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D-Pinitol Attenuated Ovalbumin-induced Allergic Rhinitis in Experimental Mice via Balancing Th1/Th2 Response

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ABSTRACT

Allergic rhinitis (AR) is a complex, chronic immunoinflammatory disorder of the membrane lining of the nasal mucosa. D-Pinitol is considered a cyclic polyol with a potential effect against various allergies. In the present study, we evaluated the anti-allergic effect of pinitol on ovalbumin (OVA)-induced AR model in mice.

BALB/c mice were initially sensitized with an intraperitoneal injection of OVA and divided into 5 groups (n=18, in each group) for a treating schedule of distilled water (DW), montelukast (10 mg/kg), and pinitol (5, 10, and 20 mg/kg) through the mouth. Two saline-injected groups were considered as controls by orally administrating DW and pinitol 20. Thereafter, test and control groups were intranasally challenged by OVA and saline, respectively.

Our results showed that the OVA challenge caused a marked elevation in AR symptoms like nasal rubbing, sneezing, and discharge which were remarkably diminished using pinitol (10 and 20 mg/kg) and the results were comparable with montelukast. Additionally, increased levels of total and OVA-specific serum Immunoglobulin (Ig) E and IgG1 were significantly attenuated by pinitol as compared to the control group but not the montelukast group. In AR-induced mice, pinitol had significant modulatory effects on representative markers of Th2 (GATA binding protein 3), signal transducer and activator of transcription-6, Interleukins (IL)-4, IL-5, IL-13, suppressors of cytokine signaling 1, Toll-like receptor 4, and myeloid differentiation factor 88), and Type 1 T helper (Th1) immune responses (T-box protein expressed in T cells and Interferon-gamma) as well as the histopathological aberrations induced in the nasal mucosa.

In conclusion, Pinitol had potential effects on OVA-induced AR mice through amelioration of nasal symptoms and balancing the Th1/Th2 immune responses during the allergic rhinitis condition.

Keywords: Allergic rhinitis; GATA3 protein; Ovalbumin; Pinitol

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INTRODUCTION

Allergic rhinitis (AR) is a complex chronic immunoinflammatory disorder of the membrane lining

of nasal mucosal depicted by itching, sneezing, rhinorrhea, nasal rubbing, congestion, drainage, eye lacrimation, and sometimes smell and taste loss.[1] Although AR is not a life-threatening disease, it significantly affects humans. According to the International Study of the Allergic Rhinitis Survey, AR prevalence has elevated 25% over the last few decades.[2] The report estimated that about 500 million individuals suffer from AR with worsening QoL (quality of life) and loss of work productivity. Thus, it is associated with a significant economic burden with treatment costs of over 6 million.[3]

The existing treatment strategies for AR include antihistamine, leukotriene inhibitors, mast cell stabilizers, anticholinergic, and intranasal corticosteroid agents.[4] Recent advanced therapeutic options such as immunotherapy and prostaglandin D2 receptor antagonists also gained significant attention for AR management.[4] Global guidelines by Allergic Rhinitis and its Impact on Asthma (ARIA) suggested topical corticosteroids as a vital first-line treatment option for managing chronic AR.[2,5,6] However, many of these therapies are known to provide symptomatic relief and are associated with several adverse effects, such as headache, mucous membrane dryness, irritation of the throat, constipation, blurring of vision, urinary retention, and sedation.[7] Thus, the development of a better and novel treatment option is the need of the hour for AR management. Recent research suggests that numerous bioactive moieties of plant origin with their multi-target strategies have proven to be beneficial in inhibiting various pathogenesis of AR.[7] Researchers have utilized various experimental models to determine these entities' therapeutic potential, and the Ovalbumin (OVA)-induced AR model is widely used during these investigations.[8-13] OVA-induced systemic sensitizations with its subsequent intranasal challenge mimics the clinical features of Immunoglobulin (Ig) E-mediated allergic inflammation.[10,14,15]

Cyclic polyols are plant metabolites and widely exist in various nuts and fruits, common in daily diet. These polyols exhibit several pharmacological actions such as anti-inflammatory, antioxidant, and anti-allergic properties. Pinitol is one such cyclic polyol present in different food such as soy, pinewood, and legumes.[16,17] It possesses numerous properties, such as antihyperlipidemic, cardioprotective, antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antiaging, and anticancer.[18,19] The inhibitory potential of pinitol against inflammation is exhibited via inhibition of the nuclear factor-Kappa B (NF-κB)/ Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha (IκBα) pathway.[18] A double-blind, randomized, placebo-controlled trial confirmed the hepatoprotective and antidiabetic properties of pinitol clinically.[17,20] Bae et al reported the anti-allergic property of pinitol through down-regulated Th2 cytokines, i.e., GATA binding protein 3 (GATA3).[21] However, its potential against allergic rhinitis has not yet been completely determined. Thus, the present investigation aimed to determine the potential of pinitol against OVA-induced AR in experimental animals.

MATERIALS AND METHODS

Drugs and Chemicals

D-pinitol (purity 95%), Ovalbumin (OVA, Grade V), aluminum hydroxide, and histamine dihydrochloride were purchased from Sigma-Aldrich Co., St Louis, MO, USA. Montelukast was obtained from Cipla Limited, Mumbai, India. Mouse OVA-specific IgE, total IgE, total IgG1, Interleukins (ILs)-4, IL-5, IL-13, Interferon-gamma (IFN-γ), and Leukotriene C4(LTC-4) enzyme-linked immunosorbent assay (ELISA) kit were obtained from Bethyl Laboratories Inc., Montgomery, TX, USA. The primary antibodies of GATA3, T-box protein expressed in T cells (T-bet), phosphorylated-Signal Transducer, and Activator of Transcription-6 (p-STAT-6), suppressors of cytokine signaling 1 (SOCS1), Toll-like receptor 4 (TLR4), and Myeloid differentiation factor 88 (MyD88) were purchased from Abcam, Cambridge, MA, USA.

Animals

Adult male BALB/c mice (18-22 g) were purchased from the animal house of Xi'an Children's Hospital, China, and kept in quarantine for one week in-house at the institute animal housed under standard laboratory conditions, i.e., a temperature of 24±1°C, relative humidity of 45–55% and 12:12 h light/dark cycle. Animals had free access to standard chaw-pelleted food and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee of Xi'an Children's Hospital, China, (approval no. XACH20202354) and performed following the

guidelines of the National Institute of Health Guide for Care and Use of Laboratory Animals and were approved by the Animal Ethics and Use.

Induction of AR and Treatment Schedule

Figure 1 depicts a schematic representation of the treatment schedule. Sensitization of BALB/c mice was done on days 1, 3, 5, 7, 9, 11, and 13 by intraperitoneal (i.p.) injection containing 500 µL of sensitization solution (50 mg of OVA and 1000 mg of aluminum hydroxide dissolved in 500 mL of saline). On day 14, mice were randomly divided into various treatment groups (n=18 mice/group) and treated for the next 7 days (day-14 to day-21) as follows:

Group I: Normal: (N): Non-sensitized and received a suspension of aluminum hydroxide in saline (intraperitoneally) followed by distilled water (10 mg/kg, per os (orally))

Group II: Allergic Rhinitis control: (AR control): OVA-sensitized and received distilled water (10 mg/kg, orally)

Group III: AR + Montelukast (10): [AR + MLT (10)]: OVA-sensitized and received standard drug treatment i.e., montelukast (10 mg/kg, orally.)

Group IV: AR + Pinitol (5): [AR + P (5)]: OVA-sensitized and received pinitol (5 mg/kg, orally.)

Group V: AR + Pinitol (10): [AR + P (10)]: OVA-sensitized and received pinitol (10 mg/kg, orally.)

Group VI: AR + Pinitol (20): [AR + P (20)]: OVA-sensitized and received pinitol (20 mg/kg, orally.)

Group VII: Per se: Non-sensitized and received pinitol (20 mg/kg, orally.)

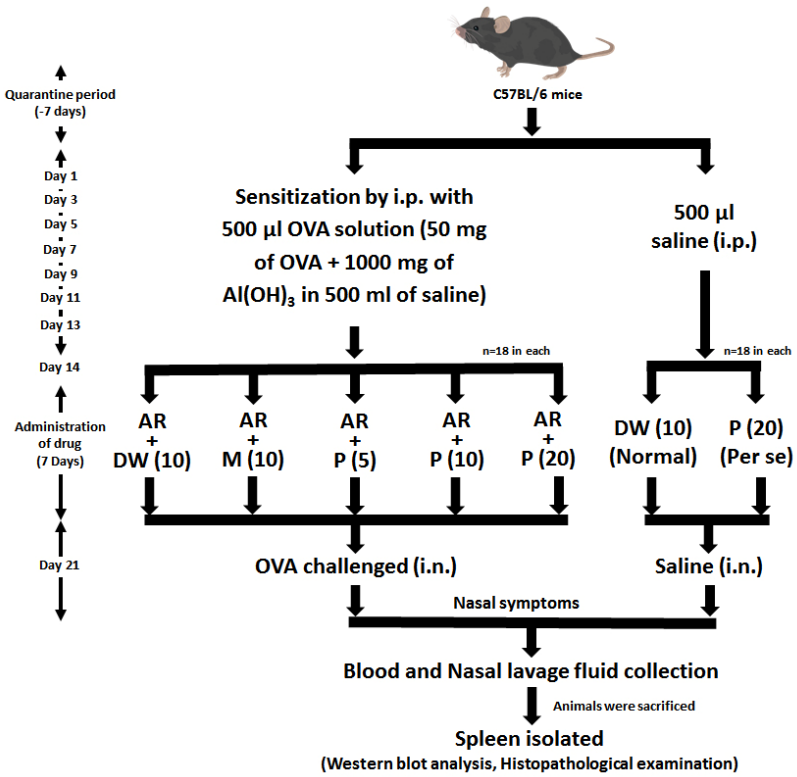

Figure 1. A schematic representation of the treatment schedule
AR: Allergic rhinitis; DW: Distilled Water; i.n.: intranasal; i.p.: intraperitoneal; M (10): Montelukast (10 mg/kg); OVA: Ovalbumin; P: Pinitol.

The three different doses of pinitol (5, 10, and 20 mg/kg, p.o.) were selected based on previous studies.[19,22] On day 21, one hour after the last dose of treatment, mice were challenged with intranasal (i.n.) administration of OVA (5%, 5 µL per nostril), and observations were recorded. The nasal symptoms were evaluated within 10 min period after the OVA challenge.[10] The histamine-induced hypersensitivity was evaluated after the challenge with histamine dihydrochloride.[10] OVA-specific IgE, total IgE, and total IgG1 in serum, while IL-4, IL-5, IL-13, IFN-γ, and LTC-4 in Nasal lavage fluid (NLF) were evaluated using respective mouse enzyme-linked immunosorbent assay (ELISA) quantitation kit (Bethyl Laboratories Inc., Montgomery, TX, USA) as per manufacturer's instructions.[23,24] After blood collection, mice were sacrificed by cervical dislocation to collect spleen and nasal mucosa. The protein expression of GATA3, T-bet, p-STAT6, SOCS1, TLR4, and MyD88 were estimated using western blot assay in spleen tissue.[25-27] The histopathology of nasal mucosal tissue was evaluated using Hematoxylin and eosin (H&E) staining. The intensity of histological aberrations in the nasal tissue was graded as Grade 0 (not present or very slight); Grade 1 (mild); Grade 2 (moderate); and Grade 3 (severe) as previously described elsewhere.[6,28]

Statistical Analysis

Data were expressed as mean ± SEM (standard error of means), and analyses were performed using GraphPad Prism 5.0 software (GraphPad, San Diego, USA). A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Body Weight and Organ Weight

Administration of OVA was associated with significant ($p=0.0035$ and $p=0.0086$) decreased body weight and increased spleen weight of AR control mice compared to normal mice. Montelukast treatment noticeably ($p=0.027$ and $p=0.011$) inhibited OVA-induced decreased body weight and increased spleen weight compared with AR control mice. Pinitol treatment (10 and 20 mg/kg) markedly ($p=0.041$ and $p=0.0229$) elevated body weight and effectively ($p=0.0372$ and $p=0.0267$) reduced weight of spleen compared to AR control mice. However, treatment with montelukast more effectively ($p=0.0113$ and $p=0.0412$) attenuated OVA-induced alterations in body weight and spleen weight compared to pinitol (20 mg/kg) treatment. Bodyweight and spleen weight did not differ significantly between normal mice and per se treated mice (Table 1).

Nasal Symptoms (Nasal Rubbing, Sneezing, and Discharge)

OVA challenges significantly increased nasal symptoms including rubbing ($p<0.0001$), sneezing ($p=0.0002$), and discharge ($p=0.0101$) in AR control mice compared with normal mice. Histamine challenges also significantly ($p<0.0001$) increased nasal rubbing and sneezing in AR control mice compared with normal mice. Montelukast treatment significantly decreased OVA-induced nasal rubbing ($p=0.0019$), sneezing ($p=0.0214$), discharge ($p=0.0346$) and histamine-induced nasal rubbing ($p=0.0019$) and sneezing ($p=0.0034$) compared to AR control mice. Pinitol (10 and 20 mg/kg) also efficiently attenuated OVA-induced nasal rubbing ($p=0.032$ and $p=0.0169$), sneezing ($p=0.012$ and $p=0.0078$), discharge ($p=0.0436$ and $p=0.0224$), and histamine-induced nasal rubbing ($p=0.03204$ and $p=0.0169$) and sneezing ($p=0.032$ and $p=0.0101$) compared to AR control mice. The nasal symptoms were non-significant in per se treated mice than normal mice (Table 1).

Serum IgE and IgG1 Levels

When compared with normal mice, OVA-specific IgE, total IgE and IgG1 levels markedly increased ($p<0.0001$, $p<0.0001$ and $p=0.0045$, respectively) in AR control mice. Montelukast noticeably reduced elevated OVA-specific IgE ($p<0.0001$), total IgE ($p<0.0001$) and IgG1 ($p=0.0438$) levels as compared with AR control mice. When compared with AR control mice, pinitol (10 and 20 mg/kg) treatment effectively attenuated elevated OVA-specific IgE ($p<0.0001$ and $p<0.0001$), total IgE ($p=0.003$ and $p<0.0001$) and IgG1 ($p=0.0126$ and $p=0.0015$) levels. However, when compared with pinitol (5, 10 and 20 mg/kg), montelukast treatment more effectively decreased these OVA-specific IgE ($p<0.0001$, $p=0.0062$ and $p=0.033$, respectively), total IgE ($p<0.0001$, $p=0.003$ and $p=0.0014$, respectively) and IgG1 levels ($p=0.0438$, $p=0.0212$ and $p=0.012$, respectively). The IgE (OVA-specific and total) and IgG1 levels did not differentiate between normal, and per se treated mice (Table 2).

Table 1. Effect of pinitol treatment on body weight, spleen weight, OVA, and histamine challenge induced nasal symptoms

Parameters	Treatment						
	Normal	AR Control	AR + MLT (10)	AR + P (5)	AR + P (10)	AR + P (20)	Per se
Body weight (gm) on day 21	34.83±1.70	25.67±1.38$^{\#}$	28.83±1.83*,$	25.17±1.66	26.00±1.63*,$	29.67±1.50*,$	30.83±1.72
Spleen wt/Body wt (mg/gm) ($X10^{-3}$) on day 21	3.33 ± 0.21	5.09±0.24$^{\#}$	4.15±0.27*,$	5.30±0.38	4.69±0.27*,$	3.90±0.16*,$	3.76±0.19
OVA challenge on day 21							
Rubbing (number)	16.00±0.45	68.00±1.13$^{\#}$	26.00±1.63*,$	66.17±1.20	45.67±0.71*,$	30.33±1.36*,$	17.17±1.45
Sneezing (number)	9.50 ± 0.72	41.00±0.45$^{\#}$	15.33±0.76*,$	38.67±0.56	31.67±0.95*,$	22.17±0.87*,$	12.17±0.48
Discharge (score)	0.83 ± 0.17	2.83±0.17$^{\#}$	0.50±0.22*,$	2.67±0.21	2.33±0.33*,$	1.17±0.17*,$	0.50±0.22
Histamine challenge on day 24							
Rubbing (number)	17.50±1.54	73.83±1.40$^{\#}$	26.83±1.40*,$	70.33±1.33	50.00±1.32*,$	36.33±1.15*,$	20.67±1.56
Sneezing (number)	11.00±1.44	52.17±1.11$^{\#}$	13.17±0.79*,$	49.00±1.46	32.00±0.82*,$	21.67±0.76*,$	14.67±1.45

Data are represented as Mean±SEM (n=4-6). Data were analyzed by one-way Analysis of variance (ANOVA) followed by Tukey's multiple range test (for body weight and relative spleen weight), non-parametric Kruskal-Wallis test ANOVA followed by Mann-Whitney's multiple comparison tests (OVA and histamine challenge number and score). For comparison with normal: $^{\#}p<0.05$, For comparison with AR control group: $*p<0.05$ and for comparison between montelukast with pinitol: $^{\$}p<0.05$.

AR: Allergic Rhinitis; MLT (10): Montelukast (10 mg/kg) treated; OVA: Ovalbumin; P: Pinitol treated; Perse: Non-sensitized pinitol treated mice.

Table 2. Effect of pinitol treatment on serum IgE (OVA-specific and total), serum IgG1, NLF ILs, IFN-γ, and LTC-4 levels

Parameters	Treatment						
	Normal	AR Control	AR + MLT(10)	AR + P (5)	AR + P (10)	AR + P (20)	Per se
OVA-specific IgE (ng/mL)	9.86±1.96	56.90±1.24$^{\#}$	18.93±1.99*,$	50.89±2.22	32.30±2.06*,$	20.66±1.40*,$	11.67±1.63
Total IgE (ng/mL)	107.00±5.94	404.60±7.12$^{\#}$	158.00±4.63*,$	415.60±6.61	311.80±8.91*,$	231.50±9.65*,$	105.40±7.59
Total IgG1 level (ng/mL)	0.31±0.05	0.66±0.06$^{\#}$	0.38±0.04*,$	0.64±0.06	0.43±0.04*,$	0.38±0.06*,$	0.27±0.03
IL-4 (pg/mL)	60.98±3.61	150.70±5.38$^{\#}$	68.41±4.60*,$	140.90±4.32	116.60±5.88*,$	84.49±6.34*,$	69.58±5.38
IL-5 (pg/mL)	38.04±5.40	103.50±6.88$^{\#}$	48.39±6.22*,$	100.80±7.22	74.19±5.92*,$	53.69±5.01*,$	38.25±3.66
IL-13 (pg/ml)	74.21±5.19	163.60±4.68$^{\#}$	75.00±3.52*,$	151.40±6.13	119.70±6.12*,$	89.24±4.63*,$	74.50±4.93
IFN-γ (pg/mL)	65.48±4.10	40.72 ± 4.43$^{\#}$	57.96±4.85*,$	43.01±5.05	50.10±3.80*,$	57.72±1.96*,$	61.46±2.72
IL-4/IFN-γ ratio	0.96±0.10	4.00±0.58$^{\#}$	1.21±0.09*,$	3.54±0.45	2.37±0.15*,$	1.47±0.11*,$	1.14±0.10
LTC-4 (pg/mL)	19.09±3.64	93.81±4.2$^{\#}$	41.41 ± 3.61*,$	85.01 ± 2.24	63.73±2.51*,$	55.33±1.85*,$	25.77±2.16

Data are represented as Mean±SEM (n=4-6) and analyzed by one-way Analysis of variance (ANOVA) followed by Tukey's multiple range test. For comparison with normal: $^{\#}p<0.05$, For comparison with AR control group: $*p<0.05$ and for comparison between montelukast with pinitol: $^{\$}p<0.05$.

AR: Allergic Rhinitis; IFN-γ: Interferon-gamma; Ig: Immunoglobulin; ILs: Interleukins; LTC-4: Leukotriene C4; MLT (10): Montelukast (10 mg/kg) treated; NLF: Nasal Lavage Fluid; OVA: Ovalbumin; P: Pinitol treated; Perse: Non-sensitized pinitol treated mice.

NLF IL-4, IL-5, IL-13, IFN-γ, and LTC-4 Levels

The NFL levels of IL-4, IL-5, IL-13 and LTC-4 were markedly (p=0.0003, p=0.0001, p<0.0001 and p<0.0001, respectively) increased, whereas the level of IFN-γ was effectively (p=0.0457) decreased in NLF of AR control mice when compared with normal mice. Montelukast treatment significantly attenuated OVA-induced alterations in NLF IL-4 (p=0.0001), IL-5 (p=0.0008), IL-13 (p=0.0001), IFN-γ (p=0.0318), and LTC-4 (p<0.0001) levels compared with AR control mice. Pinitol (10 and 20 mg/kg) administration also significantly reduced NLF levels of IL-4 (p=0.0188 and p=0.0026), IL-5 (p=0.0141 and p=0.0011), IL-13 (p=0.0116 and p<0.0001) and LTC-4 (p=0.0023 and p=0.0023), whereas IFN-γ levels were increased effectively (p=0.0416 and p=0.0413) compared to AR control mice. The ILs, IFN-γ, and LTC-4 levels did not alter in normal, and per se treated mice (Table 2).

Splenic GATA3, T-bet, p-STAT6, SOCS1, TLR-4, and MyD88 Protein Expressions

When compared with normal mice, splenic GATA3 (Figure 2A), p-STAT6 (Figure 2C), SOCS1 (Figure 2D), TLR-4 (Figure 2E), and MyD88 (Figure 2F) protein expressions were markedly (p=0.001, p=0.0019, p=0.0017, p=0.0077, and p=0.0006, respectively) up-regulated after the OVA challenge, whereas splenic protein expressions of T-bet (Figure 2B) noticeably (p=0.0088) down-regulated in AR control mice. Administration of montelukast markedly

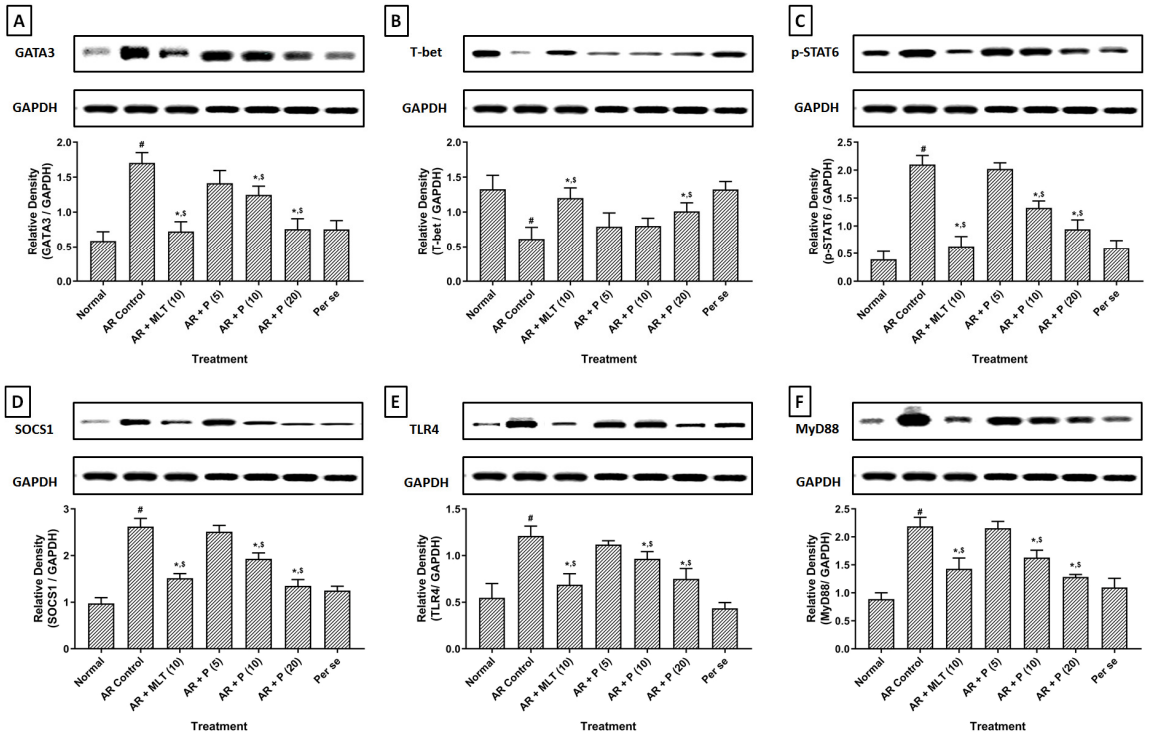

Figure 2. Effect of pinitol treatment on protein expression of GATA3 (A), T-bet (B), p-STAT6 (C), SOCS1 (D), TLR4 (E), and MyD88 (F) in the spleen. Data are represented as Mean ± SEM (n = 4) and analyzed by one-way Analysis of variance (ANOVA) followed by Tukey's multiple range test. For comparison with normal: $^{\#}p$< 0.05, For comparison with AR control group: *p< 0.05 and for comparison between montelukast with pinitol: $^{\$}p$<0.05.

AR: Allergic Rhinitis; GATA-3: GATA binding protein 3; IκBα: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha; MLT (10): Montelukast (10 mg/kg) treated; MyD88: Myeloid differentiation factor 88; NF-κB: Nuclear factor- Kappa B; OVA: Ovalbumin; P: Pinitol treated; Perse: Non-sensitized pinitol treated mice; SOCS1: Silencing of the suppressor of the cytokine signaling-1; STAT6: Signal transducer and activator of transcription 6; T-bet: T-box protein expressed in T cells; TLR4: Toll-like receptor 4.

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attenuated alterations in T-bet (p=0.032), GATA3 (p=0.0157), SOCS1 (p=0.0378), p-STAT6 (p=0.0157), MyD88 (p=0.0249), and TLR-4 (p=0.0196) protein expressions in the spleen when compared with AR control mice. Administration of pinitol (10 and 20 mg/kg) markedly inhibited up-regulated p-STAT6 (p=0.0093 and p=0.002), GATA3 (p=0.0436 and p=0.019), TLR-4 (p=0.0185 and p=0.0104), SOCS1 (p=0.0198 and p=0.0011), and MyD88 (p=0.0182 and p=0.0025) protein expressions; whereas down-regulated T-bet (p=0.0309 and p=0.0112) protein expressions in the spleen (Figure 2).

Nasal Mucosa Histopathology

Intranasal instillation of normal saline does not induce any structural perturbations in the nasal mucosa of normal and per se mice reflected by well-organized nasal architecture (Figure 3A and 3F). However, OVA-challenge resulted in the induction of histological aberrations in nasal mucosa reflected by a marked increase in the infiltration of eosinophils (p=0.0076), hyperplasia (p=0.0111), disturbance of nasal epithelium (p=0.0111), and edema (p=0.017) in AR control mice (Figure 3B). Administration of montelukast effectively reduced elevated infiltration of eosinophils (p=0.0111), hyperplasia (p=0.0111), disturbance of nasal epithelium (p=0.032), and edema (p=0.017) compared to AR control mice (Figure 3C). Pinitol (10 and 20 mg/kg) administration also showed marked inhibition in OVA-induced histological aberrations in nasal mucosa including infiltration of eosinophils (p=0.0272 and p=0.0111), hyperplasia (p=0.0272 and p=0.0076), disturbance of nasal epithelium (p=0.0111 and p=0.0076), and edema (p=0.017 and p=0.0111) (Figure 3D, 3E and 3G).

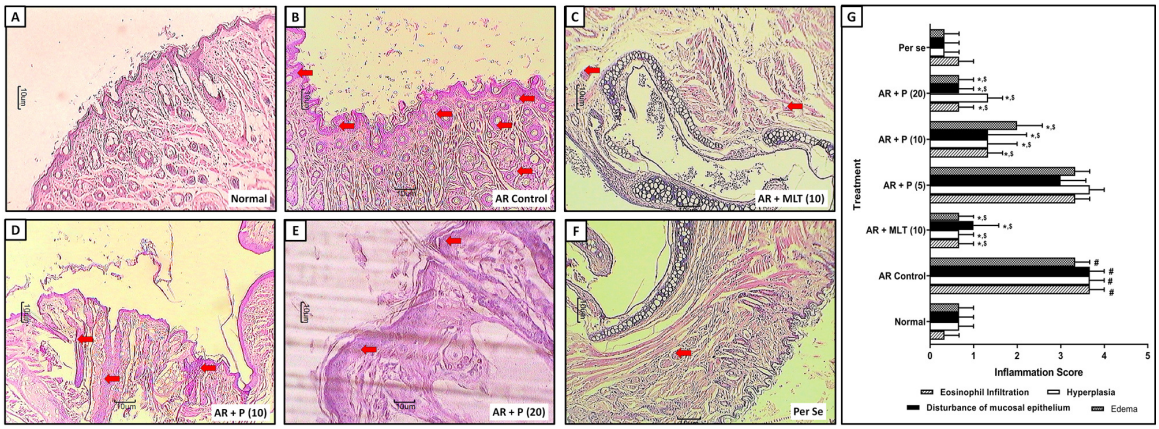

Figure 3. Effect of pinitol treatment on nasal histopathology. Photomicrograph of sections of nasal tissue from normal (A), AR control (B), montelukast (10 mg/kg) treated (C), Pinitol (5 mg/kg) treated (D), Pinitol (10 mg/kg) treated (E), and Pinitol (20 mg/kg) treated (F) mice. H&E stain at 40X. The quantitative representation of histological score (G). Data are expressed as mean±SEM (n=3), and one-way Analysis of variance (ANOVA) followed by the Kruskal-Wallis test was applied for post hoc analysis. For comparison with normal: $^{\#}p$<0.05, For comparison with AR control group: *p<0.05 and for comparison between montelukast with pinitol: $^{\$}p$<0.05.

AR: Allergic Rhinitis; MLT (10): Montelukast (10 mg/kg) treated; OVA: Ovalbumin; P: Pinitol treated; Perse: Non-sensitized pinitol treated mice

DISCUSSION

Allergic rhinitis, a common immune-inflammatory disorder of the upper respiratory system with significant prevalence, affects a person's quality of life. AR is mainly characterized by a bi-phasic allergic reaction where the initial inflammatory response phase includes mast cell activation via the allergen-IgE-dependent mechanism.[1] The activated mast cell further induces the release of cytokines, histamine, chemokines, prostaglandins, and leukotrienes, which result in sneezing, nasal secretion, and itching.[4,29] In the

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later phase, the accumulation of basophils, mast cells, and eosinophils in lamina propria and epithelium induces sustained release of various inflammatory mediators, proinflammatory cytokines, which promote late-phase reactions.[4] The current treatment options, such as leukotriene inhibitors, antihistamine, mast cell stabilizers, anticholinergic, and corticosteroids, are associated with several side effects. Thus, in the last few decades, researchers have shown interest in therapeutic moieties of herbal origin. Thus, investigators have widely implemented the AR model induced by ovalbumin to determine the potential of therapeutic moieties against IgE-mediated allergic reactions.[10,14,15] In the current study, we determined the efficacy of pinitol in OVA-induced AR and suggest that pinitol induces an anti-allergic effect by achieving Type 1 T helper (Th1)/Type 2 T helper (Th2) response balance in experimental mice (Figure 4).

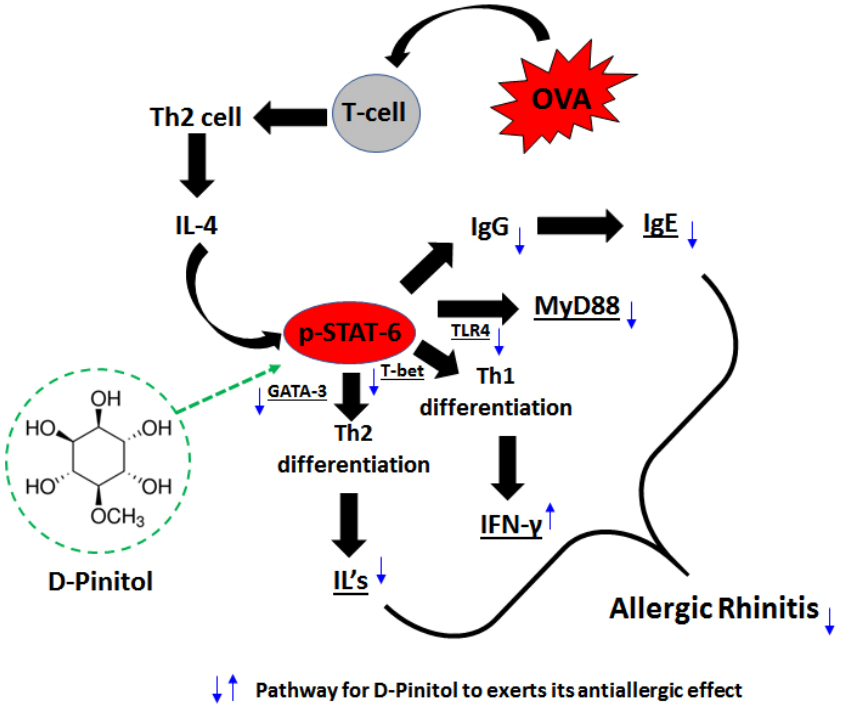

Figure 4. D-Pinitol attenuated ovalbumin-induced allergic rhinitis.
AR: Allergic rhinitis; GATA: GATA binding protein 3; IFN-γ: Interferon-gamma; Ig: Immunoglobulin; IκBα: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha; ILs: Interleukins; LTC-4: Leukotriene C4; MyD88: Myeloid differentiation factor 88; NF-κB: Nuclear factor- Kappa B; OVA: Ovalbumin; STAT-6: Signal Transducer and Activator of Transcription-6; T-bet: T-box protein expressed in T cells; Th2: Type 2 T helper; TLR4: Toll-like receptor 4; TNF-α: Tumor necrosis factor-alpha.

Previous reports suggested that Th2 cells play a significant role in developing and maintaining AR via the activation of various cytokines, such as ILs.[15,30] IL-4 causes differentiation of Th2 cells and modulation in the differentiation of Th1. IL-4, along with IL-13, is important for "class-switching" from IgE to IgM produced from B cells.[11,31-33] Furthermore, phosphorylation of STAT6 (Signal transducer and activator of transcription 6) via binding of IL-4 or IL-13 induces the development of Th2 response.[30,34] Thus, the activities of IL-4 and IL-13 during the amplification of immediate allergic responses have been established by previous researchers.[12,35] Additionally, IL-4 serves as an important mediator of mast cell growth and mast cell chemoattractants.[31,36] Th2 cytokines are also known to induce eosinophils infiltration in nasal tissue, which further aggregate the allergic response.[35,37-39] On the other hand, IFN-γ has been documented as a

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vital Th1 cytokine to inhibit the switching of B cells, which reduces IgE production.[15,40] Inhibition of IgE production by IFN-γ decreases the Th2 immune response in the nasal mucosa, further reducing the IgE-mediated nasal symptoms. In the current investigation, the challenge with OVA caused activation of IgE response, which induces production of Th2 cytokines reflected by increased production of ILs in NLFs. However, administration of pinitol inhibited these Th2 cytokines and improved the Th1 cytokine (IFN-γ) levels, thus balancing the Th1/Th2 response. This notion was further supported by histopathological evaluation of nasal mucosa where allergen-induced eosinophils recruitment was reduced by pinitol administration. A previous study also reported that pinitol attenuated allergic reaction during airway hyper-responsiveness via balancing Th1/Th2 response[19] and the outcome of the current investigation corroborates those findings.

GATA3, a transcription factor of the GATA family, has been documented to articulate Th2 cells and is responsible for STAT6 activation-dependent Th2 differentiation and modulation of Th1 cells differentiation.[31,37,41] Whereas, T-bet is another transcription factor that modulates the expression of Th1 cells; activating IFN-γ levels, thus mediating cell-mediated immunity.[15,31,37] Furthermore, the SOCS1 is the protein suggested to modulate various cytokine signaling mechanisms. Studies have documented that SOCS1 induces Janus kinase (JAK)-signal transduction via steric hindrance at signaling protein-binding sites on cytokine receptors that play a vital role in the differentiation of T helper cell and thus pathogenesis of AR.[12,36] Thus, inhibition of SOCS1 has been suggested as an essential mechanism of Th1 and Th2 cytokine balance.[12,35,42] OVA challenge resulted in up-regulation of GATA3 and SOCS1 levels whereas down-regulation of T-bet level. However, pinitol treatment inhibited OVA-induced alterations in Th1/Th2 balance, thus maintaining immunological homeostasis. The present investigation results corroborate with earlier study outcomes where pinitol induces its anti-allergic response via suppression of GATA-3 expression.[21]

Evidence suggests that OVA stimulates primarily the cell signaling cascade, which further binds to the CD14 receptor cell surface of the target cell.[43] A proximal trans-membrane receptor of the CD14, such as toll-like receptor 4 (TLR4), further activates downstream of CD14 and mediates the further signaling of endogenous antigens.[44-46] Additionally, TLR4 activation produces adaptor protein, MyD88, which can be MyD88-independent and MyD88-dependent.[44,47] MyD88 is generally known as the substance of 12 myeloid differentiation initial response genes. Being an adaptor protein, MyD88 leads to moderate signal transduction of various TLRs families.[47] This MyD88-dependent or MyD88-independent signal transduction pathway activates NF-kB, which in turn releases various cytokines and induces eosinophil infiltration.[44,47-50] Thus, the MyD88-dependent activation mechanism plays a vital role in the induction of allergic response. In the current study, OVA-induced increased levels of TLR4 and MyD88 protein were markedly reduced by pinitol depicting its vital role in the inhibition of allergic response.

Montelukast, which is Cysteinyl leukotrienes (CysLTs)-1 receptor antagonists, is the currently approved therapeutic regimen for AR management. It has proved its efficacy against rhinorrhea, sneezing, congestion, and difficulty in sleeping however, it causes an array of side effects such as hallucination, aggression, depression, insomnia, irritability, and restlessness.[7] Conversely, recent clinical studies showed that pinitol has effectively managed non-alcoholic fatty liver disorder and Type 2 Diabetes Mellitus through its antioxidant potential without any serious adverse event.[17,20] Thus, pinitol can be considered as one of the potential candidates for further clinical development to treat allergic disorders, including rhinitis.

In conclusion, present study observations suggest that pinitol exerts its potential against allergic rhinitis to ameliorate nasal symptoms. Pinitol modulated its effect via attenuation of Th2 cell activations (IL-4, IL-5, IL-13, GATA3, SOCS1, and TLR4) and improving Th1 cell activations (IFN-γ and T-bet), thus balancing Th1/Th2 response during allergic conditions.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

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