

ORIGINAL ARTICLE

Iran J Allergy Asthma Immunol
December 2012; 11(4): 316-323

Effect of Hedera Helix on Lung Histopathology in Chronic Asthma

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Received: 11 August 2011; Received in revised form: 5 February 2012; Accepted: 26 February 2012

ABSTRACT

Hedera helix is widely used to treat bronchial asthma for many years. However, effects of this herb on lung histopathology is still far from clear. We aimed to determine the effect of oral administration of Hedera helix on lung histopathology in a murine model of chronic asthma.

BALB/c mice were divided into four groups; I (Placebo), II (Hedera helix), III (Dexamethasone) and IV (Control). All mice except controls were sensitized and challenged with ovalbumin. Then, mice in group I received saline, group II 100 mg/kg Hedera helix and group III 1 mg/kg dexamethasone via orogastric gavage once daily for one week. Airway histopathology was evaluated by using light and electron microscopy in all groups.

Goblet cell numbers and thicknesses of basement membrane were found significantly lower in group II, but there was no statistically significant difference in terms of number of mast cells, thicknesses of epithelium and subepithelial smooth muscle layers between group I and II. When Hedera helix and dexamethasone groups were compared with each other, thickness of epithelium, subepithelial muscle layers, number of mast cells and goblet cells of group III were significantly ameliorated when compared with the group II.

Although Hedera helix administration reduced only goblet cell counts and the thicknesses of basement membrane in the asthmatic airways, dexamethasone ameliorated all histopathologic parameters except thickness of basement membrane better than Hedera helix.

Keywords: Airway remodeling; Asthma; BALB-c mice; Hedera helix; Microscopy electron

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INTRODUCTION

Asthma is characterized by chronic airway inflammation, which adversely affects normal lung function.¹ Corticosteroids, the most potent nonspecific

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anti-inflammatory agents, produce substantial improvement in objective lung functions of patients with asthma and are the cornerstone of asthma treatment. Many patients harbour misgivings about conventional medical treatments for asthma, particularly inhaled corticosteroid treatment^{2,3} and adherence is frequently poor.⁴ There is a need for development of additional effective treatments with fewer side effects. Recently, there has been a surge in interest in herbal medicine, possibly because they have fewer side effects than current therapy.⁵ However, well-controlled clinical trials using herbal medicine for asthma treatment are still rare.^{6,7}

Hedera helix is a well known plant as ivy plant or English ivy and is a member of the Araliaceae family. Preclinical studies showed that ivy leaf extracts have a spasmolytic, bronchodilating and antibacterial effect which is mainly attributable to the triterpene saponins contained in them.^{8,9} There was only a few preliminary studies demonstrating that ivy leaf extract preparations have some effect with respect to an improvement in respiratory functions of children with chronic bronchial asthma.¹⁰ To our knowledge, there is no study reported in English literature evaluating the effect of this herb on structural changes of chronic asthma. In the current study, we investigated whether oral administration of Hedera helix would demonstrate an ameliorating effect on lung histopathologic features and compared the effects of Hedera helix and dexamethasone on airway inflammation and remodeling in a murine model of chronic asthma.

MATERIALS AND METHODS

Experimental Animals

Pathogen free, 6- to 8-week-old, female BALB/c mice, weighing 18 to 20g, were purchased from Bornova Veterinary Control and Research Institute (Izmir, Turkey) and maintained in a pathogen-free animal laboratory of Dokuz Eylul University. They were kept in hygienic macrolene cages and in air-conditioned rooms 12 hour light/12 hour dark cycle with food and water available *ad libitum*. All animal study protocols were reviewed and approved by the Dokuz Eylul University animal care committee.

Study Groups

Twenty-eight mice were divided into four groups; Group I (Placebo), II (Hedera helix), III

(Dexamethasone) and IV (Control), each group including seven mice.

Sensitization and Inhalational Exposure

BALB/c mice were used for this study as they are high IgE responders.¹¹ The mice in the study and placebo groups (Group I, II, III) were sensitized via two intraperitoneal injections, on days 0 and 14 of the experiment, with 10 µg /0.1 mL chicken egg albumin (Ovalbumin, grade V, ≥98% pure; Sigma, St. Louis, MO, USA) together with alum as an adjuvant. The mice in the Group I, II and III were then exposed to aerosolized ovalbumin (OVA) for 30 minutes per day for three days of the week for eight weeks beginning from the 21st day of the study. The mice in control group (Group IV) received normal saline with alum intraperitoneally on days 0 and 14 of the experiment and aerosolized saline without alum for 30 minutes per day on three days of the week for eight weeks beginning from the 21st day of the study [11,12]. Exposures were carried out in a whole body inhalation exposure system. A solution of 2.5% OVA in normal saline was aerosolised by delivery of compressed air to a sidestream jet nebuliser and injected into a chamber. The aerosol generated by this nebuliser comprised >80% particles with a diameter of <4 µm. Particle concentration was maintained in the range of 10-20 mg/mm³.¹²

Study Drugs

Hedera helix was purchased from the market as standardized tablet form that contains ivy leaf powder (Enzymatic therapy, natural medicines, ivy extract, Enzymatic therapy Inc., Green Bay, WI 54311, Germany). The powder of Hedera helix was identified microscopically by B.Kivcak, The Department of Pharmacognosy, Faculty of Pharmacy, Ege University. A thick cuticle, lacks stomata on the upper epidermis and in surface view the cells were seen to have stout and strongly sinuate walls. The lower epidermis had numerous anomocytic stomata and near the stomata there were faint cuticular striations and occasional stellate trichomes were present. A cross section of the leaf was shown rows of palisade cells and porous spongy parenchyma; in both layers there were cluster of calcium oxalate crystals and a few mucilage cells. All this findings were compatible with microscopical characteristics of Folia Hederae.¹³ Aqueous extraction was performed by soaking a weighed amount of the dry

powder in distilled water and shaking for 3 hours using electric shaker. The suspension was filtered through muslin gauze and the filtrate was kept in deep freezer for 24 hour and then lyophilized. The lyophilized dry powder was collected in a stoppered sample vial, weighed and kept in a desiccator until used.

Preliminary qualitative screening for the major groups of plant secondary metabolites was conducted according to the method used by Debella.¹⁴ Dried powder of Folium Hederæ helicis were screened for the presence of triterpene saponins, flavonoids, phytosterols and phenolic compounds.

The extract of Folium Hederæ helix was given at a dose of 100 mg/kg/day once daily for 7 consecutive days via orogastric gavage. Dexamethasone was also given to the mice with a dose of 1 mg/kg/day by orogastric gavage for 7 days.

Histopathological Analysis

Animals were sacrificed by an overdose of ketamin (500 mg/kg, intraperitoneally) 24 hours after the last inhalational exposure and histopathological specimens were collected. Two investigators who were interpreting the histopathology were blinded to the treatment groups. Tissue specimens were taken from the mid zone of the left lung of mice. For electron microscopic evaluation, tissue samples of 1-2 mm³ were stocked. Histopathological samples were fixed in 10 % formaline for light microscopic evaluation. After fixation, slice from the mid zone of the left lung was embedded in paraffin. Serial sections cut at 5 µm were stained with haematoxylin and eosin (for routine histopathological examination), with Toluidine Blue (for enumeration of mast cells) and with Periodic Acid Schiff (PAS) (for enumeration of goblet cells). Photomicrographs were taken by JVC TK-890-E camera (Japan) which was adapted on Olympus BH-2 RFCA model microscope (Olympus Optical Co. Ltd, Tokyo, Japan). Serial sections were photographed at different magnifications by skipping over five fields. In order to evaluate thicknesses of epithelium and subepithelial smooth muscle layers, measurements were taken at four points of each airway and for each mouse, 20 measurements were done. Goblet cells around the airway lumina were enumerated. In order to be standardized, PAS (+) goblet cell numbers in 100µm were analyzed for each airway.

Blind histological analysis was carried out with UTHSCSA Image Tool for Windows Version 3.00

software (<http://ddsdx.uthscsa.edu/dig/itdesc.html/>; provided in the public domain by the University of Texas Health Sciences Center San Antonio, TX) after the images were transferred from a light microscope onto a computer.

Samples were fixed in 2 % glutaraldehyde for the evaluation with electron microscopic. Libra 120 Carl Zeiss electron microscope (Oberkochen, Germany) was used for this evaluation. Photomicrographs were taken by JVC TK-890-E camera. Tissues were embedded in EPON after follow-up process of electron microscopic evaluation. Respiratory tracts were marked from the semithin sections. Ultrathin sections were stained with uranyl acetate and lead citrate. Basal membrane thicknesses of samples of respiratory epithelium were examined with electron microscopy by using ITEM version 5.0 (Olympus Soft Imaging Solutions GmbH Copyright © 1986-2007) program.

Statistical Analysis

SPSS 11 package program was used in the statistical analysis. All results were presented as mean ±SD from the number of experiments indicated. For all histopathologic parameters (The thicknesses of epithelium, subepithelial smooth muscle layers, basement membrane, the number of goblet cells and mast cells) differences among four groups were determined by use of the Kruskal-Wallis. Differences between two groups were analyzed by Mann-Whitney U test. A $p < 0.05$ was considered statistically significant.

RESULTS

When compared with the control group (Group IV), the asthma group (Group I, Placebo group) had significantly higher numbers of goblet cells, mast cells, increased thicknesses of epithelium, basement membrane and subepithelial smooth muscle layers (p : 0.000, p : 0.001, p : 0.002, p : 0.000, p : 0.004, respectively). These results revealed that the chronic asthma model of mice was successfully established in this study. The mean ± SD and range of histopathologic parameters for group I, II and III groups are presented in Table 1.

Goblet cell numbers and thicknesses of basement membrane were found significantly lower in the group II (p : 0.004, p : 0.000) but there was no statistically significant difference in terms of number of mast cells

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Table 1. Comparison between asthmatic mice (Placebo group), Hedera helix and dexamethasone groups.

Topics	Placebo Group (Group I) Mean±SD (Range) (n:7)	Hedera helix (Group II) Mean±SD (Range) (n:7)	Dexamethasone (Group III) Mean±SD (Range) (n:7)
Basement membrane thickness (nm)	650.13±94.54 (514.23-768.23)	462.17±137.12 (237.68-865.17)	397.76±94.17 (278.02-598.32)
Epithelium thickness (µm)	34.00±7.07 (22.12-42.96)	30.79±6.85 (22.02-42.99)	22.74±3.18 (18.23-26.98)
Subepithelial smooth muscle layer thickness (µm)	10.61±1.57 (7.61-12.42)	9.16±3.02 (6.16-12.89)	5.23±1.02 (3.31-6.61)
Goblet cell number/100µ	5.27±3.36 (2.16-10.28)	0.80±0.68 (0.22-2.55)	0.40±0.08 (0.3-0.5)
Mast cell number (n)	18.71±3.09 (12-21)	16±2.58 (12-20)	11.42±1.01 (10-13)

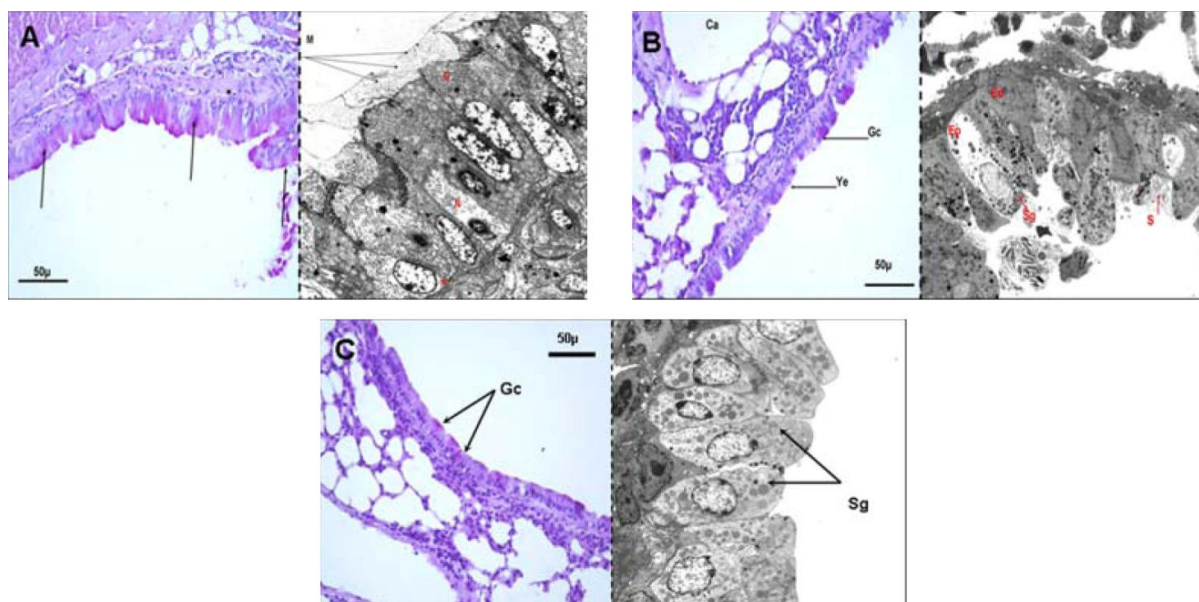


Figure 1. Light and electron microscopic findings of airways:

1A (Placebo group): In asthma group, light microscopic findings revealed irregular respiratory epithelium, some of the cell nuclei were necrotic and degenerative. Increased thickness of epithelium and high numbers of goblet cells (which were point out with arrows) were remarkable. Subepithelial smooth muscle (*) was markedly thickened. In electron microscopy, degranulation was seen in apical part of the Goblet cells. The surface of epithelium was covered with mucus. Basement membrane (*), nucleus of epithelial cell without cilia.

1B (Hedera helix group): In light microscopy, degeneration and irregularity in some of the epithelial cells (Ep) were detected. Goblet cells were decreased and protruded into the lumen. In electron microscopy, apical cytoplasm of epithelial cells were full with secretory granules. Basement membrane integrity was not disrupted.

1C (Dexamethasone group): In light microscopy, respiratory epithelium appeared regular, number of goblet cells and thickness of smooth muscle layer were decreased. In electron microscopy, epithelial cells were in secretion phase, apical cytoplasm were full with secretory granules. Subepithelial structures were seen normal.

Abbreviations: Goblet cells (Gc), mucus (M), cilia (N), epithelial cells (Ep), secretory granules (Sg), Capillary section (Ca), Ciliary structure (S).

Table 2. Comparison between placebo and Hedera helix group.

Topics	Hedera helix (Group II) Mean±SD (Range) (n:7)	Placebo Group (Group I) Mean±SD (Range) (n:7)	P value
Basement membrane thickness (nm)	462.17±137.12 (237.68-865.17)	650.13±94.54 (514.23-768.23)	0.000
Epithelium thickness (µm)	30.79±6.85 (22.02-42.99)	34.00±7.07 (22.12-42.96)	0.337
Subepithelial smooth muscle layer thickness (µm)	9.16±3.02 (6.16-12.89)	10.61±1.57 (7.61-12.42)	0.085
Goblet cell number/100µ	0.80±0.68 (0.22-2.55)	5.27±3.36 (2.16-10.28)	0.004
Mast cell number (n)	16±2.58 (12-20)	18.71±3.09 (12-21)	0.061

(p : 0.061), thicknesses of epithelium (p : 0.337) and subepithelial smooth muscle layers (p : 0.085) between placebo (Group I) and Hedera helix (Group II) groups. The mean, range and p values of histopathologic parameters are given in Table 2.

When Hedera helix (Group II) and dexamethasone (Group III) groups were compared with each other, thickness of epithelium (p : 0.018), subepithelial muscle layers (p : 0.004), number of mast cells (p : 0.004) and goblet cells (p : 0.025) of group III were significantly ameliorated when compared with the group II. There was no statistically significant difference in thickness of basement membrane (p : 0.12) between group II and

III. The mean, range and p values of histopathologic parameters are shown in Table 3.

Histopathologic views of airways are shown in figure 1. In the airways of asthmatic mice (placebo group, group I) increased goblet cell numbers, thickened epithelium, basement membrane and subepithelial smooth cell layer are seen in figure 1A. Figure 1B shows that some of the chronic changes were decreased after Hedera helix administration to asthmatic mice (Group II). In figure 1C; most of the chronic histopathologic changes were resolved after dexamethasone administration (Group III).

Table 3. Comparison between Hedera Helix and Dexamethasone group.

Topics	Hedera helix Group (Group II) Mean±SD (Range) (n:7)	Dexamethasone Group (Group III) Mean±SD (Range) (n:7)	P value
Basement membrane thickness (nm)	462.17±137.12 (237.68-865.17)	397.76±94.17 (278.02-598.32)	0.12
Epithelium thickness (µm)	30.79±6.85 (22.02-42.99)	22.74±3.18 (18.23-26.98)	0.018
Subepithelial smooth muscle layer thickness(µm)	9.16±3.02 (6.16-12.89)	5.23±1.02 (3.31-6.61)	0.004
Goblet cell number/100µ	0.80±0.68 (0.22-2.55)	0.40±0.08 (0.3-0.5)	0.025
Mast cell number (n)	16±2.58 (12-20)	11.42±1.01 (10-13)	0.004

DISCUSSION

Asthma is a chronic disease characterized by reversible airway obstruction, airway inflammation and structural alterations in the airways.¹⁵ Airway inflammation is the key factor in the pathogenesis of asthma and current strategies for the management focus on suppressing airway inflammation.^{16,17} Airway remodeling is a potentially important consequence of asthma which can be defined as the presence of persistent changes to normal airway structure involving changes in the composition, organisation and function of structural cells, as well as enhanced turnover of extracellular matrix components.¹⁸ It includes goblet cell hyperplasia, basement membrane thickening, subepithelial fibrosis, airway smooth muscle hypertrophy/hyperplasia and angioneogenesis.¹⁹ Although current asthma therapies are effective in reducing inflammation, airway remodeling is poorly responsive to current therapies.²⁰ Thus, new therapeutic modalities that can directly target the remodeling processes and reverse permanent changes in lung are essential. Herbal therapies are one of the most popular alternative treatment modalities used by asthmatic patients.⁷ Herbal medicinal products, even though in prevalent use, are of uncertain value in the treatment of asthma.⁷

The objective of this study was to evaluate the effects of Hedera helix on histopathologic changes of lungs in an allergic asthma animal model. To evaluate this, a mouse model for allergic asthma was established. Using the established model of this study, we demonstrated that sensitization and challenge with OVA significantly increased thickness of epithelium, basement membrane, subepithelial smooth muscle layers, the number of mast cells and goblet cells. Although Hedera helix administration with orogastric gavage during one week significantly reduced goblet cell numbers and thicknesses of basement membrane of asthmatic mice, dexamethasone ameliorated all histopathologic parameters except thickness of basement membrane better than Hedera helix.

Hedera helix extract is mostly used for the treatment of bronchial inflammatory diseases as well as asthma, particularly in European countries, although the drug's efficacy has not been documented with placebo controlled clinical and experimental studies that have appropriate methodology. Antiinflammatory,²¹ antibacterial,²² antihelminthic,²³ leishmanicidal,²⁴ *in-*

vitro antispasmodic⁹ and antifungal²⁵ effects of Hedera helix extracts were reported. Fazio et al reported that 9657 patients (5181 children) with bronchitis (acute or chronic bronchial inflammatory disease) were treated with a syrup containing dried ivy leaf extract in a postmarketing study and after 7 days of therapy, 95% of the patients showed improvement of their symptoms.²⁶ Hoffman et al evaluated the results of 3 studies that investigated effects of extracts from dried ivy leaves in the treatment of chronic airway obstruction suffering from bronchial asthma. They reported that ivy leaf extract preparations have effects with respect to an improvement of lung functions in children with bronchial asthma.¹⁰ Haeberlein and coworkers have recently claimed that the secretolytic and bronchodilating properties found in Hedera helix extract are due to its content in saponins, particularly alfa hederin as an inhibitor of the B2 receptors endocytosis, establishing an indirect B2 sympathomimetic action.²⁷ In the present study, analysis of the lungs showed that goblet cell hyperplasia markedly decreased in Hedera helix-treated mice. As goblet cell hyperplasia and associated mucus hypersecretion is a pathophysiological feature of asthma,²⁸ treatment with compounds that alter mucus and decrease goblet cells like Hedera helix could serve as therapeutic options for this disease.

Dexamethasone is a potent inhibitor of airway inflammation and remodeling.²⁹ Inhibition by these inhaled corticosteroids is well-documented in human asthma, and administration of corticosteroids has also been shown to inhibit the structural changes associated with airway fibrosis in other animal models.³⁰ Dexamethasone, one of the most potent corticosteroids, improved all histopathologic parameters except thickness of basement membrane more than Hedera helix group in our model.

The study had some important limitations. First of all, the level of cytokines which has an important role in asthma pathogenesis could not be evaluated in the current study. Small number of animals was used for this project (possibility that Type 1 and Type 2 errors exist). Finally, the results found in our study may not translate to positive findings in human clinical trials.

In conclusion; Hedera helix administration reduced goblet cell numbers and thickness of basement membrane, but dexamethasone ameliorated all histopathologic parameters except thickness of basement membrane better than Hedera helix in this

mouse model of chronic asthma. Further studies are needed to evaluate the effects of *Hedera helix* on asthmatic airways.

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