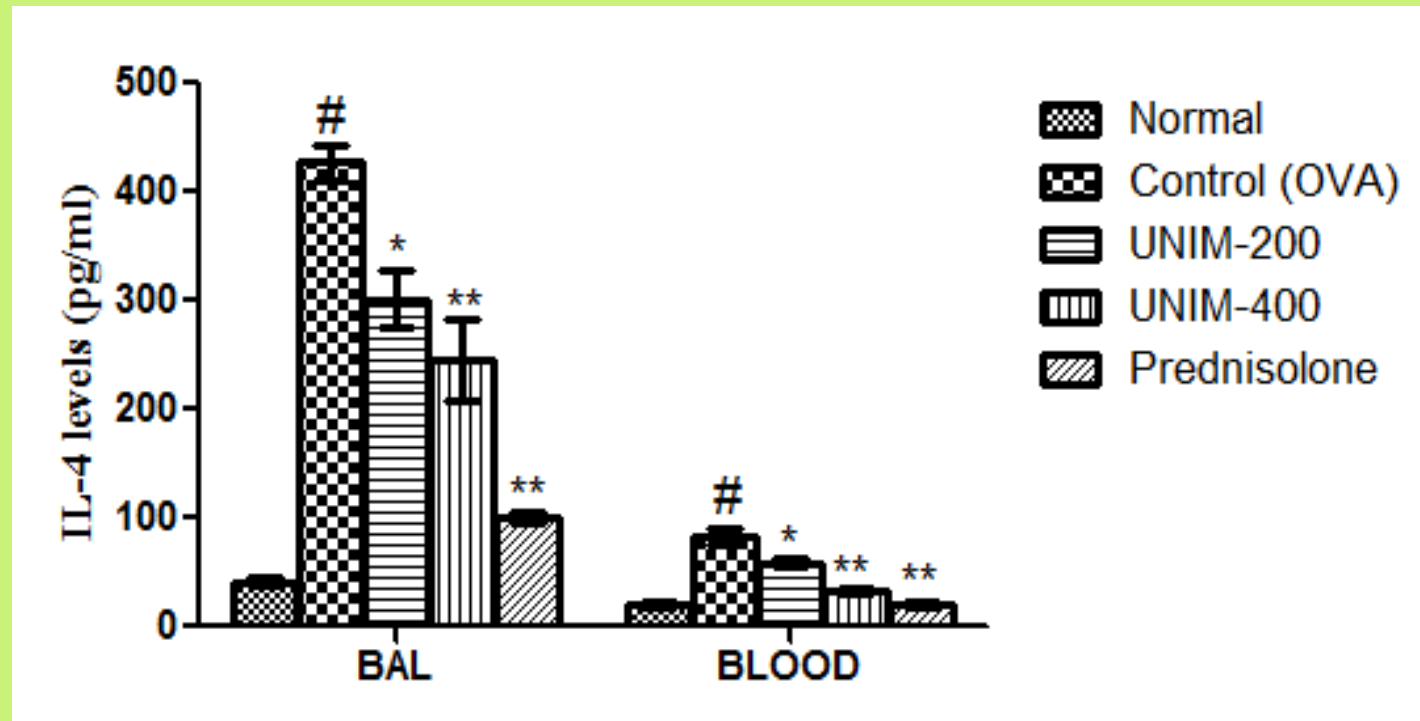
# **Effect of UNIM-352 on inflammation and immunity**

## Effect of UNIM-352 on TNF- $\alpha$ levels



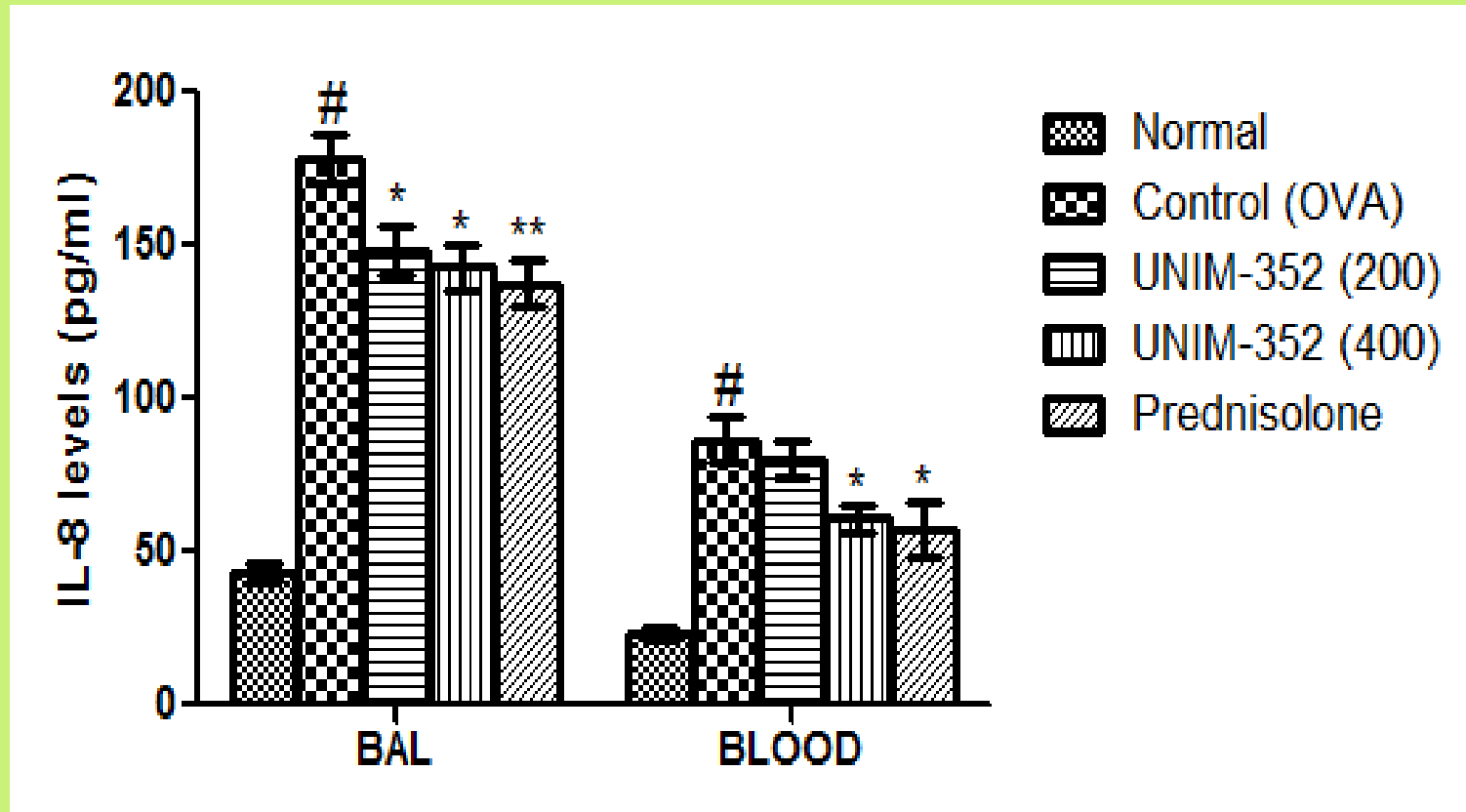
Data are expressed as Mean  $\pm$  SEM, n=6 per group. #p<0.01 vs normal group, \*p<0.05, \*\*p<0.01 vs control group

## Effect of UNIM-352 on IL-4 levels



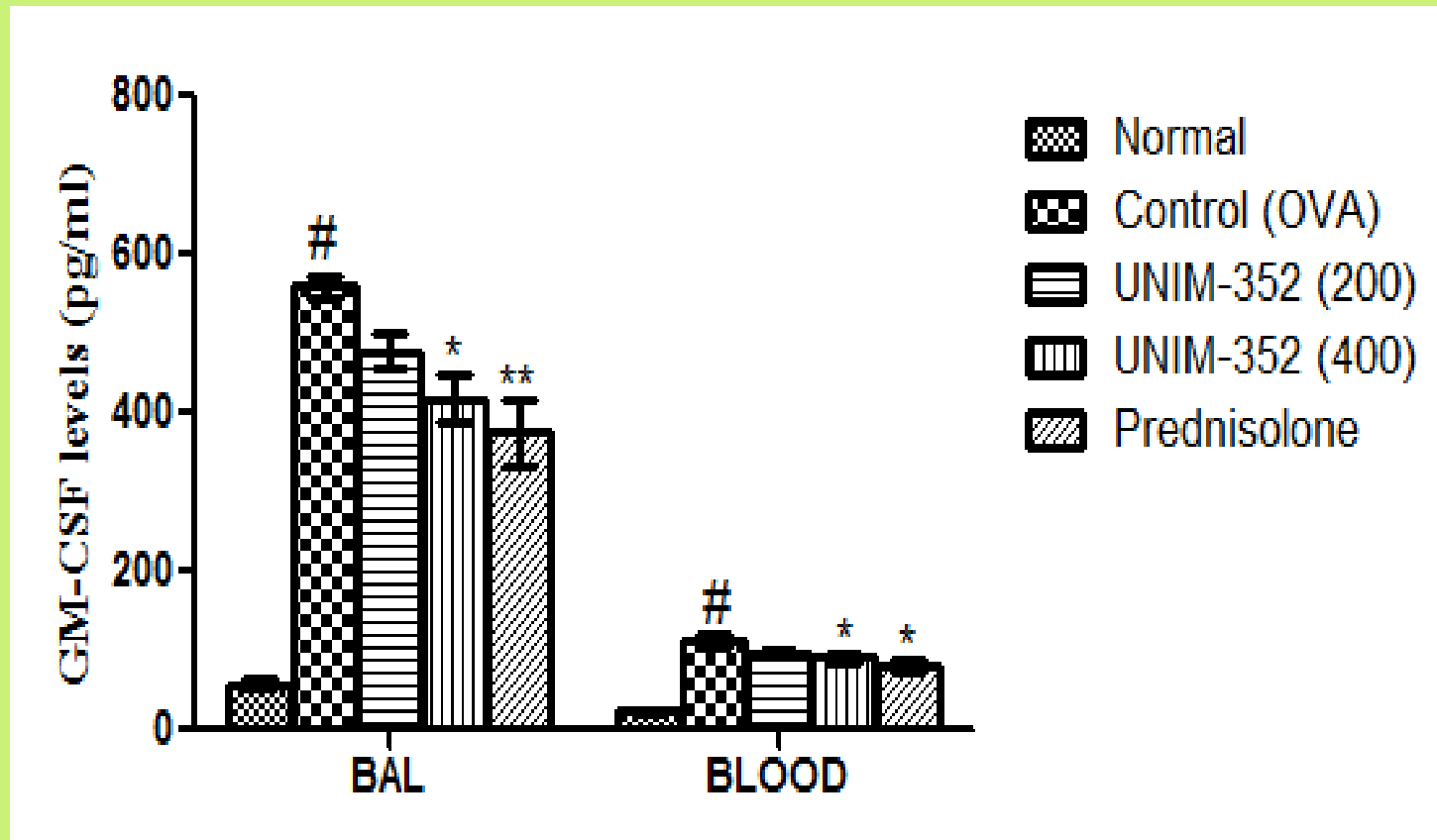
Data are expressed as Mean  $\pm$  SEM, n=6 per group. <sup>#</sup>p<0.01 vs normal group, \* p<0.05, \*\* p<0.01 vs control group

## Effect of UNIM-352 on IL-8 levels



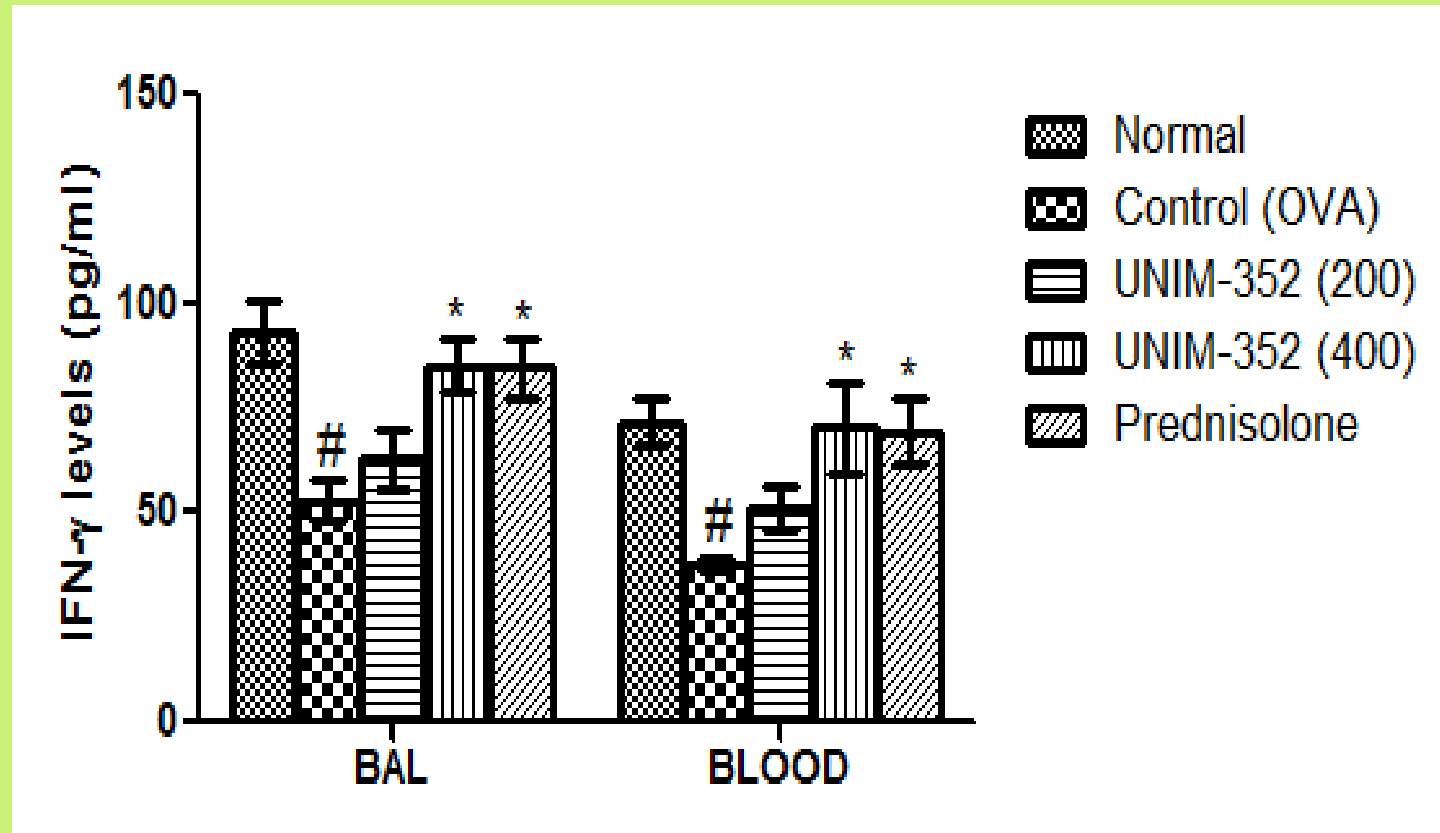
Data are expressed as Mean  $\pm$  SEM, n=6 per group. #p<0.01 vs normal group, \*p<0.05, \*\*p<0.01 vs control group

## Effect of UNIM-352 on GM-CSF levels



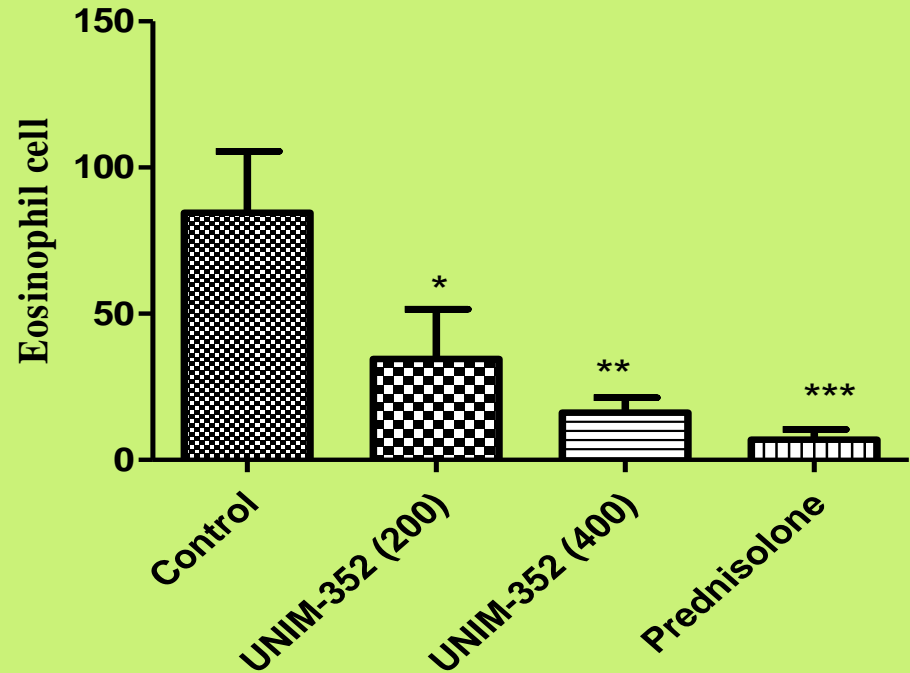
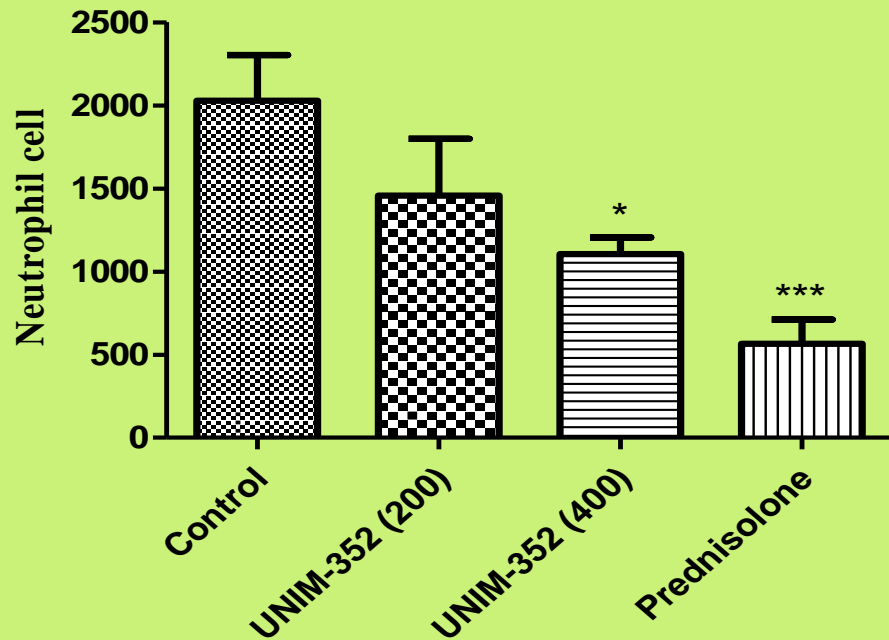
Data are expressed as Mean  $\pm$  SEM, n=6 per group. <sup>#</sup>p<0.01 vs normal group, <sup>\*</sup>p<0.05, <sup>\*\*</sup>p<0.01 vs control group

## Effect of UNIM-352 on IFN- $\gamma$ levels



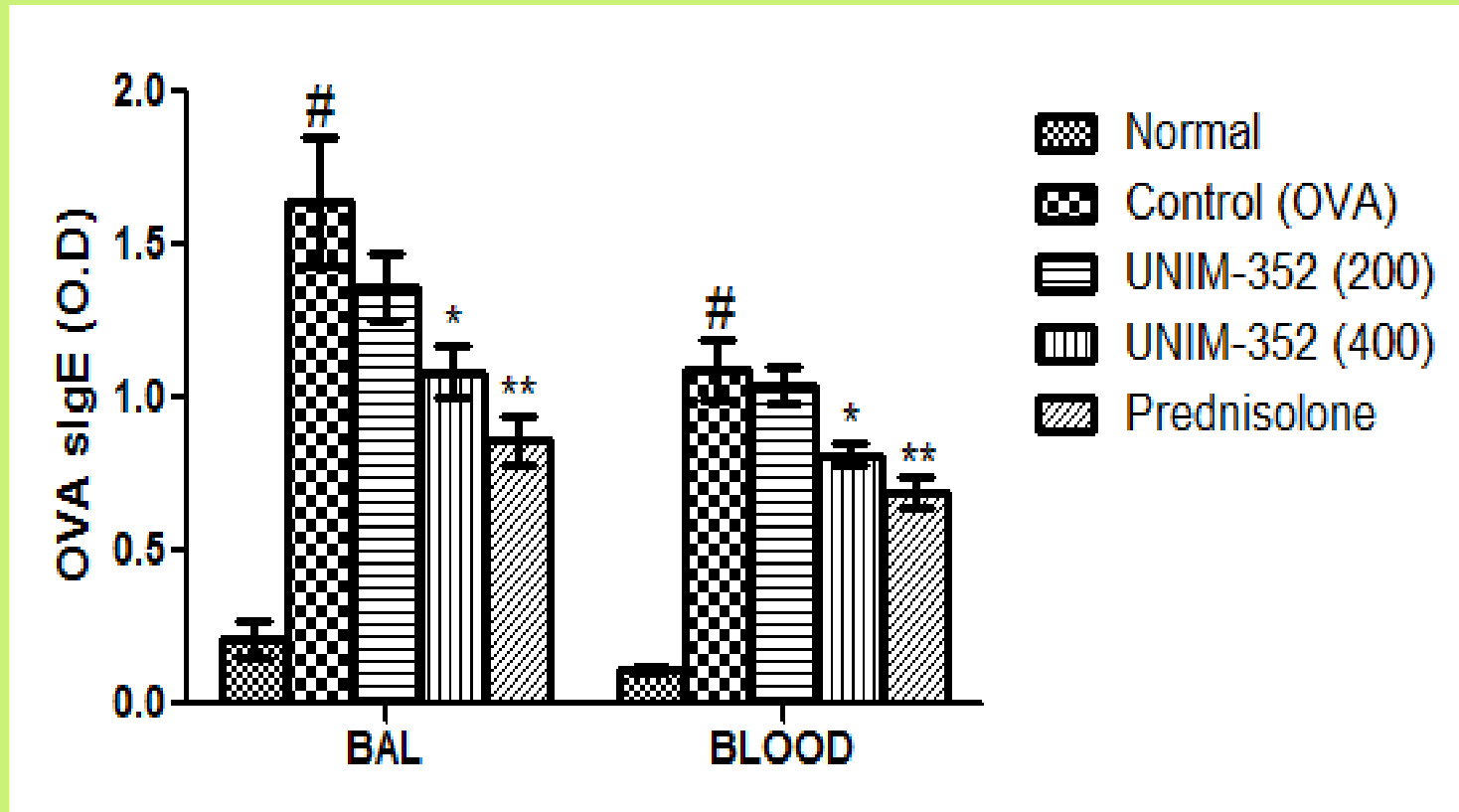
Data are expressed as Mean  $\pm$  SEM, n=6 per group. <sup>#</sup>p<0.01 vs normal group, <sup>\*</sup>p<0.05 vs control group

# Effect of UNIM-352 on neutrophils and eosinophils counts in BAL fluid of rats



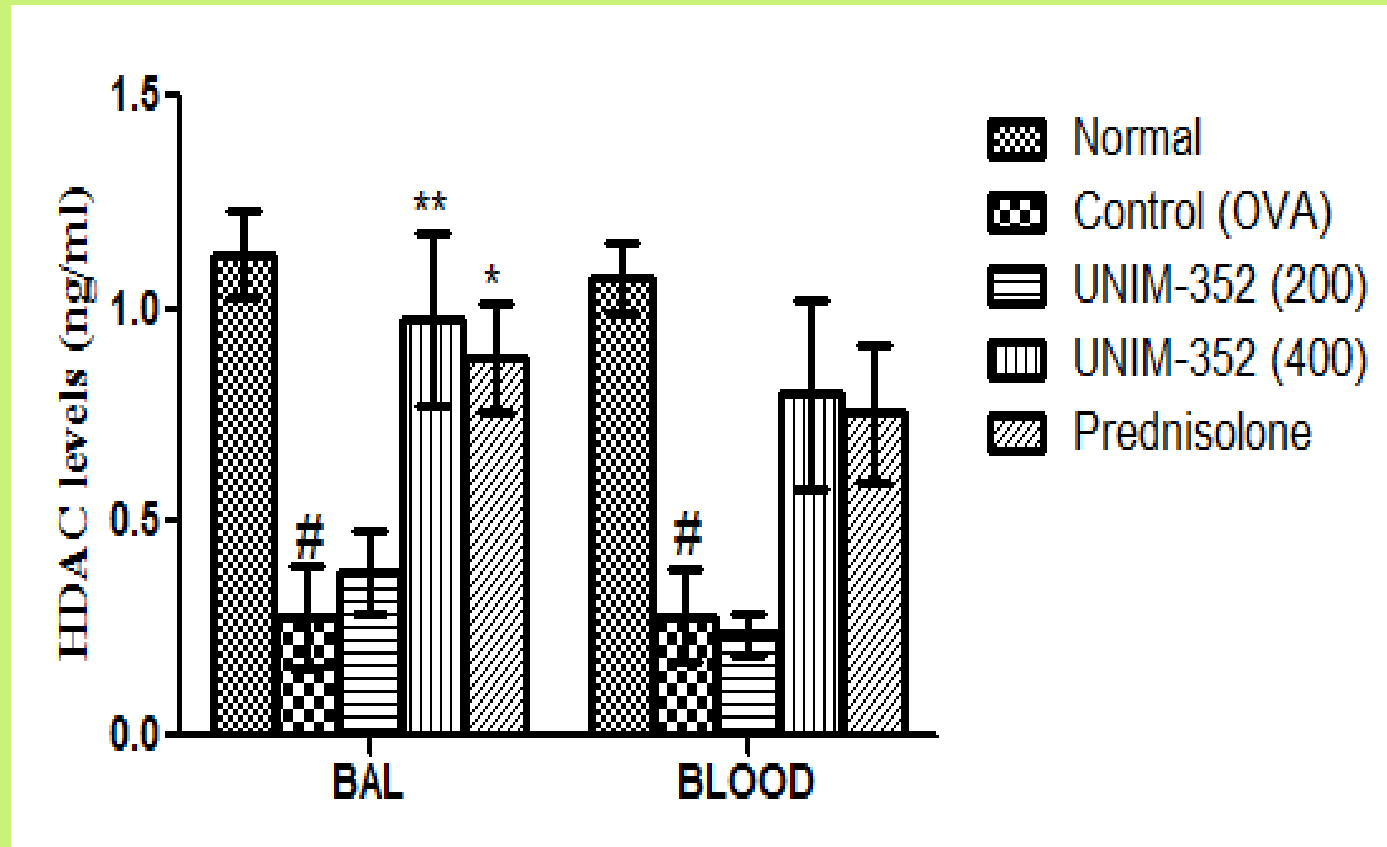


## Effect of UNIM-352 on OVA specific IgE levels



Data are expressed as Mean  $\pm$  SEM, n=6 per group. #p<0.01 vs normal group, \*p<0.05, \*\*p<0.01 vs control group

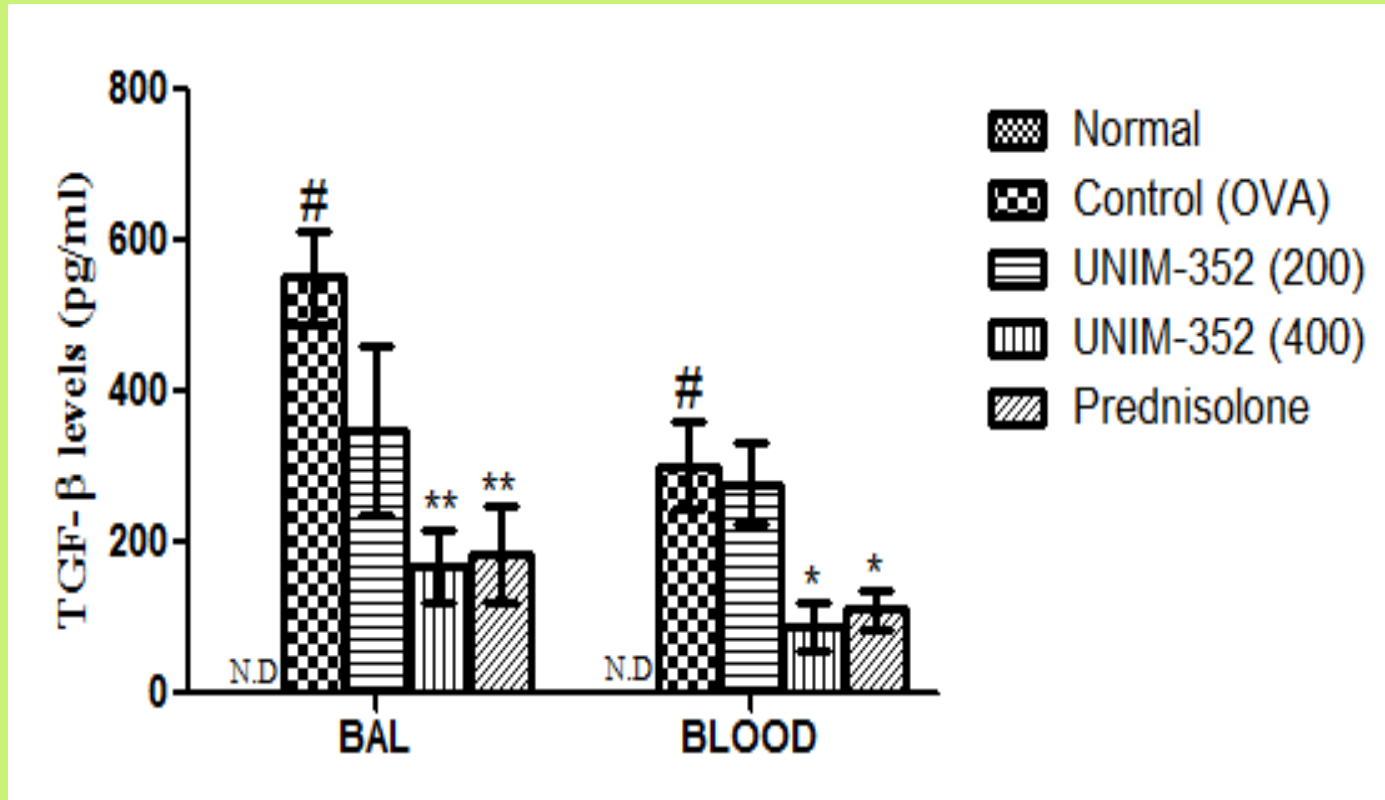
## Effect of UNIM-352 on HDAC levels



Data are expressed as Mean  $\pm$  SEM, n=6 per group. #p<0.01 vs normal group, \*p<0.05, \*\*p<0.01 vs control group

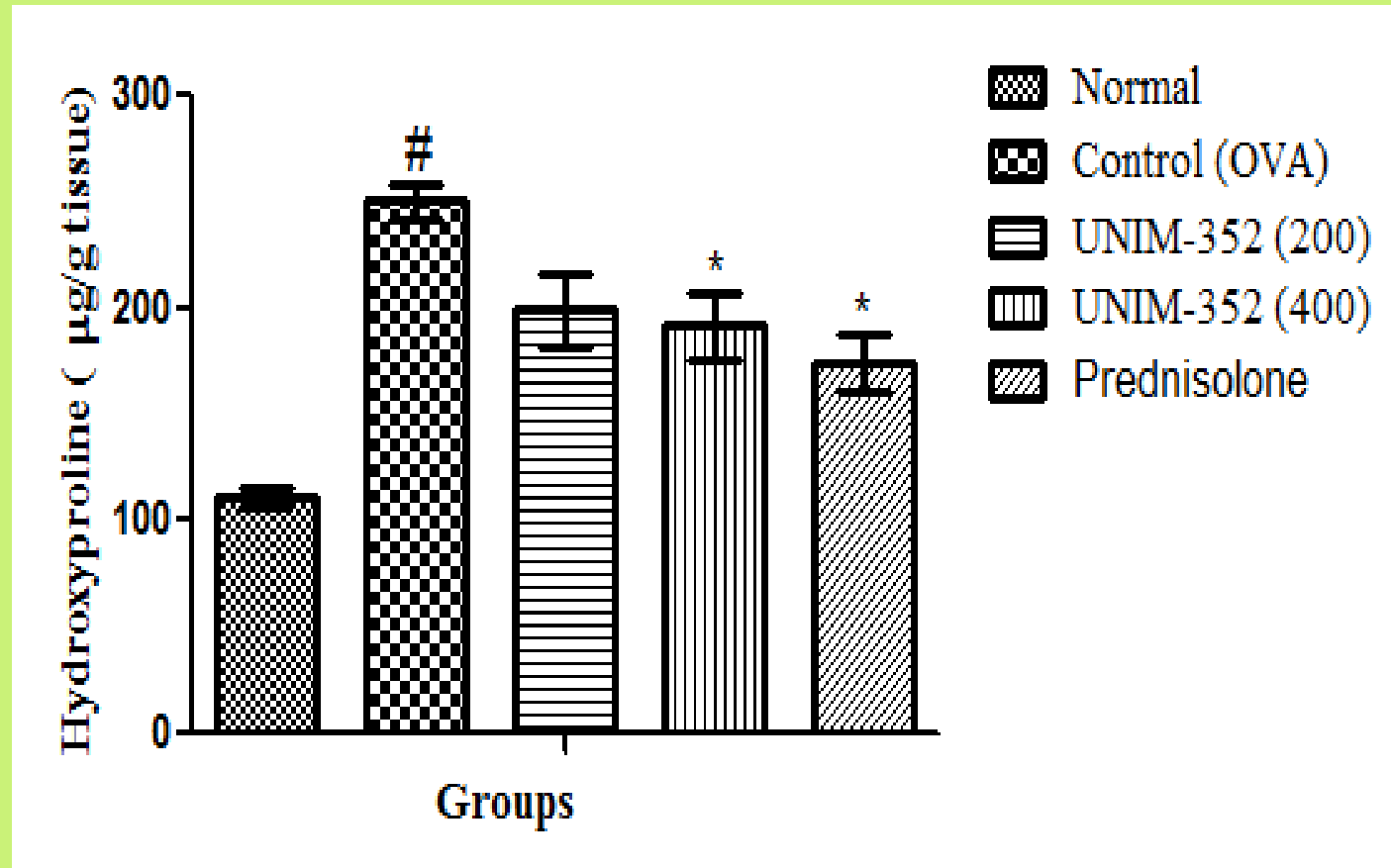
# **Effect of UNIM-352 on airway remodeling**

## Effect of UNIM-352 on TGF- $\beta$ levels



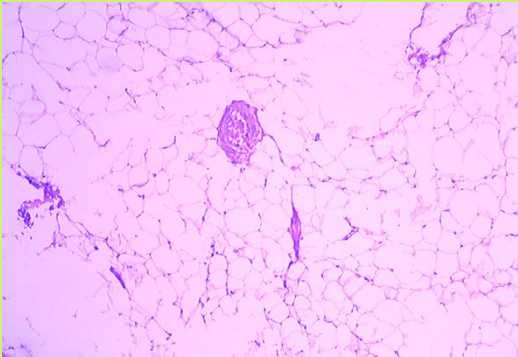
Data are expressed as Mean  $\pm$  SEM, n=6 per group. #p<0.01 vs normal group, \*p<0.05, \*\*p<0.01 vs control group

## Effect of UNIM-352 on hydroxyproline content

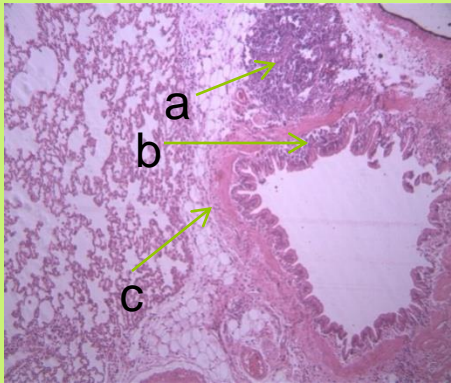


Data are expressed as Mean  $\pm$  SEM, n=6 per group. #p<0.01 vs normal group, \*p<0.05 vs control group

# Histopathological findings

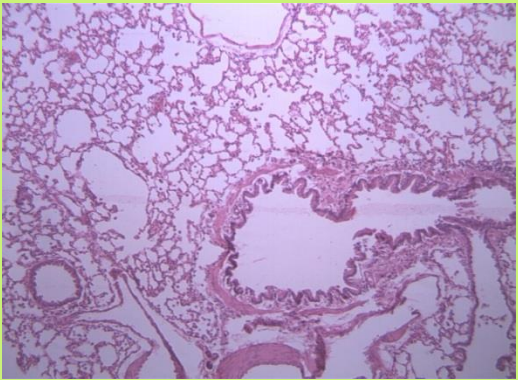


**Normal**

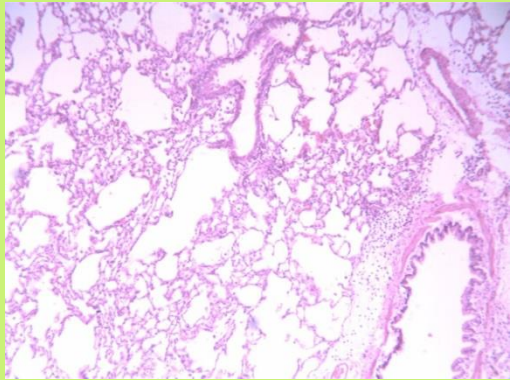


**Control**

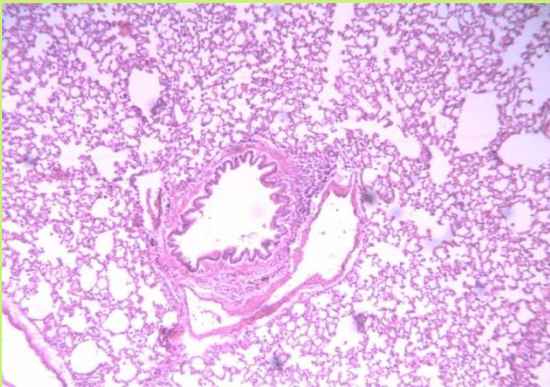
- a. Inflammatory cells
- b. Goblet cell hyperplasia
- c. Subepithelial fibrosis



**UNIM-352 (200)**



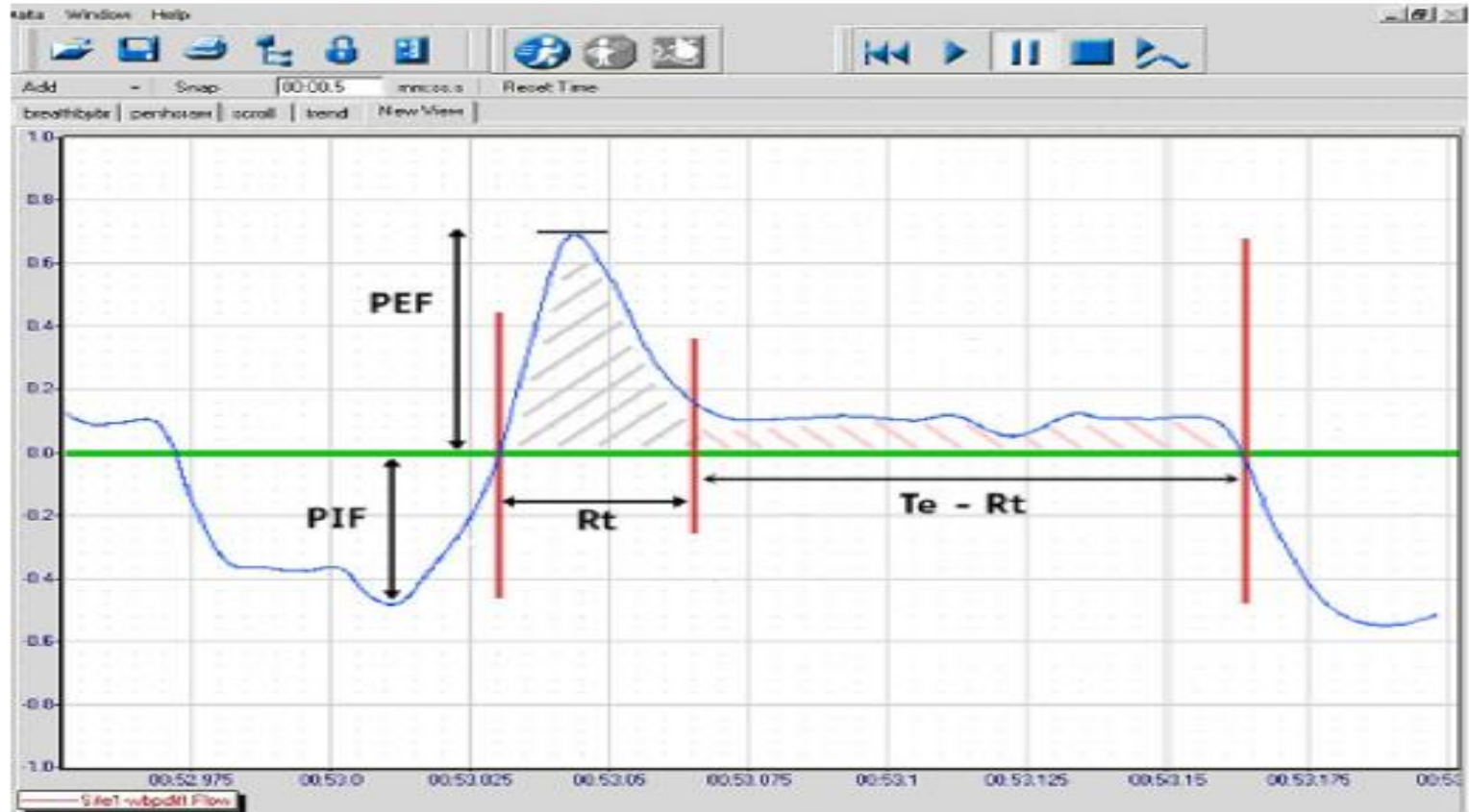
**UNIM-352 (400)**



**Prednisolone**

**Effect of UNIM-352 on bronchial  
hyperresponsiveness and airflow  
resistance**

# Effect of UNIM-352 on airway responsiveness to Methacholine

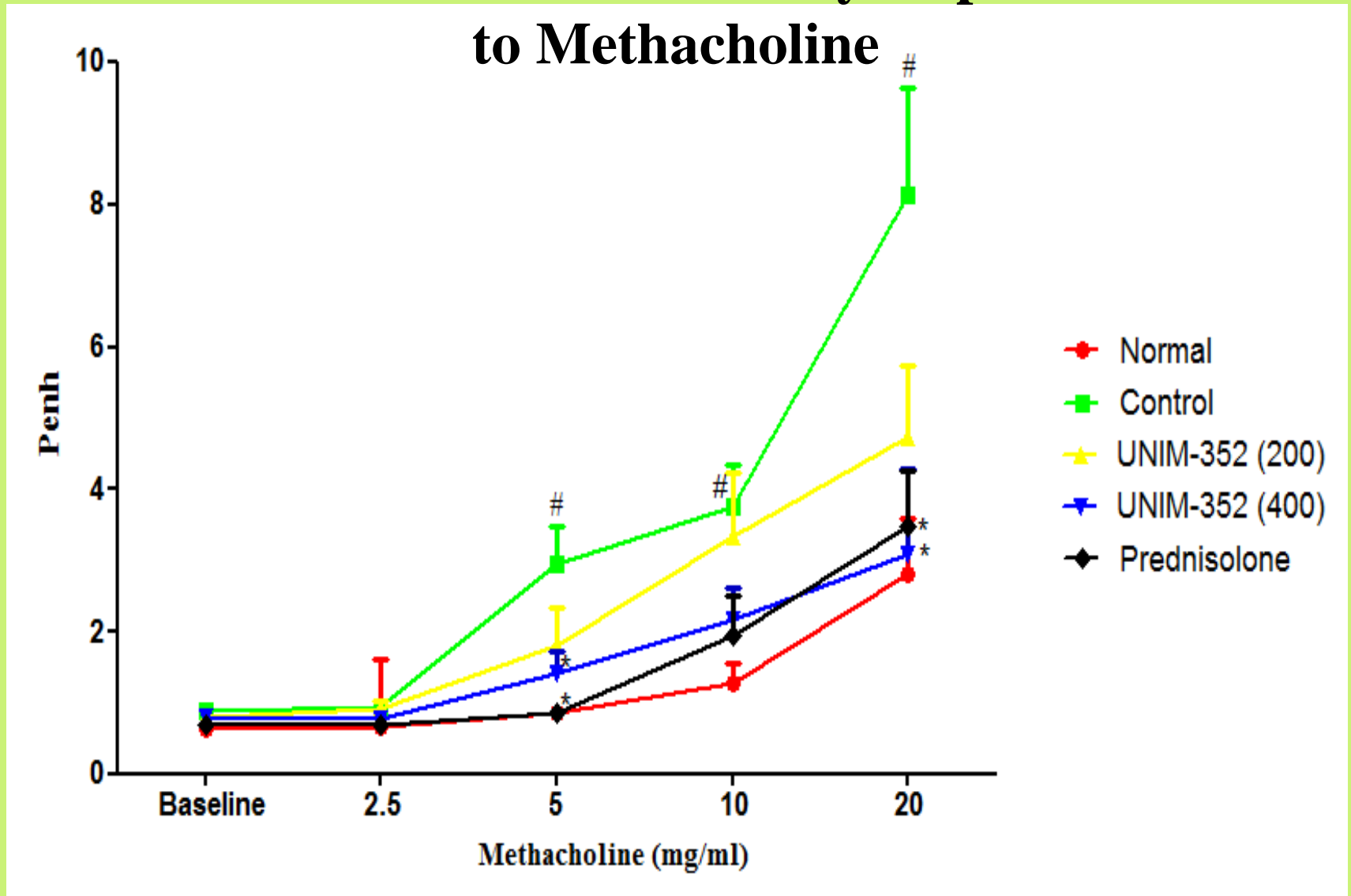


$$\text{Penh} = \text{Pause} \times \text{PEF}/\text{PIF}$$

$\text{Pause} = \text{Te}/\text{Rt} - 1$  , where  $\text{Te}$  = expiratory time,  $\text{Rt}$  = time to expire 65% of the volume



# Effect of UNIM-352 on airway responsiveness to Methacholine



Data are expressed as Mean  $\pm$  SEM, n=6 per group. \*p<0.05 vs control, #p<0.05 vs normal

# Summary

- UNIM-352 reduced the levels of TNF- $\alpha$ , IL-4, IL-8 and GM-CSF whereas IFN- $\gamma$  levels were elevated in both BAL and blood suggesting anti-inflammatory and immunomodulatory effect which is comparable to prednisolone, standard drug used for asthma
  
- Both the doses of UNIM-352 reduced the eosinophils and neutrophils counts in OVA sensitized rats. The above inflam. cytokines recruit eosinophils, the effector cells in asthma, thus results emphasize the anti-inflammatory role of the drug which may reduce the bronchial inflammation

- UNIM-352 attenuated ovalbumin specific IgE levels in both blood and BAL fluid suggesting that polyherbal agent has important immunomodulatory role
- UNIM-352 elevated HDAC levels in both blood and BAL fluid confirming anti-inflammatory effect of polyherbal agent
- UNIM-352 reduced TGF- $\beta$  (blood & BALF), hydroxyproline levels in lung homogenates, the marker of collagen formation and fibrosis, suggesting the anti-remodeling effect of UNIM-352

- Histological studies showed that UNIM-352 attenuated goblet cell hyperplasia, inflammatory cells infiltration, subepithelial fibrosis confirming anti-remodeling effect of UNIM-352
- UNIM-352 reduced the Penh in response to increasing doses of methacholine indicating attenuation of airway hyperresponsiveness and resistance to airflow
- This study showed anti-inflammatory, immunomodulatory, anti-remodeling effects of UNIM-352
- These studies help to explain the observed clinical benefits of UNIM-352 in patients of bronchial asthma

# Conclusions

- Traditional medicines have immense potentials and such logical and interactive studies will create an ideal platform to bridge the gap between traditional and modern systems of medicine
- Preclinical and clinical data with herbal drugs could complement each other and validate their use in TM
- Such translational studies will be highly conducive for herbal drug development and rationalization of therapy in many disease states

# Acknowledgements

- CCRUM, Department of AYUSH, Ministry of Health and FW (Govt. of India), New Delhi
- Prof. A Ray
- Dr. Gaurav Vishnoi
- Dr. Sreemanti Guhathakurta
- Mr. Nishant Rai

# Publications

- Rai N., Ray A., Jamil S.S., Gulati K. Cellular and molecular mechanisms of action of polyherbal preparation UNIM-352 in experimental models of bronchial asthma. Indian J of Exp Biol, 2015,53:625

Indian Journal of Experimental Biology  
Vol. 53, October 2015, pp. 625-631

## Cellular and molecular mechanisms of action of polyherbal preparation UNIM-352 in experimental models of bronchial asthma

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<sup>2</sup>Central Council for Research in Unani Medicine (CCRUM), Ministry of AYUSH, Government of India, New Delhi-110 058, India

Bronchial asthma is a chronic inflammatory disorder of the airways and pharmacotherapy is dependent on anti-inflammatory and bronchodilator agents. However, adverse effects of these agents on chronic administration and sometimes non-responsiveness to these drugs have prompted the search for viable alternatives from medicinal plant sources. UNIM-352 is a polyherbal preparation traditionally used in the Unani system of Indian medicine for the treatment of bronchial asthma. The present study defines the possible cellular and molecular mechanisms of action of UNIM-352 in experimental models of bronchial asthma and validates the observed therapeutically beneficial effects. Wistar rats were immunized and challenged with ovalbumin, and blood and bronchoalveolar lavage (BAL) fluid were assayed for cytological and biochemical markers. UNIM-352 (200 and 400 mg/kg) markedly reduced the eosinophil and neutrophil counts in both blood and BAL compared to control. The polyherbal agent also attenuated the levels of TNF- $\alpha$ , IL-4, GM-CSF and NF- $\kappa$ B whereas histone deacetylase (HDAC) levels were elevated, in both blood and BAL fluid. All effects of UNIM-352 were comparable with the standard drug, prednisolone. The results demonstrated possible cellular and molecular mechanisms of UNIM-352 and thus explain its beneficial effects in bronchial asthma.

**Keywords:** Bronchodilators, Cytokines, Herbal, Histone deacetylase, Indian medicine, Inflammation, NF- $\kappa$ B, Prednisolone, Unani.

- Gulati K., Guhathakurta S., Rai N., Siddiqui M.K., Vijayan V.K. and Ray A. Translational Studies with a Polyherbal Agent in Bronchial Asthma: A Reverse Pharmacology Approach, In: Ray A. and Gulati K. (eds.), Translational Research in New drug Development, Vidyanyam Prakashan, New Delhi, 2012, 255-268

# 15

## ***TRANSLATIONAL STUDIES WITH A POLYHERBAL AGENT IN BRONCHIAL ASTHMA: A REVERSE PHARMACOLOGY APPROACH***

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### **Abstract**

Pharmacotherapy of bronchial asthma is steroid dependent and the use of herbal agents is being increasingly explored for viable adjuncts to reduce steroid dosage. UNIM-352, a polyherbal Unani formulation, is used in traditional medicine for bronchial asthma, and the present study attempted to validate its



- Presented at **WCP 2014 (IUPHAR)** in South Africa

Rai N., Ray A., Jamil S.S., Gulati K. Experimental studies on the mechanisms of action of UNIM-352, a polyherbal agent for the treatment of bronchial asthma: a reverse pharmacology approach. **Basic & Clinical Pharmacology & Toxicology**, 2014, 115 (Suppl. 1): 301.

the hypothesis that Transient Receptor Potential Ankyrin 1 (TRPA1), an ion channel mediating nociceptive signals and neurogenic inflammation, is involved in MSU-induced responses in gout by utilizing three experimental murine models.

**Methods:** The effects of pharmacological inhibition with the selective TRPA1 antagonist HC-030031 and genetic depletion of TRPA1 were studied in MSU-induced inflammation and pain by using (i) spontaneous weight-bearing test assessing joint pain, (ii) subcutaneous air-pouch model resembling joint inflammation, and (iii) inflammatory paw edema measuring soft-tissue inflammation in the mouse. Treatment with HC-030031 was compared to the treatment with placebo and the anti-inflammatory glucocorticoid dexamethasone and the responses in TRPA1 knock-out mice were compared to those in the corresponding wild type mice. The direct effect of MSU on TRPA1 activation was studied by using Ca-imaging with Fluo3 method.

**Results:** Intra-articularly injected MSU provoked spontaneous weight shift off the affected limb in wild type but not in TRPA1 knock-out

#### 1100

#### EXPERIMENTAL STUDIES ON THE MECHANISMS OF ACTION OF UNIM-352, A POLYHERBAL AGENT FOR THE TREATMENT OF BRONCHIAL ASTHMA: A REVERSE PHARMACOLOGY APPROACH

*Rai N., Ray A., Jameel S., Gulati K.*

**Background:** Bronchial asthma is a chronic inflammatory disorder of the airways characterized by airflow obstruction, bronchial hyperresponsiveness and airway re-modelling. UNIM-352 is a polyherbal Unani preparation used in Indian traditional medicine for bronchial asthma, but the mechanism of action is not known. The present study evaluated the cellular and molecular mechanisms of action of UNIM-352 to validate its use as complimentary therapy in bronchial asthma.

**Methods:** Wistar rats (150–200 g, n = 6, per group) were used for the study. For bronchial hyper reactivity studies, animals were immu-

**THANK YOU**

# Effects of UNIM-352 on oxidative stress markers in rats

<b>Treatment (mg/rat)</b>	<b>MDA (nmol/ml)</b>	<b>SOD (U/g Hb)</b>	<b>GSH (umol/ml)</b>
Vehicle	3.1 ± 0.3	165.0 ± 12.0	45.0 ± 5.2
UNIM (200)	2.6 ± 0.2	190.0 ± 23.8	46.1 ± 6.0
UNIM (400)	2.1 ± 0.5 *	200.0 ± 28.3	53.0 ± 8.0

\*p<0.05, vs vehicle

- Guhathakurta S., Gulati K., Rai N., Jamil S.S., Ray A. An Experimental Study to Evaluate the Anti-inflammatory and Immunomodulatory Effects of UNIM-352, a Polyherbal Preparation for Bronchial asthma. Medicinal Plant Research, 2013, 3: 3-12.
- Rai N., Ray A., Gulati K. Studies on the cellular and molecular mechanisms of UNIM-352, a polyherbal preparation in experimental models of bronchial asthma. Indian Journal of Pharmacology, 2013, 45(Supp 1): S184.
- Rai N., Ray A., Gulati K. Experimental studies on UNIM-352, a polyherbal anti-asthma preparation, in rats. Indian Journal of Pharmacology, 2012, 44(Supp 1): S191.