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Original Article

Diosmetin, Methylated Flavonoid Mitigates Ovalbumin Induced Allergic Rhinitis in Mice by Attenuating Inflammatory Signaling Proteins

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ABSTRACT

Background: Allergic Rhinitis (AR) is a chronic disorder that affects about 15-20% of the global population. In severe cases, AR causes sleep deprivation and impairs work performance and quality of life. Untreated allergic rhinitis may also lead to asthma. Intranasal corticosteroids and oral antihistamines are the most commonly prescribed drugs for allergic rhinitis. The long term intake of these drugs causes various side effects; hence, an alternative drug is required to treat allergic rhinitis. Flavonoids are ubiquitous phytochemicals present in most plants and have been proven to have immense pharmacological properties. **Objectives:** In this study we assessed the ameliorating effect of one such flavonoid diosmetin against AR in mice. **Materials and Methods:** BalB/c mice were induced AR with Ovalbumin (OVA) and treated with 10, 20 mg/kg of diosmetin. Nasal symptoms in mice were assessed and then subjected to nasal lavage collection. The levels of OVA specific IgE antibody and histamine were quantified in the experimental animals. Allergic markers Prostaglandin-D2 (PGD2), Leukotriene C4 (LTC4) and Eosinophil Cationic Protein (ECP) were measured in the Nasal Lavage Fluid (NALF) of the animals. Pro-inflammatory cytokines interleukin and TNF-α were quantified in both the NALF and nasal tissue sample of the experimental animals. Eosinophilic counts in the nasal tissue were done to confirm the rhinitis induction. To assess the antioxidant property of diosmetin the levels of oxidative and antioxidant levels were quantified in the nasal mucosal tissue. **Results:** Diosmetin treatment significantly inhibited the rhinitis nasal symptoms it decreased the levels of OVA specific IgE antibody and histamine in AR induced mice. It significantly attenuated the synthesis of allergic markers thereby inhibited the allergic induction which was evidenced with eosinophilic count results. Diosmetin treatment significantly mitigated the synthesis of pro-inflammatory cytokines which observed in both NALF and nasal mucosa tissue. All together our result proves diosmetin scavenged free radicals and rendered anti-inflammatory thereby prevented mice from OVA induced allergic rhinitis. **Conclusion:** Diosmetin may be a potent drug to treat allergic rhinitis since it targets multiple signaling pathways to ameliorate allergic rhinitis. Further studies may confirm the usage of diosmetin as reliable drug for allergic rhinitis.

Keywords: Allergic reaction, Ovalbumin, Inflammatory cytokines, Diosmetin, Leukotriene C4.

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INTRODUCTION

Allergic Rhinitis (AR) is an inflammatory atopic caused due to the exposure of various allergens. This disease is basically classified into seasonal disease occurs due to the allergens exposed outside and perennial which occurs due to the indoor allergens.[1] 40% of AR occurs due to the perennial exposure of indoor allergens and 20% occurs due to seasonal variations. 40% patients are tended to be affected with both perennial allergens and seasonal variation.[2]

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Worldwide about 400-500 million population were affected with AR.[3,4] The risk factors of AR includes atopic family history, cigarette smoke exposure during early childhood, males are more prone to AR than females, increased levels of allergen specific IgE secretion in children below the age 6, early introduction of solid foods are also reported to cause AR.[5,6] Only 15% of AR condition were diagnosed by where the prevalence in about 30%.[7] The increased incidence rate of seasonal allergic rhinitis was detected children at the teenage.[8] Seasonal rhinitis were more common in children and adults are prone to chronic rhinitis.[9]

The common symptoms observed in AR patients are nasal rubbing, nasal congestion, rhinorrhea, postnasal drip, itching, lacrimation, and nasal puritis. AR patients were also presented with conjunctivitis, sinusitis, Eustachian tube dysfunction and non-productive cough.[2] Even though the mortality related to allergic rhinitis is minimal the quality of life in allergic rhinitis patients was severely affected. In 2018 review, about 3.6% adults had lost their job and 36% of adults lacked work performance due to the allergic rhinitis impact.[10] Thus AR indirectly affect financial progress both the individual and the country.[11]

Avoidance of allergens which triggers rhinitis is the first recommended treatment for the allergic rhinitis patients.[6] Intranasal corticosteroids, antihistamines, leukotriene receptor antagonists and immunotherapy are the pharmacological treatment prescribed to alleviate the symptoms of allergic rhinitis.[12] Nasal antihistamine sprays causes side effects such as pyrexia, sneezing, vomiting, drowsiness, oropharyngeal pain, epistaxis, upper respiratory tract infection, nasal burning, cough etc.[13-15] Nevertheless oral and injectable steroids ameliorates allergic rhinitis the prolonged usage causes various side effects. Hence an alternative treatment is required for the allergic rhinitis patients to lead a quality life.

Flavonoids are the unique phytochemicals with a distinctive phenolic compound structure. These flavonoids are widely distributed in plants and they are utilized in the nutraceuticals.[16,17] possess It possess pharmacological properties such as anti-inflammatory, analgesic, anticancer, neuroprotective etc. Flavonoids are the drugs with multi target hence acts on multiple pathways thereby prevents side effects.[18,19] Diosmetin (4′-methylluteolin), is a methylated flavonoid which occur in various plants however abundantly present in the citrus plants, olive leaves[20,21] and spermine.[22] Diosmetin reported to possess ant-inflammatory, antioxidant,[23] antimicrobial,[24] oestrogenic,[25] osteoblastic,[26] neuroprotective[27] and drug-drug interaction properties.[28] In this study we assessed the pharmacological effect of methylated flavonoid diosmetin against the AR-induced mice.

MATERIALS AND METHODS

Chemicals

Diosmetin, OVA, and other chemicals were collected from Sigma Aldrich, USA. The kits for the determination of biochemical markers were purchased from BioCompare, MyBiosource, and Abcam, USA, respectively.

Animals

Healthy specific pathogen free 6-8 weeks aged BALB/c mice were quarantined in the lab for 10 days before initiation of experiment. The animals were housed in the laboratory maintained with 24±2ºC temperature, 55±5% relative humidity and 12 hr light dark cycle. The animals were fed with free access laboratory pellet diet. Strict hygienic condition on animal housing was maintained as per the guidelines of animal ethical committee. All the procedure carried out in the experiment were presented before the ethical committee and obtained proper approval for conducting the procedure in animals. The procedures on animal were performed with utmost care and concern.

Ovalbumin sensitized AR model

AR mice model was induced with OVA in adult BALB/c mice. 50 µg of OVA and 1 mg of $Al(OH)_3$ were intraperitoneally injected into the mice on the 1, 8 and 15th days of treatment period. The mice were further challenged with 20 µL of OVA (10 mg/mL) instilled into nasal cavity of mice from the treatment day 22-28. The morbidity and mortality in the mice were observed through the treatment period.

Experimental Design

The acclimatized mice grouped into five each group consists of six mice. Group I the control mice were treated with saline, Group II mice are allergic rhinitis induced which were treated with $OVA+AL(OH)_3$ for 2 week and further treated with ovalbumin from treatment 22-28. Group III and IV are Ovalbumin sensitized Diosmetin treated mice which were treated with 10 and 20 mg/kg diosmetin from the day 22-28. Group V are Ovalbumin sensitized treated with dexamethasone 2.5 mg/kg from the treatment day 22-28. Both diosmetin and dexamethasone were treated through oral route. On the treatment day 28 the mice were observed for nasal symptoms and then sacrificed. Blood, nasal lavage fluid and nasal tissue were collected for further analysis.

Assessment of nasal symptoms

On 28th day of treatment the mice were housed in the home cage for 3h and then placed on to the observation chamber for 10 min to observe the nasal symptom. The sneezing and nasal rubbing counts were recorded for all the mice. After each observation the observation chamber was sterilized with alcohol and interval of 5 min were taken between two observations to avoid false positive results.

Collection of blood sample

On 29th day of treatment retro orbital blood sample collection were done on the animals. Sterile hematocrit capillary tube was gently inserted into the retro-orbital sinus of mice. The blood collected in hematocrit was transferred to centrifuge tubes and the serum samples were separated for further analysis.

Nasal lavage fluid collection

The mice were anesthetized with 1% sodium pentobarbital intraperitoneal injection. The anesthetized mice were subjected to partial tracheotomy and a catheter (22 gauge) was gently inserted to the posterior naris through the trachea along the nostrils. Gently 2.5 mL of sterile saline solution was perfused into the nasal cavities and from the anterior naris the lavage fluid was collected. The fluid collected was centrifuged at 3500 rpm for 15 min at 4ºC and the supernatant was used for further analysis.

Quantification of Ova specific IgE antibody and Histamine

Ova specific IgE antibody was quantified in both the serum and NALF of animals using the ELISA kit was procured from LS Bio Shirley, MA. Histamine levels were quantified in the serum using the detection kit procured from BioCompare, USA. TMB substrate was added to HRP enzyme to develop color formation and finally the reaction was terminated using stop solution. The final absorbance was measured at 450 nm using ELISA microplate reader. The concentration of the unknown sample was detected by comparing the OD of unknown sample with OD of standard curve.

Quantification of allergic markers

Allergic markers Prostaglandin-D2 (PGD2), Leukotriene C4 (LTC4) and Eosinophil Cationic Protein (ECP) were quantified in the nasal lavage fluid of the experimental animals using the ELISA kit procured from MyBiosource, USA. The assay was performed according the standard protocol of the manufacturer. Avidin conjugated HRP bound wells were identified with TMB substrate solution and finally stop solution was to prevent color development. The intensity of color was measured at 450 nm and the concentration was calculated with standard plot curve.

Quantification of pro inflammatory cytokines in NALF

The pro inflammatory cytokines interleukin -4, -5, -6, -33 and TNF-α were estimated in the nasal lavage fluid and the nasal tissue of the allergic rhinitis induced untreated and diosmetin treated mice. The cytokines were quantified using the ELISA kits procured from Abcam, USA. The assay was done as per the guidelines provided in the kit. Standards were prepared according to the assay protocol and assay was performed without any pipetting errors. The assay was done in triplicates to avoid false positive or false negative results. The concentrations of cytokines in the unknown samples were calculated using the standard curve plotted with OD of known concentrations.

Histopathological Analysis

Infiltration of eosinophils in nasal mucosa was assessed by histopathological analysis of nasal tissue. After NALF collection the mice were decapitated and the whole head was fixed in 10% neutral formalin for 72 hr. The head was then subjected to decalcification with ethylenediamine triacetic acid for 7 days and embedded with paraffin wax. Paraffinized tissue was section coronally into 4 micron tissue sections and then stained with Giemsa stain. The stained tissue sections were randomly viewed under light microscope. Non-overlapping area per section was randomly chosen and the eosinophils counts were done using ImageJ software.

Assessment of Oxidative stress markers

MDA assay

Lipid peroxidation in the nasal tissues of the allergic rhinitis induced untreated and diosmetin treated mice were measured by quantifying the malondialdehyde levels using Malondialdehyde (MDA) Colorimetric Assay Kit procured from Elabscience, USA. The assay was performed according to the thiobarbituric acid method and the final absorbance was measured at 532 nm.

SOD assay

Superoxide dismutase levels in the nasal tissue were quantified in the nasal tissue of experimental animals according to the hydroxylamine method using the total superoxide dismutase

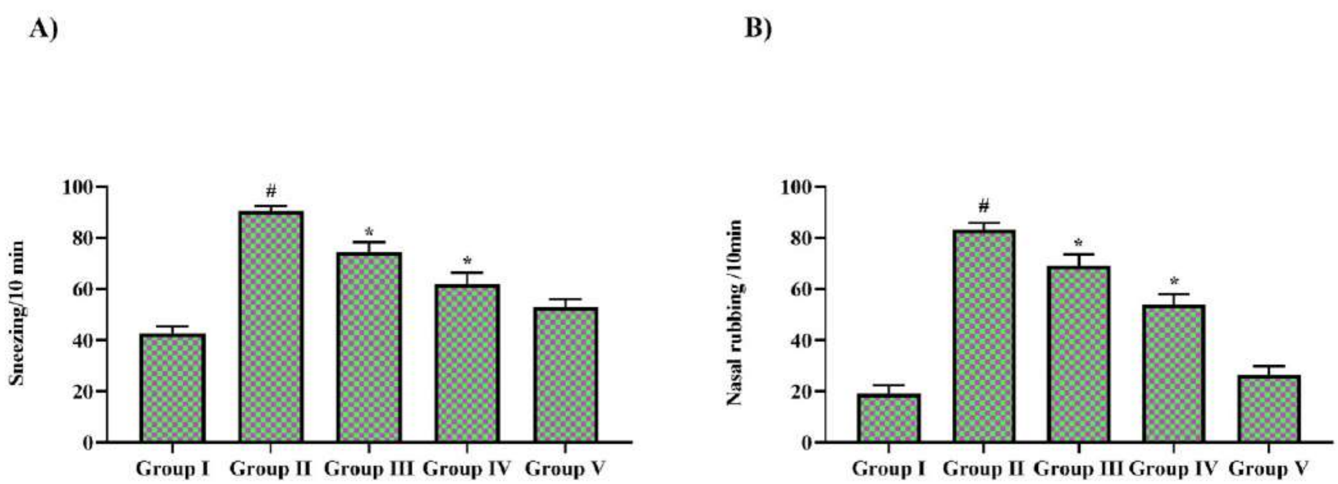

Figure 1: Flavonoid diosmetin prevented allergic nasal symptoms in AR induced mice A) Sneezing B) Nasal rubbing.

Each animal were observed for 10min. The scores were statistically analyzed with Kruskal-Wallis ANOVA for intergroup comparison followed by the *post hoc* test Whitney's multiple comparison tests for intragroup comparison. Significance was considered to be *,#p<0.5.

assay kit procured from Elabscience, USA. The final absorbance of the sample were read at 550nm using microplate reader.

ROS detection

Reactive oxygen species levels in the nasal tissues of the allergic rhinitis induced untreated and diosmetin treated mice were quantified using the ROS fluorometric assay kit procured from MyBiosource, USA. The oxidation of DCFH to DCF in the presence of ROS was estimated at the excitation 502 nm and emission wavelength of 525 nm. The ROS levels were calculated using the standard curve plot and the assay was performed in triplicates.

Statistical Analysis

All the experiments were repeated thrice and performed in triplicates. The means of the results with standard error mean were expressed as results. The data were analyzed with the statistical software GraphPad Prism version 5, USA. The data were assessed with ANOVA followed by LSD for biochemical analysis and the behavioral experiment scores were analyzed with Kruskal-Wallis ANOVA followed by Mann-Whitney's multiple comparison tests. Statistical significance was considered to be $p<0.5$ and 0.05.

RESULTS

Flavonoid diosmetin prevented allergic nasal symptoms in AR induced mice

The allergic nasal symptoms sneezing and nasal rubbing were observed for 10 min in diosmetin treated and untreated AR induced mice (Figure 1). Diosmetin significantly decreased the count of both sneezing and nasal rubbing compared to the untreated AR induced mice. Untreated AR induces mice shown 92±2 sneezing and 78±3 nasal rubbing counts. Whereas the counts were significantly decreased to 74±3 and 65±1 sneezing and 71±3 and 58±2 nasal rubbing counts respectively in 10 and 20 mg/kg diosmetin treated AR induced mice.

Flavonoid diosmetin treated inhibited Ova specific IgE antibody and Histamine secretions in AR induced mice

Diosmetin treatment significantly decreased the levels of Ova specific IgE antibody in both serum and the nasal lavage fluid of AR induced mice (Figure 2A). Significant increase in the levels of Ova specific IgE was observed in the untreated AR induced mice which shown 82±0.4 ng/mL in serum and 85±0.6 ng/mL in the NALF. Diosmetin treatment significantly reduced the levels to 64±0.6, 52±0.7 ng/mL in serum of 10 and 20 mg treatment respectively. In NALF of diosmetin treated AR induced the Ova specific IgE antibody levels were decreased to 74±0.7 and 68±0.5 ng/mL (10 and 20 mg treatment respectively).

Histamine levels were significantly increased in the AR induced untreated mice compared to control and the diosmetin treated AR induced mice. AR induced untreated mice shown 78±0.4 where it decreased to levels of 57±0.5, 49±0.6 μM in 10 and 20 mg treatment respectively. Control mice shown only 7±0.08 μM of histamine levels (Figure 2B).

Flavonoid diosmetin treatment impeded the allergic markers in AR induced mice

Allergic markers Prostaglandin-D2 (PGD2), and Leukotriene C4 (LTC4) Eosinophil Cationic Protein (ECP) were quantified in the NALF of experimental mice and results were presented in the Figure 3A-C. Control mice shown decreased level of allergic

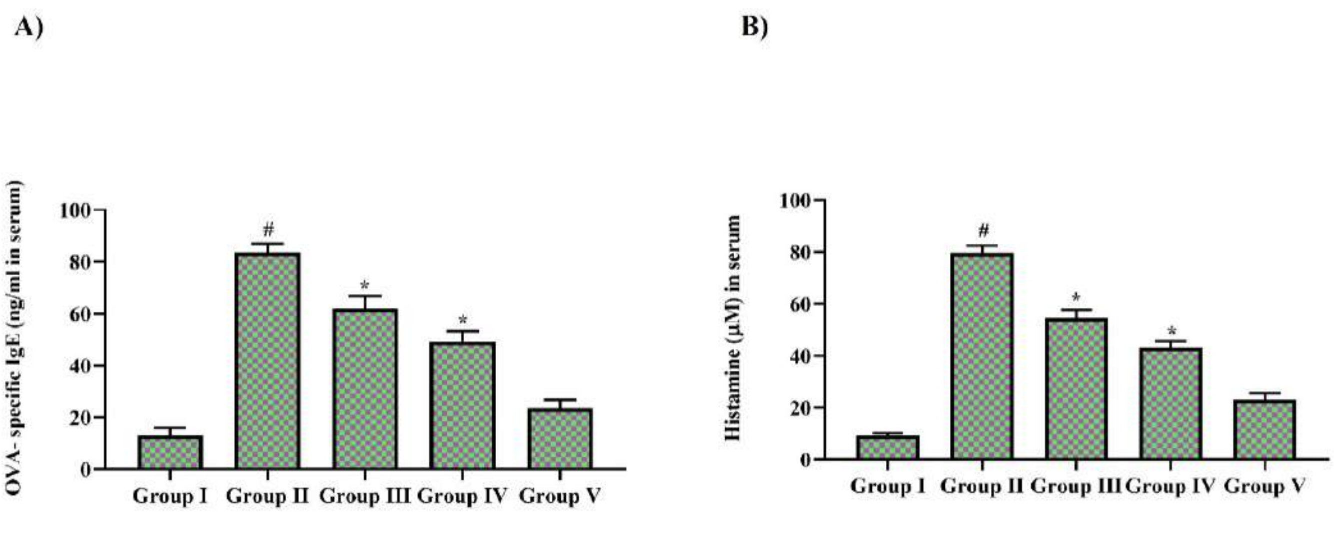

Figure 2: Flavonoid diosmetin treated inhibited Ova specific IgE antibody and Histamine secretions in AR induced mice.

A) Ova specific IgE antibody B) Histamine in the serum of experimental animals. The data were statistically analyzed with ANOVA for intergroup comparison followed by the *post hoc* test least significant difference for intragroup comparison. Significance was considered to be *,#$p<0.5$.

markers compared to all other experimental mice. The levels of PGD2 and LTC4 in control mice were 244±0.6 and 120±0.1 pg/mL respectively. Both doses of diosmetin significantly decreased the levels of PGD2 to 587±0.2 and 510±0.2 and LTC4 to 196±0.3 and 194±0.2 pg/mL respectively. Untreated AR induced mice shown increased levels of PGD2 634±0.2 and LTC4 282±0.2 pg/mL.

Diosmetin treatment significantly decreased the levels of Eosinophil Cationic Protein (ECP) in dose dependent manner. 10 mg/kg diosmetin treated mice shown ECP levels of 7.6±0.05 and it further reduced to 7.2±0.07 mg/mL in 20mg/kg diosmetin treated mice. AR induced untreated mice shown significantly increased levels of ECP 8.3±0.04 compared to all other experimental groups. Control and standard drug dexamethasone treated mice shown 2.8±0.02 and 4.3±0.02 level of ECP respectively.

Flavonoid diosmetin hindered the pro inflammatory cytokines in AR induced mice

The inhibitory effects of diosmetin against the allergen induced pro inflammatory cytokines were measured in both NALF, nasal mucosa tissue and the results were tabulated. The key inflammatory cytokines of allergic rhinitis inducers IL-4, IL-5, IL-6, IL-33 and TNF-α were estimated. Diosmetin treatment significantly decreased the pro inflammatory cytokines in dose dependent manner in both the serum and NALF. TNF-α levels were significantly increased in AR induced untreated mice compared to the other pro-inflammatory cytokines. Both the NALF and the nasal mucosa tissue samples of AR induced mice shown increased levels of TNF-α. Compared to IL-4, IL-6, IL-33 the levels of IL-5 were significantly increased in nasal tissue homogenate and decreased in NALF of AR induced mice (Figure 4A-F).

Flavonoid diosmetin obstructed the eosinophils infiltration in AR induced mice

The eosinophilic infiltration in nasal mucosal tissue of AR induced untreated and diosmetin treated mice were analyzed with stained nasal tissue section. The tissue sections were assessed the ImageJ software and the eosinophilic count in each experimental group were mentioned (Figure 5). AR induced mice shown significantly increased count of 33±0.9 eosinophils compared to control which shown only 6±0.1 eosinophils. Both diosmetin treatments decreased the levels of eosinophil count to 18±0.3 (10 mg/kg treatment) and 14±0.4 (20 mg/kg treatment). Control and dexamethasone treated mice shown eosinophil count of 4±0.09 and 9±0.08 respectively.

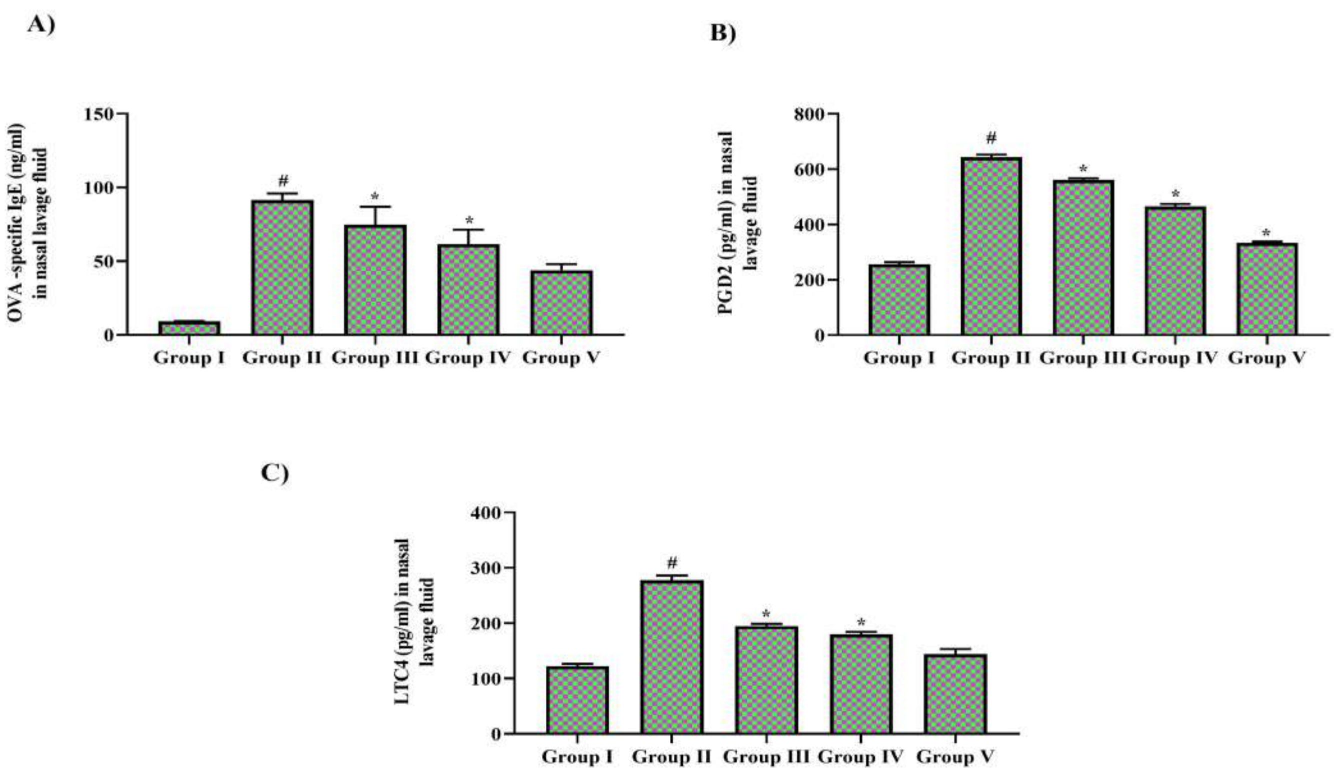

Figure 3: Flavonoid diosmetin treatment impeded the allergic markers in AR induced mice.

A) Ova specific IgE antibody B) Prostaglandin-D2 (PGD2), C) Leukotriene C4 (LTC4) in the NALF of experimental animals. The data were statistically analyzed with ANOVA for intergroup comparison followed by the *post hoc* test least significant difference for intragroup comparison. Significance was considered to be *,#*p*<0.5.

Flavonoid diosmetin scavenged ROS in AR induced mice

The antioxidant effect of diosmetin was assessed by quantifying the levels of ROS, MDA and antioxidant SOD levels in the nasal tissue of diosmetin treated AR induced mice. AR induction significantly increased the ROS production and lipid peroxidation to 12.3±0.03 and 14.6±0.08 nmol/mg protein respectively 10mg/kg diosmetin treated group shown 8.2±0.07 and 8.5±0.03 nmol/mg of ROS production and lipid peroxidation. Both the ROS and MDA levels were further reduced to 7.4±0.04 and 7.8±0.07 nmol/mg in 20 mg/kg diosmetin treated group. Control group shown decreased levels of 1.7±0.02 ROS production and 4.8±0.03 nmol/mg protein of MDA. Dexamethasone treatment also significantly decreased the to 4.7±0.02 ROS production and 5.9±0.03 nmol/mg protein of MDA compared to the AR induced untreated mice (Figure 6A-C).

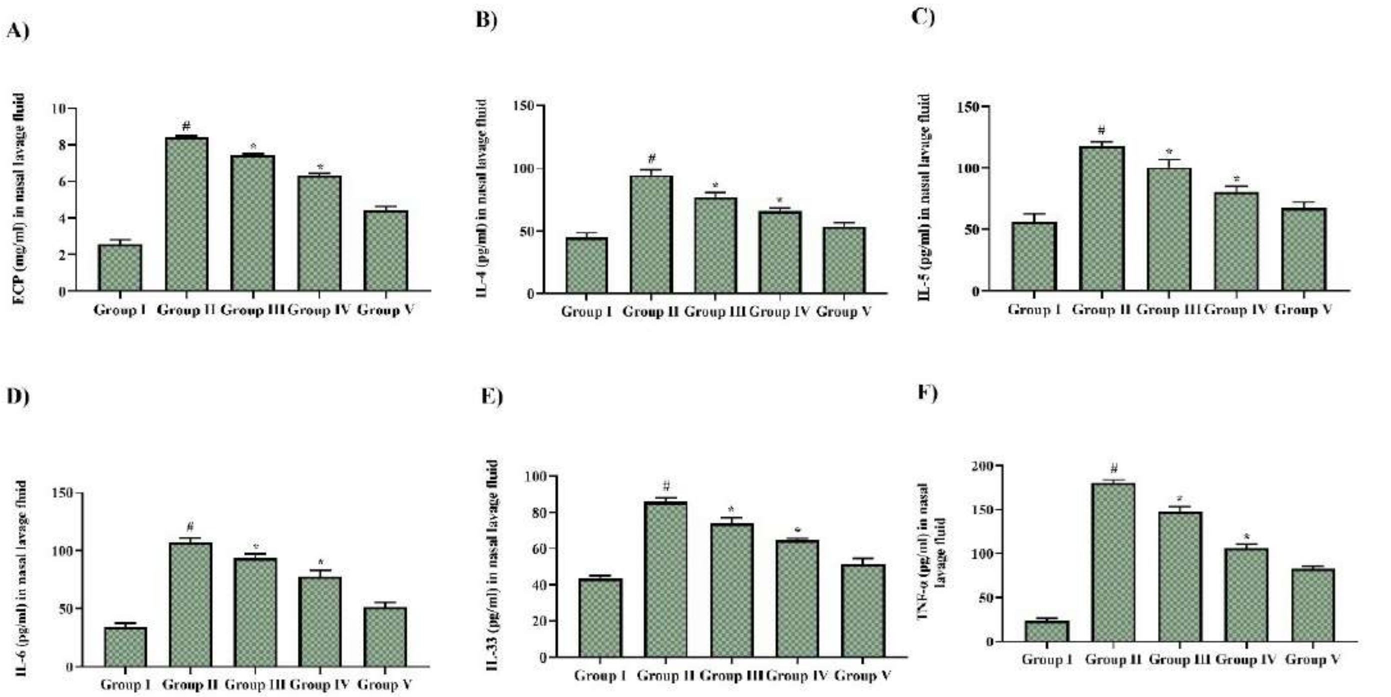

Figure 4: Flavonoid diosmetin hindered the pro inflammatory cytokines in AR induced mice.

A) Eosinophil cationic protein (ECP) B) Interleukin-4, C) Interleukin-5, D) Interleukin-6, E) Interleukin-33, F) Tumor Necrosis Factor-α in the NALF of experimental animals. The data were statistically analyzed with ANOVA for intergroup comparison followed by the *post hoc* test least significant difference for intragroup comparison. Significance was considered to be *,#p<0.05, 0.5.

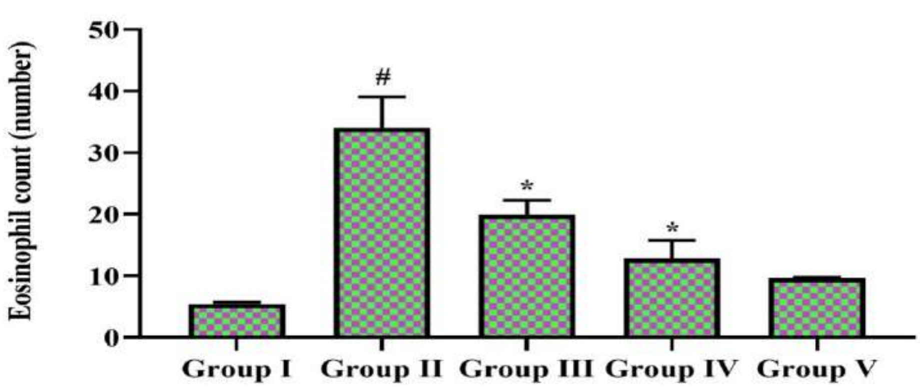

Figure 5: Flavonoid diosmetin obstructed the eosinophils infiltration in AR induced mice.

Nasal Mucosal tissue were subjected Giemsa staining and the sections were analysed with ImageJ software. The data were statistically analyzed with ANOVA for intergroup comparison followed by the *post hoc* test least significant difference for intragroup comparison. Significance was considered to be *,#p<0.5.

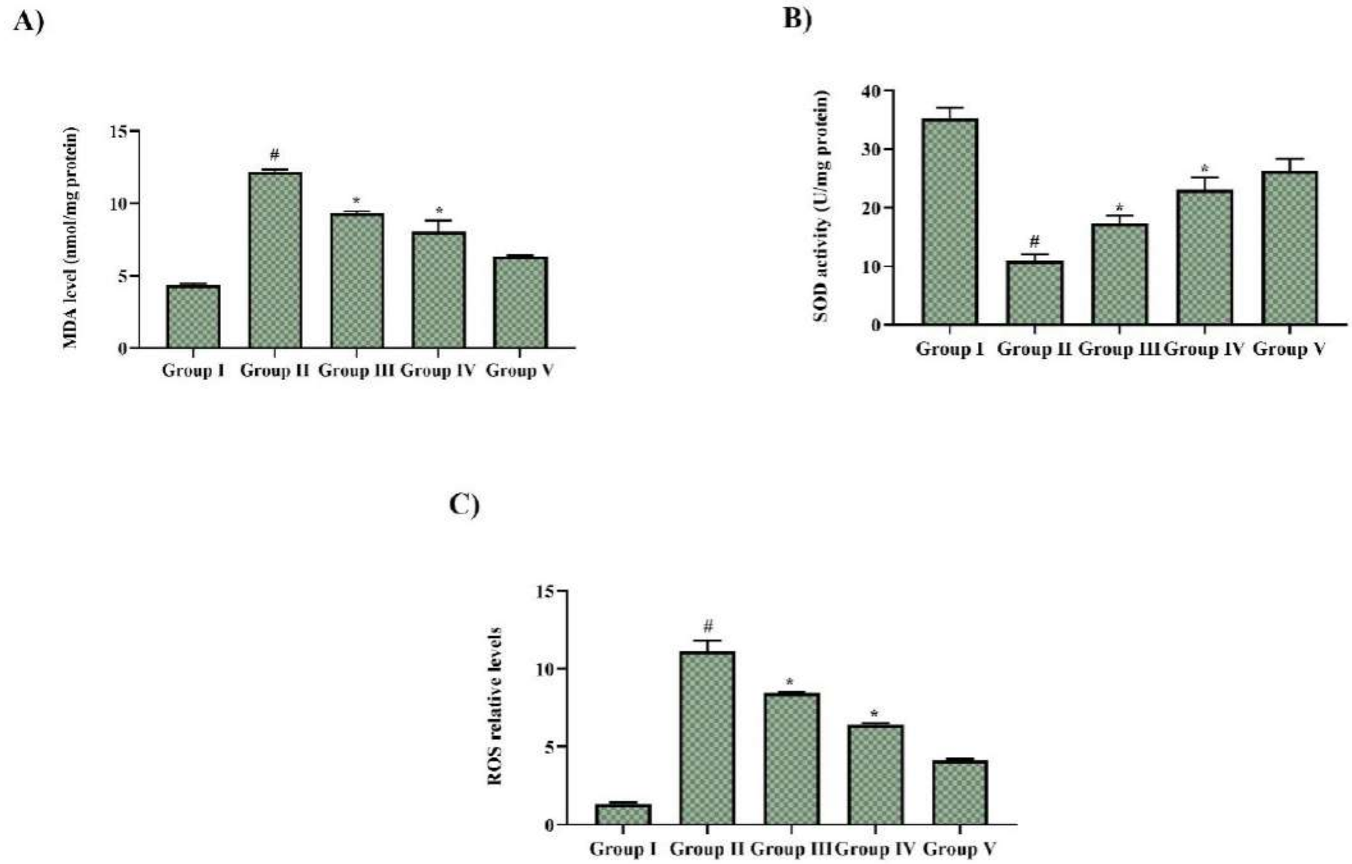

Figure 6: Flavonoid diosmetin scavenged ROS in AR induced mice.

A) Malondialdehyde, B) Superoxide dismutase, C) Reactive Oxygen Species levels in the nasal mucosal tissue of experimental animals. The data were statistically analyzed with ANOVA for intergroup comparison followed by the *post hoc* test least significant difference for intragroup comparison. Significance was considered to be *,#$p<0.05$, 0.5.

Antioxidant SOD levels were significantly increased in diosmetin and dexamethasone treated mice nasal tissue compared to AR induced untreated mice. AR induced untreated mice shown 12.2±0.07 U/mg protein whereas it is significantly increased to 15.3±0.03 and 22.7±0.04 U/mg protein in 10and20mg/kg diosmetin treated groups Dexamethasone treated AR induced mice shown 26.5±0.04 U/mg protein of SOD levels.

DISCUSSION

A prevalent modern epidemic disease which often underrated is allergic rhinitis. WHO had estimated globally about 500 million people was reported with rhinitis symptoms which also co exists with asthma. A dramatic increase in the rhinitis patients were reported in both developed and under developed countries the incidence rate has been doubled every decade from 1980 till now.[29] Even though AR is not a lethal disease the symptoms are more complicated which causes inconvenience in our day to day life.[30,31] AR can be prevented by avoiding contact of allergens to the nasal mucosal layer but with the present environmental condition and life style it is unavoidable to be away from allergens. At present drugs such antihistamines, leukotriene receptor antagonists; oral and injectable steroids were prescribed to treat AR. The necessity of long term usage of drugs and the side effects caused prevents the usage of these drugs.[6] A drug which effectively alleviates rhinitis without causing any side effects needs to be discovered. Hence in this study we assessed the potency of a phytochemical diosmetin to ameliorate the AR in mice.

Allergic rhinitis was induced in mice with the widely accepted allergen ovalbumin induced rhinitis model since the symptoms exhibited in this model mimic the same condition of humans.[32] The prime symptom of allergic rhinitis is sneezing, nasal congestion and nasal blockage hence after induction of ovalbumin induced AR in mice we assessed for the symptoms of sneezing and nasal rubbing. AR induced mice significantly shown higher count of sneezing and nasal rubbing compared to the control mice which confirmed the induction of allergic rhinitis in mice. Diosmetin treatment reduced the sneezing count and nasal rubbing in a dose dependent manner which shown the ameliorative effect of diosmetin against AR. Further to confirm we also analyzed the Ova specific IgE antibody and histamine levels in diosmetin treated and untreated AR induced mice. Ova specific IgE antibody and histamine levels were reported to be increased in the Ovalbumin induced AR mice.[4,33] In our study also the levels of Ova specific IgE antibody and the histamine levels were increased in the AR induced whereas it is decreased with diosmetin treatment.

Prostaglandin D2 are lipid mediator which specifically synthesized by the mast cells upon activation with allergens. It also produced by other immune cells such as macrophages, T helper 2 cells, dendritic cells and eosinophils during the allergic reaction.[34,35] This PGD2 prostaglandins acts as key mediator in both phases of allergic reaction and has reported in the broncho alveolar fluid of asthma induced mice model.[36] Increased levels of PGD2 were found in the asthmatic patients also.[37] Targeting PGD2 with a drug may effectively prevent induction of rhinitis therefore we assessed the efficacy of diosmetin on inhibiting the synthesis of PGD2. Diosmetin treatment significantly decreased the levels of PGD2 levels in the AR induced mice.

Prostaglandin released during allergic reaction triggers the eosinophils to synthesis cytokines including Leukotriene C4 (LTC4) causing inflammation.[38,39] Cysteine leukotrienes specifically the LTC4 were found to be elevated in nasal secretions of allergic rhinitis patients.[40] These proinflammatory lipid mediators causes constriction of bronchi. LTC4 were synthesized mainly by the eosinophils, macrophages and the mast cells during the allergen exposure.[41] In allergic asthma experimental model LTC4 was reported to mediate trafficking of eosinophils to the paratracheal lymph nodes from the lungs.[42] In our study the levels of LTC4 were significantly increased in the nasal lavage fluid and the increased eosinophilic count was observed in the nasal mucosal tissue of the AR induced untreated mice. The increase in leukotriene would have triggered the eosinophil trafficking which was evidenced in AR induced mice. Diosmetin treatment significantly inhibited the synthesis of lipid mediators PGD2 and LTC4 and prevented the eosinophil infiltration in the nasal mucosal tissue.

On exposure to allergens the activated eosinophils triggers inflammation by releasing inflammatory mediators which impairs the tissue.[43] Eosinophilic cationic protein is one such protein which is sensitive marker for the diagnosis of allergic rhinitis.[44,45] ECP and LTC4 were detected to be elevated in the nasal fluid in allergic patients.[46,47] Treatment with drugs such as mentelukast decreased the levels of ECP in the adults and pediatric allergic patients.[48,49] This correlates with our study ovalbumin induction increased the levels of ECP and LTC4 whereas diosmetin treatment significantly decreased the levels of ECP and LTC4 in AR induced mice.

AR causes inflammation in the nasal passage due to the imbalance in the TH1/TH2 cells. Allergen exposure activates TH2 cells which in turn triggers the synthesis of pro-inflammatory cytokines IL-4, IL-5 and IL-13.[50] These interleukins plays a key role in induction of pathogenesis in allergic rhinitis patients.[51] IL-4 is the initiator cytokine which induces the secretion of IgE antibody and thereby upregulates the MHC class II molecules in monocytes, mast cells, basophils.[52,53] IL-4 provokes the synthesis IL-5, IL-13 cytokines which triggers hyperresponsiveness of airways and hypersecretion of mucus.[54] Pleotropic cytokine secreted by the lung epithelium cells IL-6 was considered to be a classical marker for inflammation. IL-6 along with TNF-α regulates the cytokines and modulates the immune response.[55] Elevated levels of IL-6 was related to cause of allergic diseases and it increases the nasal secretion in the allergic rhinitis patients.[56,57] Since these cytokines plays the key role in regulating the allergic phase reactions we assessed the potency of our drug diosmetin in inhibiting these cytokine synthesis. Diosmetin treatment significantly decreased the levels of interleukin 4, IL-5, IL-6, IL-13 and TNF-α in allergic rhinitis induced a mice which confirms anti-inflammatory effect of diosmetin in ameliorating allergic rhinitis.

Alternative therapy with antioxidants had drawn attention in treating allergic rhinitis since oxidative stress plays a key role in the pathophysiology of allergic rhinitis and asthma.[58] Therefore in this study we analyzed the antioxidant potency of diosmetin against AR induced mice. Diosmetin treatment significantly increased the levels of antioxidant SOD and scavenged the reactive oxygen species thereby prevented the nasal mucosal tissue from lipid peroxidation.

CONCLUSION

Allergic rhinitis, a common inflammatory disorder that affects the quality of life of the population, is often undertreated, leading to asthma, a chronic lung disease. A potent drug that not only subsides the symptoms but also ameliorates the disease is needed today. We examined the potency of the methylated flavonoid diosmetin's ameliorative effect against allergic rhinitis in mice. Diosmetin effectively subsided the allergic rhinitis symptoms in mice by attenuating the secretion of Ova-specific IgE antibodies and histamines. Diosmetin inhibited the synthesis of the allergic markers PGD2, LTC-4, ECP, and pro-inflammatory cytokines in the AR-induced mice. It effectively prevented eosinophil trafficking in nasal mucosal tissue and scavenged free radicals, thereby preventing inflammation induction in diosmetin-treated allergic rhinitis-induced mice. Our results confirm that diosmetin is a potent antioxidant and anti-inflammatory agent that may be a potent alternative therapeutic candidate to treat allergic rhinitis. Furthermore, additional studies are still needed in the future to clearly comprehend the therapeutic role of diasmetin against AR.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AR: Allergic rhinitis; **PGD2:** Prostaglandin-D2; **LTC4:** Leukotriene C4; **ECP:** Eosinophil cationic protein; **MDA:** Malondialdehyde; **SOD:** Superoxide dismutase; **ROS:** Reactive oxygen species

SUMMARY

Allergic rhinitis is an inflammatory atopic caused due to the exposure of various allergens. AR causes inflammation in the nasal passage due to the imbalance in the TH1/TH2 cells. Diosmetin inhibited the synthesis of allergic markers PGD2, LTC-4, ECP and the pro-inflammatory cytokines in the AR induced mice.

REFERENCES

1. Wise SK, Damask C, Greenhawt M, Oppenheimer J, Roland LT, Shaker MS, *et al*. A synopsis of guidance for allergic rhinitis diagnosis and management from ICAR 2023. J Allergy Clin Immunol Pract. 2023; 11(3): 773-96. doi: 10.1016/j.jaip.2023.01.007, PMID 36894277.
2. Akhouri S, House SA. Allergic rhinitis. StatPearls [Internet]. Updated 2023 May 20: 2023 Jan.
3. Fröhlich M, Pinart M, Keller T, Reich A, Cabieses B, Hohmann C, *et al*. Is there a sex-shift in prevalence of allergic rhinitis and comorbid asthma from childhood to adulthood? A meta-analysis. Clin Transl Allergy. 2017; 7: 44. doi: 10.1186/s13601-017-0176-5, PMID 29225773.
4. Xu J, Zhang Q, Li Z, Gao Y, Pang Z, Wu Y, *et al*. *Astragalus* polysaccharides attenuate ovalbumin-induced allergic rhinitis in rats by inhibiting NLRP3 inflammasome activation and NOD2-mediated NF-κB activation. J Med Food. 2021; 24(1): 1-9. doi: 10.1089/jmf.2020.4750, PMID 33370169.
5. Liva GA, Karatzanis AD, Prokopakis EP. Review of rhinitis: classification, types, pathophysiology. J Clin Med. 2021; 10(14): 3183. doi: 10.3390/jcm10143183, PMID 34300349.
6. Trincianti C, Tosca MA, Ciprandi G. Updates in the diagnosis and practical management of allergic rhinitis. Expert Rev Clin Pharmacol. 2023; 16(7): 669-76. doi: 10.1080/17512433.2023.2225770, PMID 37314373.
7. Mortada MM, Kurowski M. Challenges in local allergic rhinitis diagnosis, management, and research: current concepts and future perspectives. Medicina (Kaunas). 2023; 59(5): 929. doi: 10.3390/medicina59050929, PMID 37241161.
8. Tenero L, Vaia R, Ferrante G, Maule M, Venditto L, Piacentini G, *et al*. Diagnosis and management of allergic rhinitis in asthmatic children. J Asthma Allergy. 2023; 16: 45-57. doi: 10.2147/JAA.S281439, PMID 36636703.
9. Varshney J, Varshney H. Allergic rhinitis: an overview. Indian J Otolaryngol Head Neck Surg. 2015; 67(2): 143-9. doi: 10.1007/s12070-015-0828-5, PMID 26075169.
10. Vandenplas O, Vinnikov D, Blanc PD, Agache I, Bachert C, Bewick M, *et al*. Impact of rhinitis on work productivity: A systematic review. J Allergy Clin Immunol Pract. 2018; 6(4): 1274-86.e9. doi: 10.1016/j.jaip.2017.09.002, PMID 29017832.
11. Pawankar R, Mori S, Ozu C, Kimura S. Overview on the pathomechanisms of allergic rhinitis. Asia Pac Allergy. 2011; 1(3): 157-67. doi: 10.5415/apallergy.2011.1.3.157, PMID 22053313.
12. Yáñez A, Rodrigo GJ. Intranasal corticosteroids versus topical H1 receptor antagonists for the treatment of allergic rhinitis: a systematic review with meta-analysis. Ann Allergy Asthma Immunol. 2002; 89(5): 479-84. doi: 10.1016/S1081-1206(10)62085-6 , PMID 12452206.
13. Bernstein JA. Azelastine hydrochloride: a review of pharmacology, pharmacokinetics, clinical efficacy and tolerability. Curr Med Res Opin. 2007; 23(10): 2441-52. doi: 10.1185/030079907X226302, PMID 17723160.
14. van Bavel J, Howland WC, Amar NJ, Wheeler W, Sacks H. Efficacy and safety of azelastine 0.15% nasal spray administered once daily in subjects with seasonal allergic rhinitis. Allergy Asthma Proc. 2009; 30(5): 512-8. doi: 10.2500/aap.2009.30.3284, PMID 19747429.
15. Howland WC, Amar NJ, Wheeler W, Sacks H. Efficacy and safety of azelastine 0.15% nasal spray administered once daily in patients with allergy to Texas mountain cedar pollen. Int Forum Allergy Rhinol. 2011; 1(4): 275-9. doi: 10.1002/alr.20065, PMID 22287431.
16. Perez-Vizcaino F, Fraga CG. Research trends in flavonoids and health. Arch Biochem Biophys. 2018; 646: 107-12. doi: 10.1016/j.abb.2018.03.022, PMID 29580946.
17. Wen K, Fang X, Yang J, Yao Y, Nandakumar KS, Salem ML, *et al*. Recent research on flavonoids and their biomedical applications. Curr Med Chem. 2021; 28(5): 1042-66. doi: 10.2174/0929867327666200713184138, PMID 32660393.
18. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016; 5:e47. doi: 10.1017/jns.2016.41, PMID 28620474.
19. Ferraz CR, Carvalho TT, Manchope MF, Artero NA, Rasquel-Oliveira FS, Fattori V, *et al*. Therapeutic potential of flavonoids in pain and inflammation: mechanisms of action, pre-clinical and clinical data, and pharmaceutical development. Molecules. 2020; 25(3): 762. doi: 10.3390/molecules25030762, PMID 32050623.
20. Amanpour A, Kelebek H, Selli S. LC-DAD-ESI-MS/MS-based phenolic profiling and antioxidant activity in Turkish cv. Nizip Yaglik olive oils from different maturity olives. J Mass Spectrom. 2019; 54(3): 227-38. doi: 10.1002/jms.4326, PMID 30593706.
21. Contreras MDM, Lama-Muñoz A, Espínola F, Moya M, Romero I, Castro E. Valorization of olive mill leaves through ultrasound-assisted extraction. Food Chem. 2020; 314: 126218. doi: 10.1016/j.foodchem.2020.126218, PMID 31982857.
22. Patel K, Gadewar M, Tahilyani V, Patel DK. A review on pharmacological and analytical aspects of diosmetin: a concise report. Chin J Integr Med. 2013; 19(10): 792-800. doi: 10.1007/s11655-013-1595-3, PMID 24092244.
23. Yang Y, Gong XB, Huang LG, Wang ZX, Wan RZ, Zhang P, *et al*. Diosmetin exerts anti-oxidative, antiinflammatory and anti-apoptotic effects to protect against endotoxininduced acute hepatic failure in mice. Oncotarget. 2017; 8(19): 30723-33. doi: 10.18632/oncotarget.15413, PMID 28430612.
24. Meng JC, Zhu QX, Tan RX. New antimicrobial mono-and sesquiterpenes from Soroseris hookeriana subsp. erysimoides. Planta Med. 2000; 66(6): 541-4. doi: 10.1055/s-2000-8607, PMID 10985081.
25. Yoshikawa M, Uemura T, Shimoda H, Kishi A, Kawahara Y, Matsuda H. Medicinal foodstuffs. XVIII. Phytoestrogens from the aerial part of *Petroselinum crispum* Mill. (parsley) and structures of 6"-acetylapiin and a new monoterpene glycoside, petroside"-acetylapiin and a new monoterpene glycoside, petroside. Chem Pharm Bull (Tokyo). 2000; 48(7): 1039-44. doi: 10.1248/cpb.48.1039, PMID 10923837.
26. Hsu YL, Kuo PL. Diosmetin induces human osteoblastic differentiation through the protein kinase C/p38 and extracellular signalregulated kinase 1/2 pathway. J Bone Miner Res. 2008; 23(6): 949-60. doi: 10.1359/jbmr.080219, PMID 18269307.
27. Bhatt PR, Benzeroual KE. Neuroprotective effects of fisetin and diosmetin, alone and in combination, on lipopolysaccharide-induced neuronal cells. FASEB J. 2013; 27(S1):1175-8. doi: 10.1096/fasebj.27.1_supplement.1175.8.
28. Bajraktari G, Weiss J. The aglycone diosmetin has the higher perpetrator drug-drug interaction potential compared to the parent flavone diosmin. J Funct Foods. 2020; 67. doi: 10.1016/j.jff.2020.103842: 103842. Ballard CR, Junior MR. Health benefits of flavonoids. In: Segura MR, editor. Bioactive compounds. Cambridge, UK: Woodhead Publishing; 2019. p. 185-201.
29. Zvezdin B, Hromis S, Kolarov V, Milutinov S, Zarić B, Jovancević L, *et al*. Allergic asthma and rhinitis comorbidity. Vojnosanit Pregl. 2015; 72(11): 1024-31. doi: 10.2298/vsp140605099z, PMID 26731978.
30. Lakhani N, North M, Ellis AK. Clinical manifestations of allergic rhinitis. J Allergy Ther. 2012;S5: 007.
31. Rosati MG, Peters AT. Relationships among allergic rhinitis, asthma, and chronic rhinosinusitis. Am J Rhinol Allergy. 2016; 30(1): 44-7. doi: 10.2500/ajra.2016.30.4252, PMID 26867529.
32. Bui TT, Piao CH, Song CH, Chai OH. Skullcapflavone II attenuates ovalbumin-induced allergic rhinitis through the blocking of Th2 cytokine production and mast cell histamine release. Int Immunopharmacol. 2017; 52: 77-84. doi: 10.1016/j.intimp.2017.08.029, PMID 28886581.
33. Shao YY, Zhou YM, Hu M, Li JZ, Chen CJ, Wang YJ, *et al*. The anti-allergic rhinitis effect of traditional Chinese medicine of Shenqi by regulating mast cell degranulation and Th1/Th2 cytokine balance. Molecules. 2017 Mar 22; 22(3): 504. doi: 10.3390/molecules22030504, PMID 28327534.
34. Pettipher R, Hansel TT. Antagonists of the prostaglandin D2 receptor CRTH2. Drug News Perspect. 2008; 21(6): 317-22. doi: 10.1358/dnp.2008.21.6.1246831, PMID 18836589.
35. Schuligoi R, Sturm E, Luschnig P, Konya V, Philipose S, Sedej M, *et al.* CRTH2 and D-type prostanoid receptor antagonists as novel therapeutic agents for inflammatory diseases. Pharmacology. 2010; 85(6): 372-82. doi: 10.1159/000313836, PMID 20559016.
36. Herrerias A, Torres R, Serra M, Marco A, Pujols L, Picado C, *et al.* Activity of the cyclooxygenase 2-prostaglandin E prostanoid receptor pathway in mice exposed to house dust mite aeroallergens, and impact of exogenous prostaglandin E2. J Inflamm. 2009; 6(1): 30. doi: 10.1186/1476-9255-6-30.
37. Balzar S, Fajt ML, Comhair SA, Erzurum SC, Bleecker E, Busse WW, *et al*. Mast cell phenotype, location, and activation in severe asthma. Data from the severe asthma research program. Am J Respir Crit Care Med. 2011; 183(3): 299-309. doi: 10.1164/rccm.201002-0295OC, PMID 20813890.
38. Fujitani Y, Kanaoka Y, Aritake K, Uodome N, Okazaki-Hatake K, Urade Y. Pronounced eosinophilic lung inflammation and Th2 cytokine release in human lipocalin-type prostaglandin D synthase transgenic mice. J Immunol. 2002; 168(1): 443-9. doi: 10.4049/jimmunol.168.1.443, PMID 11751991.
39. Mesquita-Santos FP, Vieira-de-Abreu A, Calheiros AS, Figueiredo IH, Castro-Faria-Neto HC, Weller PF, *et al*. Cutting edge: prostaglandin D2 enhances leukotriene C4 synthesis by eosinophils during allergic inflammation: synergistic *in vivo* role of

endogenous eotaxin. J Immunol. 2006; 176(3): 1326-30. doi: 10.4049/jimmunol.176.3.1326, PMID 16424158.

40. Figueroa DJ, Borish L, Baramki D, Philip G, Austin CP, Evans JF. Expression of cysteinyl leukotriene synthetic and signaling proteins in inflammatory cells in active seasonal allergic rhinitis. Clin Exp Allergy. 2003; 33(10): 1380-8. doi: 10.1046/j.1365-2222.2003.01786.x, PMID 14519144.

41. Thompson-Souza GA, Gropillo I, Neves JS. Cysteinyl leukotrienes in eosinophil biology: functional roles and therapeutic perspectives in eosinophilic disorders. Front Med (*Lausanne*). 2017; 4: 106. doi: 10.3389/fmed.2017.00106, PMID 28770202.

42. Wang HB, Akuthota P, Kanaoka Y, Weller PF. Airway eosinophil migration into lymph nodes in mice depends on leukotriene C4. Allergy. 2017; 72(6): 927-36. doi: 10.1111/all.13094, PMID 27874209.

43. Peters-Golden M, Gleason MM, Togias A. Cysteinyl leukotrienes: multi-functional mediators in allergic rhinitis. Clin Exp Allergy. 2006; 36(6): 689-703. doi: 10.1111/j.1365-2222.2006.02498.x, PMID 16776669.

44. Urrutia-Pereira M, Mocelin LP, Ellwood P, Garcia-Marcos L, Simon L, Rinelli P, *et al*. Prevalence of rhinitis and associated factors in adolescents and adults: a Global Asthma Network study. Rev Paul Pediatr. 2023; 41:e2021400. doi: 10.1590/1984-0462/2023/41/2021400, PMID 36888752.

45. Stevens WW, Peters AT, Tan BK, Klingler AI, Poposki JA, Hulse KE, *et al.* Associations between inflammatory endotypes and clinical presentations in chronic rhinosinusitis. J Allergy Clin Immunol Pract. 2019; 7(8): 2812-2820.e3. doi: 10.1016/j.jaip.2019.05.009, PMID 31128376.

46. Staudacher AG, Peters AT, Kato A, Stevens WW. Use of endotypes, phenotypes, and inflammatory markers to guide treatment decisions in chronic rhinosinusitis. Ann Allergy Asthma Immunol. 2020; 124(4): 318-25. doi: 10.1016/j.anai.2020.01.013, PMID 32007571.

47. Perić A, Mirković CŠ, Đurđević BV, Perić AV, Vojvodić D. Eosinophil chemokines and Clara cell protein 16 production in nasal mucosa of patients with persistent allergic rhinitis. Eurasian J Med. 2017; 49(3): 178-82. doi: 10.5152/eurasianjmed.2017.17203, PMID 29123440.

48. Hayashi H, Fukutomi Y, Mitsui C, Kajiwara K, Watai K, Kamide Y, *et al.* Omalizumab for aspirin hypersensitivity and leukotriene overproduction in aspirin-exacerbated respiratory disease. A Randomized Controlled Trial. Am J Respir Crit Care Med. 2020; 201(12): 1488-98. doi: 10.1164/rccm.201906-1215OC, PMID 32142372.

49. Tutluoglu B, Tosun GA, Akbas I, Yaman M. Effects of montelukast on serum ECP and bronchial hyperreactivity in mild asthmatics. Paper presented at the World Congress on Lung Health and 10th Eur Respir Soc Annual Congress, Florence, Italy, 2000 [CD-ROM] Accompanied European Respiratory Journal 17. 2000; 3 [abstr].

50. Zhao C, Yu S, Li J, Xu W, Ge R. Changes in IL-4 and IL-13 expression in allergic-rhinitis treated with hydrogen-rich saline in guinea-pig model. *Allergol Immunopathol* (Madr). 2017; 45(4): 350-5. doi: 10.1016/j.aller.2016.10.007, PMID 28215576.

51. Nur HSM, Tan HT, Shukri Md. N, Mohd Ashari NS, Wong KK. Allergic rhinitis: A clinical and pathophysiological overview. Front Med (*Lausanne*). 2022; 9: 874114.

52. Yamanishi Y, Miyake K, Iki M, Tsutsui H, Karasuyama H. Recent advances in understanding basophil-mediated Th2 immune responses. Immunol Rev. 2017; 278(1): 237-45. doi: 10.1111/imr.12548, PMID 28658549.

53. Yip W, Hughes MR, Li Y, Cait A, Hirst M, Mohn WW, *et al.* Butyrate shapes immune cell fate and function in allergic asthma. Front Immunol. 2021; 12: 628453. doi: 10.3389/fimmu.2021.628453, PMID 33659009.

54. Sahoo A, Wali S, Nurieva R. T helper 2 and T follicular helper cells: regulation and function of interleukin-4. Cytokine Growth Factor Rev. 2016; 30: 29-37. doi: 10.1016/j.cytogfr.2016.03.011, PMID 27072069.

55. Kamimura D, Ishihara K, Hirano T. IL-6 signal transduction and its physiological roles: the signal orchestration model. Rev Physiol Biochem Pharmacol. 2003; 149: 1-38. doi: 10.1007/s10254-003-0012-2, PMID 12687404.

56. Gentile DA, Yokitis J, Angelini BL, Doyle WJ, Skoner DP. Effect of intranasal challenge with interleukin-6 on upper airway symptomatology and physiology in allergic and nonallergic patients. Ann Allergy Asthma Immunol. 2001; 86(5): 531-6. doi: 10.1016/S1081-1206(10)62901-8, PMID 11379804.

57. Zhang Z, Xiao C, Gibson AM, Bass SA, Khurana Hershey GK. EGFR signaling blunts allergen-induced IL-6 production and Th17 responses in the skin and attenuates development and relapse of atopic dermatitis. J Immunol. 2014; 192(3): 859-66. doi: 10.4049/jimmunol.1301062, PMID 24337738.

58. Han M, Lee D, Lee SH, Kim TH. Oxidative stress and antioxidant pathway in allergic rhinitis. Antioxidants (Basel). 2021; 10(8): 1266. doi: 10.3390/antiox10081266, PMID 34439514.

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