Ameliorative potential of galangin in murine model of ovalbumin-induced allergic rhinitis: a role of PI3K-PKB pathway

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OBJECTIVE
To examine the potential of galangin in a mouse model of ovalbumin (OVA)-induced allergic rhinitis (AR), as chronic AR, induced by immunoglobulin-E (IgE), leads to histamine release and nasal inflammation, and although galangin exhibits antiasthmatic and anti-inflammatory potential, its effect on AR is yet to be investigated.

ANIMALS
126 BALB/c mice.

METHODS
AR induction involved sensitizing female mice with OVA (5%, 500 µL, IP) for 14 days. Post OVA challenge, the mice were divided into 7 groups (n = 18/group), including normal, AR control, montelukast (10 mg/kg), galangin (5, 10, and 20 mg/kg), and per se (galangin [20 mg/kg] treatment. Various outcomes were evaluated, including nasal symptoms, histopathology, biochemistry, and nasal lavage fluid inflammatory cytokines and signaling pathways in nasal mucosal to assess galangin potential in AR.

RESULTS
In AR mice, galangin (10 and 20 mg/kg) significantly (P < .05) reduced sneezing, rubbing, and nasal discharge post-OVA challenge. Galangin treatment attenuated (P < .05) elevated serum histamine, β-hexosaminidase, IgE, and Immunoglobulin G1 levels in AR control mice. Additionally, galangin significantly (P < .05) decreased OVA-induced alterations in IL-4, IL-6, IL-13, and interferon-γ levels in nasal lavage fluid compared to AR control mice. Western blot analysis demonstrated that galangin lowered OVA-induced AR by significantly (P < .05) downregulating the phosphorylated protein kinase B  and mammalian target of rapamycin-protein expressions while markedly (P < .05) upregulating the glycogen synthase kinase-3β protein expressions in nasal mucosal. Galangin also significantly ameliorated (P < .05) the OVA-induced histological aberrations in the nasal mucosa, reflected by reduced eosinophil infiltration, hyperplasia, and edema.

CLINICAL RELEVANCE
Galangin exhibits antihistaminic and anti-inflammatory effects in AR mice by regulating IgE-mediated histamine and inflammatory release and modulating the phosphatidylinositol 3-kinase/Ak strain transforming/mammalian target of rapamycin pathways.

Keywords: allergic rhinitis; galangin; interleukins; ovalbumin; PI3K

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results in significant societal expenses. In China, the total expenses for adults with AR and non-AR were €195.6 per patient/year and €185.3 per patient/year, respectively. The total societal cost for adults with AR and non-AR is approximately €440.9 million and €671.9 million per year, respectively.1

Although chronic AR presents distinct clinical features, the comprehensive understanding of its underlying pathophysiological mechanisms, particularly in relation to immune mediators, genetics, and environmental factors, remains unclear.2 The primary cause of AR is the histamine discharge from mast cells, which triggers the secretion of IL-4 and IL-5, leading to the development of T helper type 2 (Th2) cytokine responses.3 The histamine H1 receptor (H1R) and histamine H4 receptor (H4R) play crucial roles in allergic inflammation, even though reports4 indicate that H1R antagonists may not yield satisfactory outcomes in the management of AR. Hence, managing histamine pathways is essential in the treatment of AR. There has been an increased use of novel drugs that target the H4R for treating allergic diseases. Combined therapy of H1R and H4R antagonists results in greater relief from allergic symptoms than using either type of antagonist alone.5 Additionally, the regulation of H4R expression in mast cells is influenced by the phosphatidylinositol 3-kinase (PI3K) pathway.6 This pathway can control the release of cytokines, including tumor necrosis factor-α and IL-8. Studies7,8 have verified that the H4R exhibits functional effects by modulating cytokine production, specifically by downregulating interferon-γ (IFN-γ) and upregulating IL-6 in cells derived from a monocyte cell line when exposed to histamine stimulation. The mammalian target of rapamycin (mTOR), a key regulator of autophagy, stands as a down-stream target of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PI3K-PKB) pathway.9 Activation of the PI3K-PKB can directly phosphorylate mTOR at serine 2448, impeding autophagy and fostering cell survival.10,11 A recent study12,13 found that activating the PI3K/Akt strain transforming (AKT)/mTOR pathway inhibits pulmonary autophagy, attenuating allergic asthma airway inflammation. However, inhibiting this pathway increases inflammation.

Current treatment regimens for managing AR include mast cell stabilizers, anticholinergics, antihistamines, leukotriene receptor antagonists, corticosteroids, and allergen immunotherapy.14 Nevertheless, these medications come with significant adverse effects, including blurred vision, dry mouth, constipation, urinary retention, tachycardia, and sedation, compromising their uses in a clinical setting.15 Moreover, an expanding number of individuals are exploring alternative and complementary approaches, such as incorporating medicinal herbs and their bioactive products, to manage symptoms associated with AR.16

Flavonoids have been demonstrated in experiments to prevent the proteolytic enzyme β-hexosaminidase and histamine production from mast cells.17 Galangin, a major flavonoid found naturally in Alpinia and Propolis species, has a variety of biological actions, including anti-inflammatory, antibacterial, antioxidative stress, antiaging, antifibrosis, antihypertensive, and anticancer activities.18 Research depicted that galangin alleviates ovalbumin (OVA)-induced airway inflammation by regulating the nuclear factor-κB (NF-κB) pathway, reducing total cell and eosinophil counts, lowering IL-4, IL-5, and IL-13 in bronchoalveolar lavage fluid, and decreasing OVA-specific IgE in serum.19 Although galangin has shown promising antiasthmatic properties, its AR mechanism has not yet been investigated. Consequently, this study seeks to explore the pharmacological effects of galangin on OVA-induced AR in mice by assessing nasal mucosal behavior, conducting biochemical analyses, and examining histological studies. Additionally, the research evaluates the impact of galangin on protein expression levels of PI3K, anti-AKT antibody (p-PKB), mTOR, and glycogen synthase kinase-3β (GSK3β) in the nasal mucosa.

Methods

Experimental animals

BALB/c mice (female, 18 to 22 g) were used to induce AR, and the experimental protocol (B2056668) was approved by the Jinan Zhihe Medical Science & Technology Medical Research, which is in line with animal protection, animal welfare, and ethical principles and is in line with the National Institute of Health Guide for Care and Use of Laboratory Animals and were approved by the Animal Ethics and Use Committee.

Drugs and chemicals

Galangin (purity ≥ 97%), OVA (grade V), aluminum hydroxide, and histamine dihydrochloride were purchased from Sigma-Aldrich Co. Montelukast was obtained from Cipla Ltd. Mouse OVA-specific IgE, total IgE, total Immunoglobulin G1 (IgG1), β-hexosaminidase, IL-4, IL-6, IL-13, and IFN-γ ELISA kit were obtained from Bethyl Laboratories Inc. The primary antibodies of PI3K (ab283852; 1:500), p-PKB (ab38449; 1:500), mTOR (EPR390[1]; ab134903; 1:1,000), GSK3β (ab93926; 1:2,000), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; ab8245; 1:500) were purchased from Abcam.

Induction of AR and treatment

Mice were sensitized on alternate days by administration of OVA (500 μL, 5%, IP) till day 14.20 Sensitized mice were randomly divided (n = 18 mice/group) into various groups AR control (received distilled water [DW; 10 mg/kg, PO]), montelukast (10 mg/kg, PO),16,21 and galangin (5, 10, and 20 mg/kg, PO)22 and received respective treatments till day 21. The normal group of mice received aluminum hydroxide IP without OVA and was treated with DW (10 mg/kg), whereas the per se group received galangin (20 mg/kg) only till day 21. On day 21, nasal symptoms were evaluated using a method reported elsewhere.23 The solutions of galangin were freshly prepared daily by dissolving in DW (1 mg/ml) and administrated orally for biological evaluations.
Biochemical and molecular assessment
Post this, blood specimens were collected to obtain serum, whereas nasal lavage fluid (NLF) was collected using the method reported elsewhere, and both were stored at −20 °C for biochemical assessments. Briefly, mice underwent partial tracheotomy under deep anesthesia by intraperitoneal injection of 1% sodium pentobarbital (50 mg/kg). A 22-gauge catheter was inserted into the posterior naris from the opening of the trachea and along the direction of the nostrils. Sterile saline solution (3 mL) was perfused gently into the nasal cavities, lavage fluid was collected from the anterior naris, centrifuged at 220 X g and 4 °C for 10 min, and the supernatant was stored at −20 °C. A respective mouse-specific ELISA quantitation kit was used to determine IgE (OVA-specific and total), histamine, IgG1, and β-hexosaminidase in serum, whereas IL-4, IL-6, IL-13, and IFN-γ in NLF. Mice were sacrificed by cervical dislocation, and nasal mucosal specimens (n = 6) were collected and maintained at −70 °C. Nasal tissue protein was extracted using a reagent kit (Thermo Fisher Scientific) and was used to determine the expressions of PI3K, p-PKB, mTOR, and GSK3β using the Western blot method. Protein bands were visualized using the Chemiluminescent kit (Bio-Rad Laboratories Inc), and GAPDH served as the loading control.

Histological analysis
Another section of the nasal mucosa was stored for 24 hours in 10% formalin for histological examination using H&E. These sections were examined under a light microscope (Olympus DP71, DP-BSW Ver.03.03; Olympus Medical Systems India Private Ltd) for various histopathology features described previously. The intensity of histological aberrations in the nasal tissue was graded as grade 0 (not present), grade 1 (minimal), grade 2 (mild), grade 3 (moderate), and grade 4 (severe).

Statistical analysis—The sample size was calculated based on the power analysis method considering 30% expected attrition using a formula: corrected sample size = sample size/(1 − [% attrition/100]). Data analysis was conducted using GraphPad Prism 5.0 software (GraphPad Inc) and reported as mean with SEM and median with quartile range. A value of P less than .05 is considered statistically significant. A post hoc analysis was conducted using Tukey multiple ranges (for parametric tests) and the Kruskal-Wallis, followed by Mann-Whitney’s multiple comparison tests (nonparametric tests) during 1-way ANOVA.

Results
Effect of galangin on body weight and relative spleen weight
Results indicated that there was a significant (P < .05) decrease in body weight and a notable (P < .05) increase in relative spleen weight in AR control compared to normal and per se (galangin, 20 mg/kg)–treated mice (Table 1). Conversely, mice treated with montelukast (10 mg/kg) significantly (P < .05) mitigated the OVA-induced decrease in body weight and increase in relative spleen weight compared to the AR control group. Similarly, compared to the AR control, mice treated with galangin (10 and 20 mg/kg) exhibited a significant (P < .05) increase in animal body weight along with a noticeable (P < .05) reduction in the relative spleen weight, although no significant effect in the body and spleen weights was observed in mice treated with galangin (5 mg/kg) in contrast to AR control. Furthermore, the increase in body weight was more significant (P < .05) with the galangin treatment compared to the montelukast-treated group.

Effect of galangin on nasal symptoms
The effect of galangin on nasal symptoms (rubbing, sneezing, and discharge) in OVA-induced AR mice is provided (Table 1). In contrast to the normal and per se–treated group, AR control mice post OVA administration showed a marked (P < .05) increase in nasal symptoms (rubbing, sneezing, and discharge) in OVA-induced AR mice (n = 6).
in the nasal symptoms as depicted by an elevated frequency of sneezing, rubbing, and nasal discharge. The OVA-induced alterations in these nasal symptoms were significantly \((P < .05)\) decreased in the mice treated with galangin (10 and 20 mg/kg) compared to AR control mice. However, no significant effect was observed with the low dose of galangin (5 mg/kg) treatment. Furthermore, a more prominent effect \((P < .05)\) in reducing nasal sneezing, rubbing, and discharge was observed following montelukast intervention in contrast to galangin treatment. Additionally, per se–treated mice did not show any induction of nasal rubbing, sneezing, or discharge after the intranasal OVA challenge.

**Effect of galangin on serum histamine, \(\beta\)-hexosaminidase, IgE, and IgG1 levels**

In comparison to normal and per se–treated mice, AR control mice exhibited a significant \((P < .05)\) elevation in serum concentration of histamine, \(\beta\)-hexosaminidase, IgG1, OVA-specific IgE, and total IgE. However, treatment with galangin (10 and 20 mg/kg) significantly \((P < .05)\) mitigated the OVA-induced increase in serum histamine, \(\beta\)-hexosaminidase, OVA-specific IgE, total IgE, and IgG1 levels compared to AR control mice. Notably, the group of mice treated with montelukast demonstrated a remarkable and more pronounced effect in reducing \((P < .05)\) the serum histamine, IgE, IgG1, and \(\beta\)-hexosaminidase levels compared to those treated with galangin. Additionally, none of these serum biochemical parameters showed alterations in mice treated per se, and no significant effect was observed with the low dose of galangin (5 mg/kg) (Table 2).

**Effect of galangin on nasal mucosal IFN-\(\gamma\), IL-4, IL-6, IL-13, and IFN-\(\gamma\) levels**

Following the OVA challenge, there was a notable \((P < .05)\) reduction in IFN-\(\gamma\) concentration and a significant \((P < .05)\) elevation in IL-4, IL-6, IL-13 levels, and IL-4/IFN-\(\gamma\) ratio in the NLF of AR control mice compared to normal and per se–treated mice (Table 2). Treatment with galangin (10 and 20 mg/kg) in contrast to the AR control group significantly mitigated \((P < .05)\) these changes in IL levels and IL-4/IFN-\(\gamma\) ratio, along with an increase \((P < .05)\) concentration of IFN-\(\gamma\). However, the montelukast (10 mg/kg)-treated group showed a more prominent effect in suppressing \((P < .05)\) the OVA-induced alterations in IL-4, IL-6, IL-13, IFN-\(\gamma\), and IL-4/IFN-\(\gamma\) ratio levels in NLF when compared to the galangin-treated group.

**Effect of galangin on nasal mucosal p-PKB, PKB, GSK3\(\beta\), and mTOR protein expressions**

Following OVA challenge, there was a significant upregulation \((P < .05)\) in the expressions of p-PKB and mTOR proteins along with a notable downregulation \((P < .05)\) in the expression of GSK3\(\beta\) protein in the nasal mucosa within AR control group as compared to the normal and per se–treated mice (Figure 1). Treatment with montelukast (10 mg/kg) in contrast to the AR control mice significantly \((P < .05)\) upregulated the expression of GSK3\(\beta\) protein and downregulated \((P < .05)\) the expression of p-PKB and mTOR proteins. However, no significant effect was observed with the expression of PKB protein. Similarly, mice treated with galangin (10 and 20 mg/kg) exhibit significant attenuation \((P < .05)\) in OVA-induced alterations in expressions of p-PKB, GSK3\(\beta\), and mTOR proteins in nasal mucosal compared to the AR control group. However, mice treated with a low dose of galangin (5 mg/kg) show no significant effects on p-PKB, GSK3\(\beta\), and mTOR protein expressions.

Furthermore, these findings are supported by a positive correlation between the frequency of nasal sneezing and protein expression of p-PKB \(\left(R^2 = 0.8377; \text{Figure 2}\right)\) and mTOR \(\left(R^2 = 0.8339\right)\) in OVA-challenged AR mice. Similarly, a positive correlation was observed between the OVA challenge nasal sneezing and the \(\beta\)-hexosaminidase levels \(\left(R^2 = 0.903\right)\). However, a negative correlation was noted between frequencies of nasal sneezing and p-GSK3\(\beta\) protein expression \(\left(R^2 = 0.6406\right)\).

**Table 2—Effect of galangin (G) on serum biochemistry and IL-4, IL-6, and IL-13, and interferon-\(\gamma\) (IFN-\(\gamma\)) levels in nasal lavage fluid in allergic rhinitis (AR) mice \((n = 6)\)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>AR control</th>
<th>MLT (10 mg/kg)</th>
<th>G (5 mg/kg)</th>
<th>G (10 mg/kg)</th>
<th>G (20 mg/kg)</th>
<th>Per se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine ((\mu g/mL))</td>
<td>67.16 ± 8.43</td>
<td>366.10 ± 5.95*</td>
<td>105.70 ± 10.58*</td>
<td>339.00 ± 10.94</td>
<td>255.20 ± 10.03*</td>
<td>143.30 ± 12.41*</td>
<td>73.16 ± 9.78</td>
</tr>
<tr>
<td>(\beta)-Hexosaminidase (ng/mL)</td>
<td>12.16 ± 1.92</td>
<td>45.59 ± 1.68*</td>
<td>17.80 ± 1.99*</td>
<td>43.05 ± 1.37*</td>
<td>31.27 ± 1.53*</td>
<td>24.56 ± 2.31*</td>
<td>16.26 ± 2.43</td>
</tr>
<tr>
<td>OVA-specific IgE (ng/mL)</td>
<td>9.20 ± 1.67</td>
<td>55.09 ± 1.59*</td>
<td>12.93 ± 1.81*</td>
<td>48.84 ± 1.56*</td>
<td>32.34 ± 0.89*</td>
<td>21.10 ± 1.58*</td>
<td>17.03 ± 2.25</td>
</tr>
<tr>
<td>Total IgE (ng/mL)</td>
<td>117.20 ± 6</td>
<td>418.70 ± 7.50*</td>
<td>142.10 ± 8.16*</td>
<td>407.20 ± 8.83*</td>
<td>322.10 ± 5.93*</td>
<td>220.70 ± 6.94*</td>
<td>96.35 ± 9.54</td>
</tr>
<tr>
<td>Total IgG1 level (ng/mL)</td>
<td>0.26 ± 0.03</td>
<td>0.60 ± 0.03*</td>
<td>0.28 ± 0.02*</td>
<td>0.54 ± 0.02*</td>
<td>0.44 ± 0.02*</td>
<td>0.37 ± 0.02*</td>
<td>0.31 ± 0.02*</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>46.37 ± 3.96</td>
<td>146.40 ± 5.48*</td>
<td>58.90 ± 5.29*</td>
<td>136.50 ± 4.44</td>
<td>113.70 ± 4.29*</td>
<td>78.32 ± 4.79*</td>
<td>78.05 ± 3.10</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>134.00 ± 10.14</td>
<td>290.20 ± 10.73*</td>
<td>147.50 ± 9.35*</td>
<td>289.50 ± 8.75</td>
<td>228.30 ± 9.80*</td>
<td>188.90 ± 7.23*</td>
<td>144.00 ± 8.47</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>1.55 ± 0.31</td>
<td>37.55 ± 0.82*</td>
<td>7.71 ± 0.71*</td>
<td>35.44 ± 2.15</td>
<td>23.35 ± 0.70*</td>
<td>11.68 ± 0.71*</td>
<td>4.67 ± 0.55</td>
</tr>
<tr>
<td>IFN-(\gamma) (pg/mL)</td>
<td>58.36 ± 2.99</td>
<td>34.82 ± 4.27*</td>
<td>53.51 ± 4.91*</td>
<td>45.24 ± 3.77</td>
<td>47.65 ± 4.76*</td>
<td>54.43 ± 3.72*</td>
<td>66.94 ± 3.43</td>
</tr>
<tr>
<td>IL-4/IFN-(\gamma) ratio</td>
<td>0.69 ± 0.07</td>
<td>4.58 ± 0.64*</td>
<td>1.14 ± 0.11*</td>
<td>3.14 ± 0.32*</td>
<td>2.51 ± 0.29*</td>
<td>1.49 ± 0.18*</td>
<td>1.19 ± 0.10</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SE and analyzed by 1-way ANOVA followed by Tukey post hoc test. Per se group received G (20 mg/kg) without exposure to ovalbumin (OVA).

\(\text{IgE} = \text{immunoglobulin E, IgG1 = immunoglobulin G1, MLT = Montelukast.}\)

\(\#P < .05, \dagger P < .05, \ddagger P < .05\) compared with the normal and AR control groups.
Effect of galangin on histopathology of the nasal mucosa

It is evident (Figure 3) that the nasal mucosal tissue in the normal mice exhibits a well-organized histological structure characterized by no eosinophil infiltration (grade 0), hyperplasia (grade 0), no alterations in the mucosal epithelial layers (grade 0), and an absence of edema (grade 0). However, intranasal challenge with OVA results in noticeable (P < .05) histological aberrations, characterized by abnormal infiltration of eosinophils (grade 3), hyperplasia (grade 4), edema (grade 4), and disruption of the mucosal epithelium in the nasal mucosal tissue (grade 4) of AR control mice, compared to the normal group. On the contrary, mice treated with montelukast (10 mg/kg), when compared to AR control, exhibited notable (P < .05) protection against histological abnormalities in the nasal mucosa induced by OVA challenge. This is evident through a considerable (P < .05) reduction in eosinophil infiltration (grade 1), hyperplasia (grade 1), and edema (grade 0), with minimal mucosal epithelium disruption (grade 1). A similarly significant (P < .05) but less pronounced protective effect is observed in the nasal mucosal tissue of mice treated with galangin (10 and 20 mg/kg) compared to AR control mice. Nevertheless, per se (galangin [20 mg/kg] alone)-treated mice did not exhibit any histological changes. This observation indicates that per se–treated mice did not have any histological alterations such as eosinophil infiltration (grade 0),

Figure 1—Effect of galangin (G) on nasal mucosal phosphorylated protein kinase B (p-PKB; A), PKB (B), glycogen synthase kinase 3β (GSK3b; C), and mammalian target of rapamycin (mTOR; D) protein expression (n = 6). The data are expressed as mean ± SE and analyzed by 1-way ANOVA followed by Tukey post hoc test. #P < .05, *P < .05, and $P < .05 compared with the normal, allergic rhinitis (AR), and one another (G and MLT) control groups. Per se group received G (20 mg/kg) without exposure to ovalbumin. GAPDH = Glyceraldehyde-3-phosphate dehydrogenase. MLT = Montelukast.
Figure 2—A simple regression of nasal mucosal protein expression of phosphorylated protein kinase B (p-PKB; A), PKB (B), glycogen synthase kinase 3β (GSK3β; C), and mammalian target of rapamycin (mTOR; D) with ovalbumin (OVA) challenge-induced nasal sneezing in allergic rhinitis mice. Correlation coefficients were determined using a 2-sided Fisher test. GAPDH = Glyceraldehyde-3-phosphate dehydrogenase.

Figure 3—A–F—Effect of galangin (G) on nasal histopathology using H&E staining (40X; n = 3). Eosinophil infiltration (black arrow), hyperplasia (blue arrow), and edema (yellow arrow). Per se group received G (20 mg/kg) without exposure to ovalbumin. AR = Allergic rhinitis. MLT = Montelukast.
hyperplasia (grade 0), edema (grade 0), and only minimal disruption in the epithelial mucosa (grade 1).

Discussion

Allergies are abnormal immune reactions triggered by ingesting, inhaling, or coming into contact with toxic foreign substances and are intended to remove foreign contaminants. Despite its crucial role in the body’s defense system, the hypersensitivity reactions in allergies can be potentially harmful if left untreated. Although AR poses a lower mortality risk than asthma, treating it is crucial to improving the health of aging populations worldwide. Even though present medications provide some comfort for AR, a comprehensive cure remains difficult, necessitating the discovery of novel medicines. Natural products have garnered interest in drug research in recent years due to their unique and favorable attributes, which provide a viable solution to overcome the limits of synthetic molecules. Considering that AR is characterized by nasal membrane inflammation and hyperreactivity, resulting in symptoms such as itching, sneezing, nasal congestion, and discharge, and recognizing galanin’s antiasthma and anti-inflammatory properties, this study investigated the role of galangin in OVA-induced AR in mice. According to findings, galangin alleviated AR symptoms, modulated serum biomarkers, and mitigated inflammatory cytokines induced by OVA. Furthermore, galangin regulates AR-induced changes in the protein expression of p-PKB, PKB, GSK3β, and mTOR in the nasal mucosa.

AR occurs in 2 stages of an allergic response. During the early phase, mast cells play an essential role in regulating the immune system and allergic inflammation, which starts within minutes of allergen exposure. IgE-mediated cross-linking of the membrane-bound high-affinity IgE receptor (FcεRI) initiates mast cell degranulation during this early stage. This degranulation triggers intracellular signaling, rapidly releasing mediators involving histamine, cytokines, chemokines, and proteases. Histamine secretion during this phase causes early symptoms featuring sneezing, itching, rubbing, congestion, and nasal discharge. Our current investigation depicted that sensitization of the mice through the intranasal challenge of OVA resulted in AR nasal symptoms (sneezing, rubbing, and nasal discharge). Notably, our findings also demonstrated an increased histamine level, as observed in the serum of AR control mice after OVA challenge, indicating the role of histamine in the onset of nasal symptoms. Mice treated with galangin suppressed histamine release and stabilized mast cells, resulting in a noticeable decrease in sneezing, rubbing, and nasal discharge frequency. Research has demonstrated that galangin potentially inhibited the histamine release in mast cell–mediated allergic inflammation by regulating intracellular calcium levels and mitigating the inflammatory cytokines via modulating NF-κB, caspase-1, and mitogen-activated protein kinase.

The pathogenesis of AR is complex, where inflammatory cytokines play a central role in various events, including cell survival and differentiation, activation of immune cells, cell migration, and cell death. Research on AR pathogenesis focuses primarily on the classical Th1/Th2 balance. As a result of Th2-cell activation, IL-4, IL-5, and IL-13 are produced, contributing to allergy symptoms. As part of allergic responses, IL-4 promotes B-cell–induced production of IgE, and when stimulated, it quickly produces additional allergen-specific IgE and promotes mast cell maturation and differentiation. It also works in tandem with IL-13 to enable B-cell transformation into plasma cells for immunological memory. Conversely, IL-13 is released by activated T cells, B cells, and mast cells. Research demonstrated that galangin has potential anti-inflammatory effects with a long history of modulating the proinflammatory mediators involved in chronic disease, including arthritis, bronchitis, nephritis, stroke, cognitive dysfunction, and inflammatory bowel disease. In general, galangin showed diverse anti-inflammatory effects via (1) decreasing the expression of tumor necrosis factor-α, IL-6, IL-8, and IL-1β in asthma mast cell–mediated allergic inflammation; (2) reducing the IL-4 and IL-13 levels in atopic dermatitis; (3) reducing the IL-6 levels in inflammatory bowel diseases involving ulcerative colitis; and (4) downregulating the infiltration of mast cells triggering the release of IL-5, IL-13, IL-31, and IFN in atopic dermatitis. These data are in accordance with our results stating that galangin exhibits promising anti-inflammatory effects by inhibiting proinflammatory cytokines. This finding reinforces our observations, demonstrating a significant reduction in inflammatory infiltration in the histological architecture of mouse nasal tissue following galangin therapy.

Protein kinase B, or AKT, is a serine/threonine kinase that participates in various cellular activities, including signaling downstream of growth factors, cytokines, and other cellular stimuli. It is often associated with signaling pathways involved in inflammatory disease pathogenesis. In IgE-induced early allergy reactions, the PI3K–PKB pathway plays a crucial role in the acute rise in mast cell activation and vascular permeability. The mTOR protein, conversely, is a serine/threonine protein kinase belonging to the PI3K-associated protein kinase family. Research in allergic diseases has shown that mTOR functions as a sensor of the immune microenvironment and controls the function and differentiation of immune cells owing to its ability to regulate various immune cells and limit proinflammatory molecules. Under inflammatory conditions caused by allergens, the mRNA and protein levels of PI3K, AKT, and mTOR were upregulated, and this condition was observed in a present investigation where the intranasal challenge of the allergenic OVA showed upregulation of p-PKB and mTOR proteins in the nasal mucosa. Interestingly, this upregulation in p-PKB and mTOR proteins was suppressed by galangin intervention, signifying that it may contribute to antiallergic function in AR via suppression of mast cell PI3K/AKT/mTOR pathway. Furthermore, our results are consistent with earlier research showing the benefits of
galangin in blocking the PI3K/AKT pathways in uric acid-induced renal inflammation in the NRK-52E cell line\(^{49}\) and cardiac remodeling.\(^{50}\)

As a serine/threonine kinase, GSK3 comprises 2 similar paralogs, which play key roles in the inflammation and pathogenesis of diseases like diabetes, cancer, and neurological conditions.\(^{51}\) Specifically, GSK3\(\beta\) is involved in various cellular processes, including those related to allergic asthma.\(^{52}\) Studies\(^{53}\) have shown that inactivating the PKB, PI3K, and mTOR complex (mTORC)1 axis resulted in nuclear accumulation of both GSK3\(\alpha\) and GSK3\(\beta\). This suggests that PI3K-Akt-mTORC1 activation promotes GSK3\(\beta\) retention in the cytosol, implying a cellular localization interaction.\(^{54}\) Our results align with these findings, indicating that activation of p-PKB and mTOR in OVA-challenged mice retains GSK3\(\beta\) in the nasal mucosa. Galangin treatment explicitly upregulates GSK3\(\beta\), highlighting its active role in inhibiting nasal mucosa inflammation.

Although many studies have sought to examine the antiallergic properties of herbal medicine in treating AR through in vivo research, only a limited number of plants have demonstrated success in clinical applications through randomized, double-blind studies. Additionally, some promising bioactive phytochemicals (luteolin-7-O-rutinoside, mangiferin, methylwarifetine, okicamelliaside, petasin, shikonin, tussilagone, and warifiteine) have been identified as potential AR treatment candidates, their clinical efficacy in human subjects remains unexplored.\(^{54}\) In the present study, galangin also showed its antiasthmatic and anti-inflammatory potential in the experimental model of AR; thus, it can be considered for further investigation in clinical subjects for treating allergic disorders, including AR.

In conclusion, findings show that galangin has an antiallergic, antiasthmatic, and anti-inflammatory potential in AR mice via modulating the production of histamine, \(\beta\)-hexosaminidase, proinflammatory cytokines (IL-4, IL-6, IL-13, and IFN-\(\gamma\)), and immunoglobulins (IgE and IgG1). Galangin also alleviate OVA-induced AR in mice by modulating the PI3K/AKT/mTOR pathway and reducing mast cell activation. These findings imply that galangin might be an effective AR therapy. Meanwhile, these findings provide an underlying mechanism supporting the therapeutic usage of galangin to treat AR.

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**References**


**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org.