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# Anti-inflammatory effect of N-(trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonic acid amide and gluconic acid on allergic rhinitis and asthma controlling

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**Abstract**
Allergic rhinitis and asthma are the main airway diseases with a higher prevalence. Eosinophilic inflammation, airway hyperresponsiveness, mucus hypersecretion, and reversible airflow obstruction are immunopathogenesis symptoms of rhinitis and asthma. Crotonic acid has bioactivity on the inflammation, and gluconic acid as chelator may protect crotonic acid activity in airway and together may control allergic rhinitis and asthma.
Allergic rhinitis and asthma mice models were treated with crotonic and gluconic acids. The total IgE, histamine, IL-4, IL-5, and IL-13 levels were measured. In lung tissues, goblet cell hyperplasia, mucus hypersecretion, and inflammation were evaluated.
The level of IL-5, goblet cell hyperplasia, and perivascular and peribronchial inflammation were controlled by crotonic acid in asthma and allergic rhinitis groups. But, total IgE, histamine, IL-4, and IL-13 levels, and mucus hypersecretion had no significant changes between treated and nontreated asthma and rhinitis groups.
Crotonic acid can control eosinophilic inflammation via harnessing IL-5 and preventing goblet cell hyperplasia. When used with gluconic acid, it had a strong effect on the control of allergic rhinitis and asthma immunopathologies.


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## Introduction

Allergic rhinitis and asthma are complex and multifactorial airway diseases with an increased prevalence in the developed world, characterized by airway inflammation, bronchoconstriction, dyspnea, wheezing, and rhinorrhea. Allergy is an important problem affecting public health and afflicting more than 350 million individuals globally. A heightened allergo-immune response to environmental triggers is the dominant feature in patients with a genetic predisposition. In allergic subjects, Th2 response to the specific allergen leads to increased Th2 cytokines and total IgE levels. These factors can sensitize mast cells and basophils, and after retrigger of allergen, clinical and allergic symptoms are initiated.[1,2]

Allergic reactions are the main orchestra of asthma. Asthma is a complicated bronchial disease affecting respiration, which is characterized by cough, breathlessness, and wheezing. The immunopathogenesis of asthma include infiltration of eosinophils around bronchi and vessels, airway hyperresponsiveness, mucus hypersecretion, and reversible airflow obstruction, and harnessing of these problems are necessary in control of asthma.[3,4]

However, currently, there is no complete cure for allergic diseases. Worldwide, heavy economic burden is imposed on countries' national budget annually for providing healthcare services for patients with allergy and asthma. Some natural acids which have significant effects on the immune system may be important in the development, severity, and course of allergy and asthma, can modulate innate and adaptive immune system responses, and may therefore influence the development and the course of allergic rhinitis and asthma.[1,3]

Crotonic acid (2E-but-2-enoic acid) is a C4 short-chain, unsaturated carboxylic acid. Crotonic acid is an oligosaccharide, low-molecular component, and its bioactivity on inflammation has been reported, but there are no published studies on using of this component on inflammation of airways.[5,6] Gluconic acid is a nontoxic, nonvolatile, noncorrosive, and biodegradable chemical, which is highly soluble in water, and its aqueous solution has found application as a catalytic medium for organic synthesis. Gluconic acid is also a powerful chelator.[7,8] The chelating ability of gluconic acid may protect crotonic acid activity in airway and leads to high potent bioactivity of crotonic acid in the respiratory system. So, in this study, we used crotonic and gluconic acids for the treatment and control of allergic rhinitis and asthma.

## Material and Methods

### Animal models and treatment schedule

BALB/c mice (7–8 weeks old) were divided into seven groups (n = 15), as follows: (1) negative control group or healthy group that received no treatment (N), (2) asthma group with no treatment (A), (3) asthma group treated with N-(trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonic acid amide (crotonic acid [A.Cro)]), (4) asthma group treated with crotonic and gluconic acids (A.Cro.Glu), (5) allergic rhinitis group with no treatment (R), (6) allergic rhinitis group treated with crotonic acid (R.Cro), (7) and allergic rhinitis group treated with crotonic and gluconic acid (R.Cro.Glu). Allergic asthma and allergic rhinitis were induced by ovalbumin according to a previously described protocol.[3,4] All treatments were administered via nebulization on Days 25, 27, and 29 after first sensitization (Day 1). The total volume of nebulizing liquid was 10 ml for both crotonic acid (200 µg/ml) and gluconic acid (30 µg/ml) each day, nebulized for 30 min (0.3 ml/min) separately at 0.6 mPa pressure. The blood, bronchoalveolar lavage fluid (BALF), and lung tissue samples were collected on Day 31.

### Immunoglobulin E (IgE)

Blood samples were collected, centrifuged, and sera were separated. Total IgE level was measured by using specific mouse ELISA kit.

### Histamine level

The histamine level was determined in sera of the studied mice. After the last challenge, collected blood samples were used to measure the histamine level using specific ELISA kits.

### Cytokine levels

After anesthetization of mice, BALF samples were collected via catheter, centrifuged, and supernatant was stored for cytokine assay. Levels of IL-4, IL-5, and IL-13 were measured using specific mouse ELISA kits.

### Histopathology

At the end of challenging period, the lung tissues were isolated, and histopathological sections were prepared. The tissues were then stained using hematoxylin and eosin (H&E) and periodic acid–Schiff stains, and goblet cell hyperplasia, mucus hypersecretion, and peribronchiolar and perivascular inflammation were evaluated by using a point scoring system as described before.[4]

### Statistical analysis

The experimental tests were repeated thrice. Results were presented as mean ± SD, and SPSS was used for analyses. GraphPad prism was used for designing and presentation of curves. Student's t-test was used to analyze the differences between treated and nontreated groups. $P < 0.05$ was considered to be significant.

## Results

### IgE level

The total IgE was significantly increased in A and R groups (2301 ± 21.2 and 2526.6 ± 38.2 ng/ml, respectively)

compared to Group N (211.9 ± 9.2 ng/ml; P < 0.05). The total IgE level had no significant difference (P > 0.05) between nontreated and treated asthma and rhinitis groups (A.Cro: 2284.3 ± 27.9, A.Cro.Glu: 2277.6 ± 29.1, R.Cro: 2490.1 ± 33.2, and R.Cro.Glu: 2484.5 ± 26.3 ng/ml).

### Histamine level

The histamine level was increased in A and R groups (579 ± 28 and 595 ± 19 ng/ml, respectively) significantly (P < 0.05) compared to Group N (81 ± 5 ng/ml). There was no significant difference (P > 0.05) between nontreated and treated asthma and rhinitis groups (A.Cro: 522 ± 32, A.Cro.Glu: 526 ± 27, R.Cro: 553 ± 25, and R.Cro.Glu: 549 ± 36 ng/ml).

### Cytokine levels

The levels of Th2 cytokines in A and R groups (IL-4: 96.2 ± 4.4 and 98.2 ± 5.5 pg/ml; IL-5: 89.1 ± 6.2 and 76.4 ± 6.2 pg/ml; IL-13: 136.3 ± 20.2 and 130.5 ± 21.1 pg/ml, respectively) increased significantly (P < 0.05) when compared with N group (IL-4: 41.6 ± 3.9, IL-5: 37.7 ± 4.8, IL-13: 66.2 ± 8.1 pg/ml). The levels of IL-4 and IL-13 had no significant difference (P > 0.05) in nontreated and treated groups of asthma and rhinitis, but the levels of IL-5 decreased significantly in treated groups of asthma and allergic rhinitis (P < 0.05) compared with nontreated groups. Also, IL-5 level decreased significantly in A.Cro.Glu group (P < 0.05) when compared with A.Cro group (Figure 1).

### Histopathology

Perivascular and peribronchial inflammation, goblet cell hyperplasia, and mucus secretion were increased in A group significantly (P < 0.05) when compared with N group. Goblet cell hyperplasia and perivascular and peribronchial inflammation were significantly decreased in A.Cro and A.Cro.Glu groups compared to A group (Figure 2). Perivascular inflammation was controlled significantly (P < 0.05) in A.Cro.Glu group compared to A.Cro group (P < 0.05). There was no significant difference in mucus hypersecretion between the nontreated and treated groups (P > 0.05).

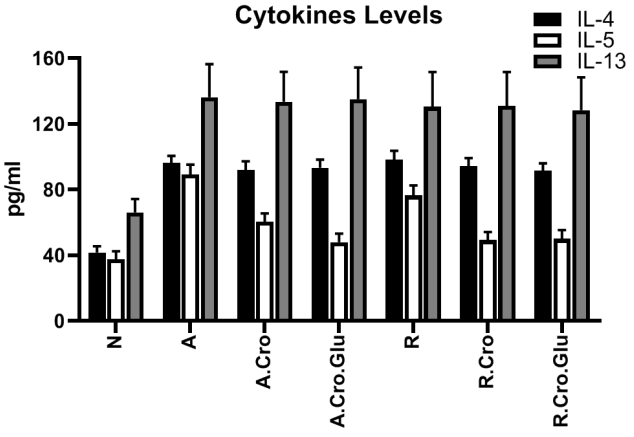


**Figure 1** Cytokines levels. The IL-4, IL-5, and IL-13 levels in BALF were evaluated by ELISA method in all groups.

## Discussion

Asthma is a public health problem worldwide, and its economic burden on healthcare is very high in terms of both direct and indirect costs. The main problems associated with asthma include mortality, morbidity, and health system involvement. On the other hand, allergic rhinitis as nasal and upper airways inflammation can reduce quality of life, especially in children. Therefore, the control of rhinitis and asthma attack is the main goal of asthma treatment.[1,4] Asthma is a complex inflammatory airway disease, characterized by eosinophilic infiltration, mucus hypersecretion, goblet cell hyperplasia, airway hyperresponsiveness, and reversible airflow obstruction. Allergy is one of the main causes of asthma.[2,3] In our study, there was no significant difference between treated and nontreated asthma and rhinitis groups. This may be due to the weak effect of crotonic acid on the allergopathology mediators.

Airway inflammation heterogeneity in asthma indicates there are different mechanisms involved. Inflammation, allergic reaction, and immune response dysregulation are the main mechanisms. The main symptoms of asthma are mucus oversecretion, bronchoconstriction, and airway inflammation that lead to cough, airways obstruction, wheezing, breathlessness, and chest tightness.[2,4] Crotonic acid was employed in immunomycin production by *Streptomyces hygroscopicus* var. *ascomyceticus* as immunosuppressant agent.[9] N-(trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonic acid amide (A77 1726) has antiproliferative and anti-inflammatory effects in animal models. Also, in psoriasis clinical trials, it has effect on the epidermal hyperproliferation and inflammatory cells infiltration.[10,11] Goblet cell hyperplasia and perivascular and peribronchial inflammation were controlled by crotonic acid treatment in asthmatic mice. Also, perivascular inflammation was better controlled in A.Cro.Glu group than in A.Cro group, and when crotonic acid was used in combination with gluconic acid, it had a strong effect on the control of inflammation. However, it had antiproliferative and antihyperplasia effects on the goblet cells, which is important in asthmatic patients.

Environmental triggers in genetically predisposed people activate Th2 immune response and orchestrate asthma pathophysiology. Some infections such as those caused by *Linguatula serrata* can change the expression of some related molecules and immune responses that should be noted.[12,13] IL-13 plays an important role in mucus secretion and goblet cell hyperplasia. IL-5 activates eosinophils and their migration to airways (bronchial inflammation). IL-4 induces IgE isotype switching and activation of mast cells. IL-4, IL-5, and IL-13 levels were high in the asthmatic patients.[1,3,4] The gamma-aminobutyric acid (GABA) receptors express in granule cells and its agonist cis-4-amino-crotonic acid evokes currents in these cells.[14] Honaucin A consists of 4-chlorocrotonic acid and (S)-3-hydroxy-g-butyrolactone that are connected via an ester linkage and can inhibit lipopolysaccharide-stimulated nitric oxide production. The decrease in nitric oxide levels is accompanied by the decrease in transcription of several proinflammatory cytokines. It can be applied in therapeutic areas related with inflammation, such

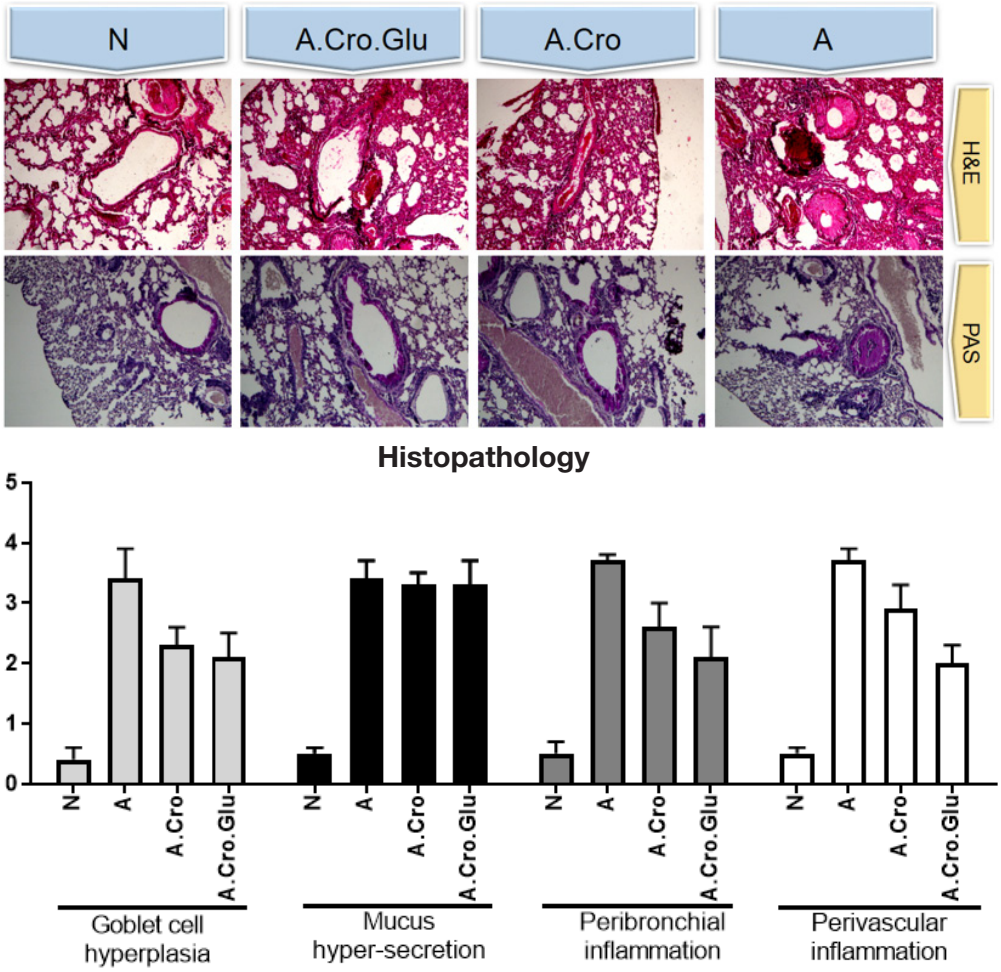


**Figure 2** Lung histopathology. Lung tissues after staining (with H&E and PAS), were evaluated for the perivascular and the peribronchiolar inflammation, goblet cell hyperplasia, and mucus hypersecretion.

as asthma.[15,16] On the other hand, cell signaling pathways are important targets for asthma treatment, and the recognition of new ligands (agonists or antagonists) for adaptor molecules provides a new therapeutic approach.[1,3] Crotonic acid may have targeted effect on cell signaling and may control signaling pathways that are related to inflammation, cell migration, and cell proliferation.

It should be considered that the development of new anti-inflammatory therapies that target cyclooxygenases (COX) 1 and 2 in patients with allergic diseases is necessary. The crotonic acid agents can be new modulators of inflammation.[17,18] However, some metabolites can attenuate immune response by inhibition of the transcriptional activity of peroxisome proliferator-activated receptor gamma, which may explain the role in immunomodulation and anti-inflammatory effects.[19-21] In this study, the level of IL-5 was decreased in asthma and allergic rhinitis groups that were treated with only crotonic acid and combination of crotonic and gluconic acids. IL-5 level was decreased significantly in A.Cro.Glu group compared with A.Cro group, which showed that when crotonic acid was used in combination with gluconic acid, it had powerful effect on the control of release IL-5. Gluconic acid had protective effect on crotonic acid in the airway that led to the increase in stability and efficacy of crotonic acid. Crotonic acid can control inflammation (especially eosinophilic inflammation) via harnessing of IL-5 around bronchi and bronchial vessels and also prevent goblet cell proliferation and hyperplasia. When was used with gluconic acid, it had strong effect on the control of allergic rhinitis and asthma immunopathologies. Our study showed that crotonic acid had anti-inflammatory and immunomodulatory effects, and its effect on control of inflammation is more powerful than antiallergic effect, even when used with gluconic acid.

This study also had some limitations. It did not include Th1 cytokines levels and airway hyperresponsiveness. Some cellular signaling pathways, such as NF-κB and the MAP kinase, and inflammatory factors, such as COX-2, iNOS, and PGs, that have effect on asthma pathophysiology were not studied, which should be noted in future researches. Also, we could not find similar studies (which used crotonic acid for treatment of allergy) and could not compare our results with publicized studies.

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