Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Kirenol Ameliorated Ovalbumin-Induced Allergic Rhinitis in Mice via Suppression of Oxidative Stress and Inflammatory Response

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Submitted: 26-Nov-2020 Revised: 02-Mar-2021 Accepted: 22-Apr-2021 Published: 24-Jan-2022

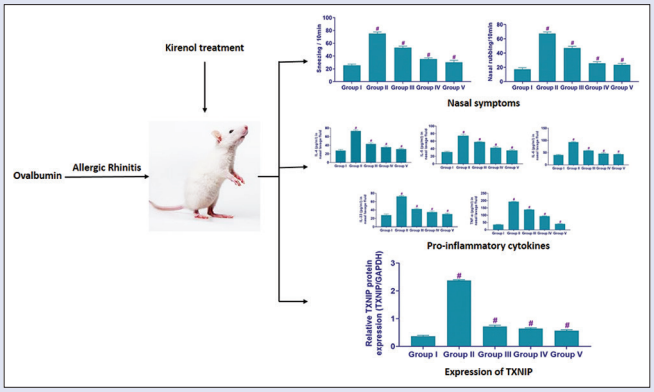
ABSTRACT

Background: Allergic rhinitis (AR) is a type-1 inflammatory ailment characterized by the recurrent nasal rubbings, sneezing, and other unpleasant symptoms. AR is an imperative airway inflammatory ailment that affects nearly 20%–30% of the global population. Kirenol is a bioactive diterpenoid with numerous beneficial properties. **Objectives:** In this study, we aimed to assess the therapeutic role of kirenol against the ovalbumin (OVA)-induced AR in mice via suppression of oxidative and inflammatory responses. **Materials and Methods:** AR was induced in BALB/c mice via administration of OVA. Subsequently, the animals were administered with 20 and 30 mg/kg of kirenol. Dexamethasone was utilized as the standard drug. The frequencies of nasal rubbings and sneezing of the mice were recorded. The status of histamine, OVA-specific immunoglobulin E (IgE), prostaglandin-D2 (PGD2), and leukotriene C4 (LTC4) was examined by using commercial assay kits. The levels of eosinophil cationic protein (ECP), interleukin (IL)-4, IL-5, IL-6, IL-33, and tumor necrosis factor (TNF)-α were investigated through the marker-specific assay kits. The mononuclear cells were isolated from the spleen of the animals of untreated AR group and cultured in an RPMI-1640 medium. The mRNA expression of a thioredoxin-interacting protein (TXNIP) in the spleen cells was studied by real-time polymerase chain reaction technique. The status of oxidative stress and inflammatory cytokines was examined by using assay kits. **Results:** Kirenol (20 and 30 mg/kg)-administered AR mice exhibited reduced frequency of nasal rubbing and sneezing. Kirenol effectively reduced the status of OVA-specific IgE and histamine in AR mice. The status of PGD2, LTC4, IL-4, IL-5, IL-6, IL-33, TNF-α, ECP status, and eosinophil count was diminished by the kirenol. The level of malondialdehyde and reactive oxygen species was reduced and the activity of superoxide dismutase markedly was improved by the kirenol. The kirenol-supplemented spleen-derived mononuclear cells showed downregulation of mRNA expression of TXNIP. **Conclusion:** These results demonstrate the curative role of kirenol against the OVA-mediated AR in mice, which can be a promising agent to treat AR in humans in future.

Key words: Allergic rhinitis, immunoglobulin E, inflammation, kirenol, ovalbumin

SUMMARY

- Allergic rhinitis is an important risk factor for allergic asthma which decreases the quality of life of a patient with allergic rhinitis
- The malondialdehyde and reactive oxygen species levels were reduced and the activity of superoxide dismutase was markedly increased by the kirenol
- Kirenol-supplemented cells downregulated the mRNA expression of a thioredoxin-interacting protein.



Abbreviations used: AR: Allergic rhinitis; OVA: Ovalbumin; DEX-dexamethasone; TXNIP: Thioredoxin-interacting protein; SOD: Superoxide dismutase; NLF: Nasal lavage fluid; IgE: Immunoglobulin-E; Camp: Cyclic adenosine monophosphate; DCFH-DA: 2, 7-diacetyl dichlorofluorescein; IL: Interleukin; PGD2: Prostaglandin-D2; LTC4: Leukotriene C4; ECP: Eosinophil cationic protein; MDA: Malondialdehyde; ROS: Intracellular reactive oxygen species.

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DOI: 10.4103/pm.pm_513_20

Access this article online
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INTRODUCTION

Allergic rhinitis (AR) is an immunoglobulin E (IgE)-regulated, upper airway hypersensitivity reaction to various allergens such as dust mites, molds, pollen, and animal dander.[1] Recently, there has been an increase in the cases of AR each year, which shows that AR is one of the biggest public health threats. It leads to chronic inflammatory upper respiratory disorders, which affects nearly 20%–30% of the global population.[2] Most importantly, AR is an imperative risk factor for allergic asthma, which affects most of the people and decreases their quality of life.[3] AR is characterized by increased serum levels of IgE, inflammation in

Cite this article as: Liu T, Gao H, Gao Y, Guo D, Si W, Du H. Kirenol ameliorated ovalbumin-induced allergic rhinitis in mice via suppression of oxidative stress and inflammatory response. Phcog Mag 2021;17:893-9.

the nasal mucosa, and elevated counts of eosinophils. The symptoms of AR include nasal rubbing, rhinorrhea, frequent sneezing, lacrimation and nasal congestion, and irritation.[4] Progression of AR often involves complex pathological mechanisms that is distinguished by IgE-mediated inflammation and the participation of Th2 cells, eosinophils, and mast cells.[5] There are two stages involved in the progression of AR: initial and late stages. The initial stage involves the binding of allergen and IgE receptors on the mast cell surface, which leads to the release of histamine and increased vascular permeability.[6] The late stage involves in the accumulation of inflammatory cells such as macrophages, eosinophils, lymphocytes, and neutrophils. The penetration of leukocytes into the inflammatory site results in the reduced airway width and secretion of mucus that leads to the demonstration of bronchial and nasal signs and symptoms.[7,8]

Chronic exposure to various allergens stimulates allergen-specific Th2 cells that causes increased secretion of Th2 cytokines (e.g., interleukin [IL]-4 and IgE) which, in turn, stimulates the hyper-responsiveness in the nasal mucosa.[9] The binding of IgE to mast cells after allergic stimuli stimulates the secretion of various regulators (e.g., leukotrienes and histamine), which was accountable for arteriolar dilation, itching, and increased permeability of the vasculum of lungs. During an allergic response, the inflammatory cells such as mononuclear cells, basophils, mast cells, and eosinophils arrive at the nasal mucosa and liberate a variety of regulators such as leukotrienes, histamine, IL-4, IL-6, and tumor necrosis factor (TNF)-α, thereby stimulating the inflammatory processes. Despite extensive therapeutic options available to treat AR, there are no successful recoveries and nearly 20% of the patients with AR do not respond well to the current treatment approaches.[10]

The oxidative stress serves a crucial function in the pathological progression of inflammation in the airway which was extensively studied.[11] During oxidative stress, eosinophils liberate the cytotoxic regulators such as eosinophilic peroxidase and lead to severe injuries to the airway epithelium. The intracellular antioxidants such as superoxide dismutase (SOD) play an important role in the protection of the cells against oxidative stress via eliminating superoxide radicals.[12] Even though AR is not a fatal disorder, it often affects the quality of life of patients with AR. In addition, there are no surgical procedures performed to treat AR. Patients with AR suffer the most of both physical uneasiness and psychological stress which decrease their quality of life.[13] The current treatment option available for AR is based on the existing information about different regulators implicated in the stimulation of allergic reactions. Clinically, the therapeutic approaches for AR consist of administration of mast cell stabilizers, antibiotics, glucocorticosteroids, antihistamines, cyclic adenosine monophosphate inducer, leukotriene receptor antagonist, Th2 cytokine inhibitor, and anti-inflammatory agents,[14,15] although these aforementioned approaches remain unsatisfied and have restricted use as they cause economic burden and various adverse effects such as blurring of vision, nasal dryness, headache, throat irritation, tachycardia, urinary retention, and sedation. Hence, there is an urgent need to expand the understanding of AR development and progression to find a promising treatment agent for AR.[16]

So far, there are various animal models which help in the exploration and development of novel therapeutic approaches for AR. The ovalbumin (OVA)-induced AR animal model is one of them and is most suitable for the study of AR.[17] It is an easy, extensively utilized, noninvasive investigational model for the study of IgE-regulated allergic respiratory disorders. This model possesses close similarities with the pathological signs and symptoms of AR such as nasal rubbing, sneezing, mucosal edema, congestion, and increased levels of IgE.[18,19] Kirenol, a bioactive diterpenoid, is derived from *Herba siegesbeckiae*, and it possesses numerous beneficial properties that are utilized in the treatment of various ailments. Some of the previous studies have shown that kirenol possessed potent antiarthritic activity,[20,21] cardioprotective activity;[22] antiphotoaging activity,[23] anti-inflammatory and diabetic wound healing activity,[24] and anticancer activity.[25] However, to the best of our knowledge, the curative potential of kirenol against OVA-induced AR has not been studied yet. Hence, in this study, we planned to assess the therapeutic role of kirenol against the OVA-induced AR in mice.

MATERIALS AND METHODS

Chemicals

Kirenol, OVA, 2,7-diacetyl dichlorofluorescein (DCFH-DA), and other chemicals were procured from Sigma Aldrich, USA. The OVA-specific IgE and histamine assay kits were purchased from Biocompare, CA, USA. The prostaglandin-D2 (PGD2) and leukotriene C4 (LTC4) kits were purchased from Biovision, Milpitas, USA. The assay kits for inflammatory markers were obtained from MyBioSource, San Diego, USA. The TRIzol reagent and real-time polymerase chain reaction (RT-PCR) kits were procured from Thermo Fisher, USA. Malondialdehyde (MDA) and SOD assay kits were acquired from Elabscience, USA.

Animals

BALB/c mice weighing more than 18–20 g were procured from the institutional animal house, Yanan University, Yanan, China. All animals were kept in specific disease-free cages and the temperature was maintained at 22°C–24°C. The experimental procedures on the mice were utilized by strictly following the protocols suggested by the Institutional Animal Ethical Committee (YAU-0722). Animals were acclimatized for 1 week and were provided with a pellet diet.

Induction of allergic rhinitis and treatment procedures

The AR was induced in mice via administration of OVA as described previously by Ikeda *et al.*[26] Animals were divided into five groups: control, AR control, AR + 20 mg kirenol, AR + 30 mg kirenol, and AR + 2.5 mg dexamethasone (DEX). The control animals were intraperitoneally (i. p.) administered with phosphate-buffered saline (PBS) and then intranasally (i. n.) administered with PBS. The AR control animals were sensitized via i. p. administration of 0.2 mL suspension of 0.5 mg/mL OVA and 20 mg/mL aluminum hydroxide on days 0, 7, and 14. Then, the animals were administered (i. n.) with 100 μg of OVA daily from days 21–28. In the treatment groups, animals were stimulated with AR as stated above and then administered (i. n.) with 20 and 30 mg/kg of kirenol, respectively, on days 21, 23, 25, and 27. On day 28, the mice were sacrificed and the samples were collected for additional assays.

Collection of nasal lavage fluid

On day 28, mice were subjected to partial tracheotomy under anesthesia via administration (i. p.) of 40 mg/kg of sodium pentobarbital. The 24-gauge catheter was utilized to insert it into the posterior nasopharynx from the trachea. Then, 3 mL of sterile PBS was utilized to perfuse nasal cavities. The perfusion was executed twice, and the nasal lavage fluid (NLF) was collected and centrifuged at 2000 rpm for 10 min. The supernatant was collected for further analysis.

Frequency of nasal rubbing and sneezing

The total incidences of nasal rubbings and sneezing of the mice from all the groups were assessed after 24 h of the last OVA induction. The events

of nasal rubbing and sneezing incidences of animals during the 15 min interval were monitored in a blind manner and recorded.

Measurement of interleukin and inflammatory markers

The level of OVA-specific IgE and histamines in the serum of both control and experimental mice were measured using enzyme-linked immunosorbent assay kits based on the guidelines of manufacturer (Biocompare, CA, USA). The level of IgE, PGD2, and LTC4 was examined in the NLF with the aid of kits according to the manufacturer instructions (Biovision, Milpitas, USA). The levels of eosinophil cationic protein (ECP), IL-4, IL-5, IL-6, IL-33, and TNF-α in the NLF were investigated through the marker-specific assay kits (MyBioSource, San Diego, USA). Every sample was analyzed in triplicate.

Measurement of eosinophil count in the nasal mucosa

The nasal mucosal tissues of every mouse were collected after sacrificing the animals under anesthesia. The samples were embedded in paraffin and sectioned (5 μm size). Then, the sections were deparaffinized and stained using hematoxylin and eosin for counting the eosinophils in nasal tissue samples. The total number of eosinophils was counted under the optical microscope, and the results were tabulated.

Culture of spleen mononuclear cells and stimulation

The spleen mononuclear cells were isolated from animals of AR group. Then, 4×10^6 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). The cells were then sensitized, briefly, the control cells were incubated with PBS, and the cells in treatment group were incubated with 20 and 30 μM of kirenol along with OVA (10 μg/mL) challenge for 24 h. In this study, 10 μM of DEX was utilized as the standard control. Subsequently, the cell supernatants were collected and utilized for further experiments.

Real-time polymerase chain reaction

Total RNA was extracted from the spleen mononuclear cells with the aid of TRIzol reagent and by the following manufacturer's instructions (Thermo Fisher, USA). The extracted RNA was utilized for the construction of cDNA by the commercial kit. The RT-PCR was performed to identify the mRNA expression of thioredoxin-interacting protein (TXNIP). The primer sequences for TXINP sense 5′-GCCACACTTACCTTGCCAAT-3′ and antisense 5′-TTGGATCCAGGAACGCTAAC-3′ were utilized (Macrogen, Seoul, Republic of Korea). β-*Actin* was utilized as the reference for normalization, and the results were presented as fold induction.

Measurement of malondialdehyde and superoxide dismutase activity

The amount of MDA formed and SOD activity in the spleen mononuclear cell supernatant was examined with the help of commercial MDA and SOD assay kits and by following the manufacturer's guidelines (Elabscience, USA).

Intracellular level of reactive oxygen species

The level of reactive oxygen species (ROS) formed in the cells was identified by DCFH-DA fluorescence staining technique. After 24 h OVA challenge and kirenol treatment, the cells were suspended in PBS and 1 μL of DCFH-DA dye (1 mg/mL) was added to the cells and incubated at 37°C for 30 min in the dark. Then, ROS generation was examined.

Measurement of inflammatory markers

Followed by the 24 h OVA challenge and kirenol treatment, the cell supernatants were collected and the status of pro-inflammatory regulators (IL-4, IL-5, IL-6, IL-33, and TNF-α) was quantified with the help of commercial marker-specific assay kits and by following manufacturer's protocols (MyBioSource, San Diego, USA).

Statistical analysis

The data were presented as mean ± standard deviation of triplicate measurements. The obtained results were statistically analyzed via using GraphPad Prism software. One-way analysis of variance was calculated, and Tukey's *post hoc* assessment was performed to analyze the differences between groups. $P < 0.05$ was regarded as significant.

RESULTS

Kirenol decreased nasal symptoms in allergic rhinitis mice

The incidences of nasal rubbing and sneezing increased in the AR mice when compared to normal mice. Interestingly, AR mice treated with kirenol (20 and 30 mg/kg) demonstrated low ($P < 0.05$) incidence of both nasal rubbing and sneezing. According to our results, 30 mg/kg of kirenol was more effective in reducing sneezing and nasal rubbing than that of 20 mg/kg. The standard drug DEX also reduced the frequency of rubbing and sneezing in the AR mice ($P < 0.05$) [Figure 1]. There was no significant variation between 30 mg/kg and 2.5 mg kirenol in DEX-treated mice.

Kirenol decreased the ovalbumin-specific immunoglobulin E and histamine status in the serum of allergic rhinitis mice

The untreated OVA-induced AR mice illustrated the drastic increase in the level of OVA-specific IgE and histamine when compared to normal mice [Figure 2]. Kirenol (20 and 30 mg/kg) notably reduced the levels of IgE and histamine in the AR mice ($P < 0.05$). This shows that the kirenol attenuated ($P < 0.05$) the OVA-induced augmentation in the levels of IgE and histamine in the AR mice [Figure 2]. The standard drug DEX also reduced the IgE and histamine in AR mice.

Kirenol reduced the levels of immunoglobulin E, prostaglandin-D2, and leukotriene C4 in the nasal lavage fluid of allergic rhinitis mice

As depicted in Figure 3, the level of IgE, PGD2, and LTC4 was increased severely in the NLF of untreated OVA-induced AR mice when compared to the control mice. Surprisingly, the administration of 20 and 30 mg/kg of kirenol to the AR mice drastically reduced the elevated levels of IgE, PGD2, and LTC4 in the NLF ($P < 0.05$). In particular, 30 mg/kg kirenol effectively reduced the levels IgE, PGD2, and LTC4 in the AR mice when compared to 20 mg/kg kirenol [Figure 3]. DEX treatment also demonstrated the remarkable diminution in the IgE, PGD2, and LTC4 in the AR mice.

Kirenol decreased the levels of pro-inflammatory cytokines in the nasal lavage fluid of allergic rhinitis mice

The status of pro-inflammatory cytokines, i.e., IL-4, IL-5, IL-6, IL-33, TNF-α, and ECP was increased in the NLF of the OVA-induced AR mice,

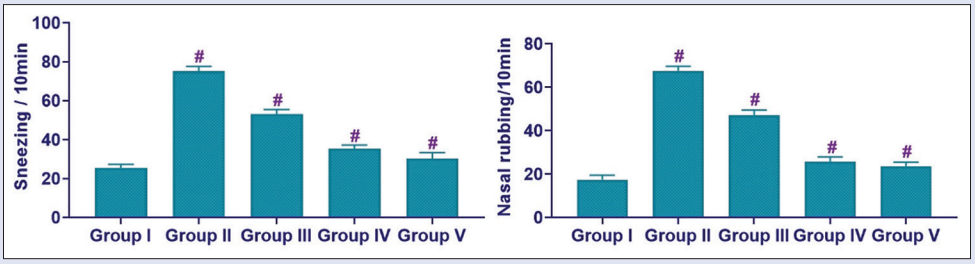

Figure 1: Effect of kirenol on the nasal symptoms in allergic rhinitis mice. The kirenol (20 and 30mg/kg) supplemented allergic rhinitis mice illustrated the reduced incidences of both nasal rubbing and sneezing. Data were portrayed as mean ± standard deviation of triplicate measurements. The significance was determined via analysis of variance successively Tukey's *post hoc* study; note: $^{\#}P < 0.05$ compared with control and $^{\#}P < 0.05$ compared with ovalbumin-group

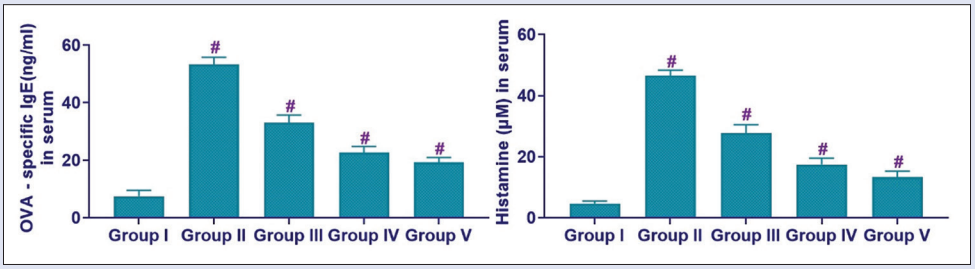

Figure 2: Effect of kirenol on the ovalbumin-specific immunoglobulin-E and histamine status in the serum of allergic rhinitis mice. The 20 and 30mg/kg of kirenol treatment demonstrated the marked reduction in the ovalbumin-specific immunoglobulin-E and histamine status in the allergic rhinitis mice. Data were portrayed as mean ± standard deviation of triplicate measurements. The significance was determined via analysis of variance successively Tukey's *post hoc* study; note: #$P < 0.05$ compared with control and $^{\#}P < 0.05$ compared with ovalbumin-group

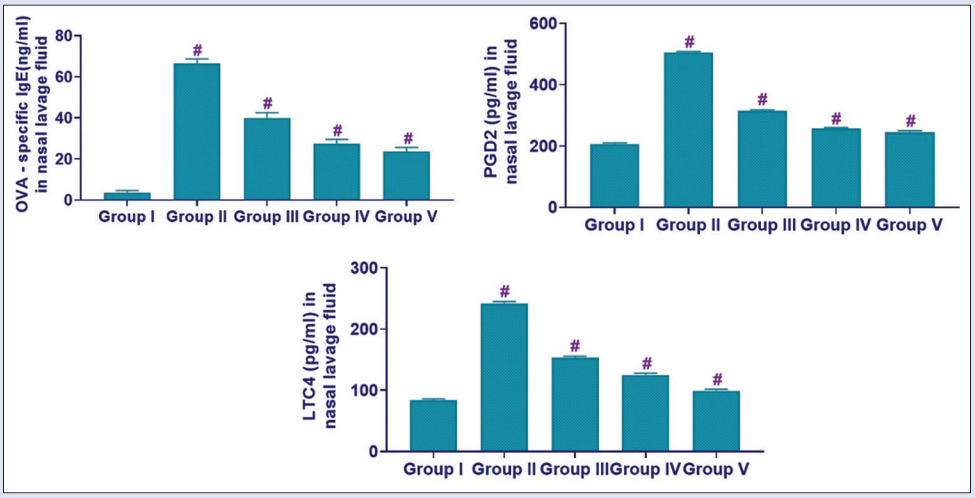

Figure 3: Effect of kirenol on the ovalbumin-specific immunoglobulin-E, prostaglandin-D2 and leukotriene C4 status in the nasal lavage fluid of allergic rhinitis mice. As depicted in the Figure 3, the status of ovalbumin-specific immunoglobulin-E, prostaglandin-D2 and leukotriene C4 was markedly lessened through the kirenol supplementation. Data were portrayed as mean ± standard deviation of triplicate measurements. The significance was determined via analysis of variance successively Tukey's *post hoc* study; note: $^{\#}P < 0.05$ compared with control and $^{\#}P < 0.05$ compared with ovalbumin-group

which indicates severe inflammatory condition. Surprisingly, kirenol (20 and 30 mg/kg) significantly reduced the level of pro-inflammatory cytokines in the NLF of the AR mice ($P < 0.05$) [Figure 4]. This shows the anti-inflammatory property of kirenol against the OVA-induced AR in mice. Kirenol at 30 mg/kg dosage showed efficacy similar to the standard DEX-treated AR mice.

Kirenol retained the eosinophil count in the nasal tissues of allergic rhinitis mice

The untreated OVA-induced AR in mice demonstrated an increased total count of eosinophils in the nasal tissue samples, which is in contrast to the control mice. Kirenol (20 and 30 mg/kg) significantly decreased the total eosinophil

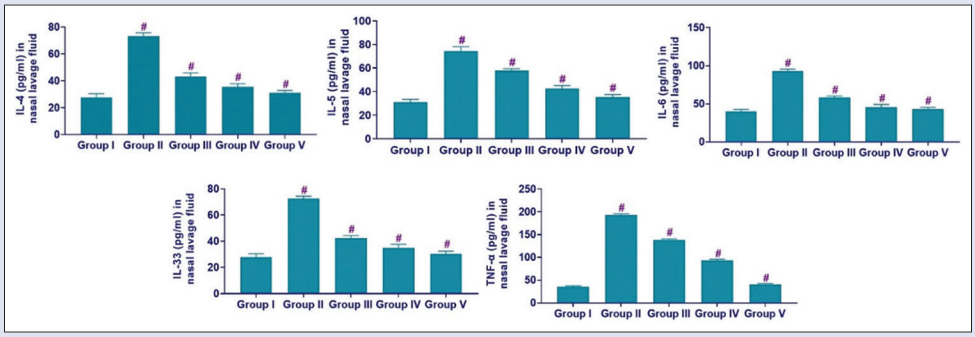

Figure 4: Effect of kirenol on the pro-inflammatory cytokines level in the nasal lavage fluid of allergic rhinitis mice. The supplementation of 20 and 30mg/kg of kirenol was noticeably repressed the status of the pro-inflammatory regulators i.e., interleukin-4, interleukin-5, interleukin-6, interleukin-33, tumor necrosis factor-α and eosinophil cationic protein in the nasal lavage fluid of the allergic rhinitis mice. Data was portrayed as mean ± standard deviation of triplicate measurements. The significance was determined via analysis of variance successively Tukey's *post hoc* study; note: $^{\#}P < 0.05$ compared with control and $^{\#}P < 0.05$ compared with ovalbumin-group

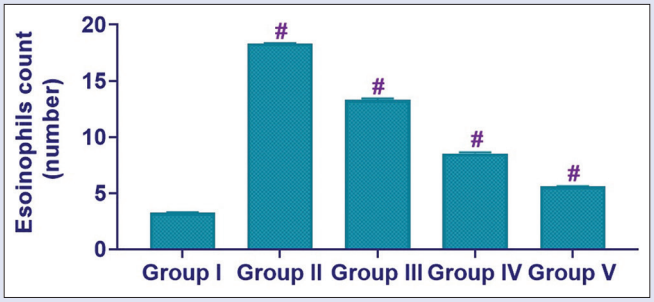

Figure 5: Effect of kirenol on the eosinophil count in the nasal tissues of allergic rhinitis mice. The 20 and 30mg/kg of kirenol supplemented allergic rhinitis mice demonstrated the noticeably reduced amount of eosinophils in the nasal tissues of allergic rhinitis mice. Data was portrayed as mean ± standard deviation of triplicate measurements. The significance was determined via analysis of variance successively Tukey's *post hoc* study; note: $^{\#}P < 0.05$ compared with control and $^{\#}P < 0.05$ compared with ovalbumin-group

count in the nasal tissue samples when compared to the AR mice ($P < 0.05$). The effect of 30 mg/kg of kirenol was similar to that of DEX [Figure 5].

Kirenol reduced the oxidative stress and inflammatory markers in the ovalbumin-induced spleen mononuclear cells

The level of MDA, ROS, IL-4, IL-5, IL-6, IL-33, and TNF-α was elevated and the activity of SOD was reduced in the OVA-induced spleen mononuclear cells [Figure 6]. This result shows that the OVA increases oxidative and inflammatory responses. Surprisingly, kirenol (20 and 30 μM)-induced spleen mononuclear cells showed a marked reduction in the levels of MDA and ROS and also suppressed the levels of IL-4, IL-5, IL-6, IL-33, and TNF-α ($P < 0.05$). The activity of SOD was markedly increased after kirenol supplementation. Kirenol and DEX demonstrated similar kind of outcomes [Figure 6].

Kirenol downregulated the expression of thioredoxin-interacting protein in the ovalbumin-induced spleen mononuclear cells

The effect of kirenol on the TXNIP expression in OVA-induced spleen mononuclear cells was studied via RT-PCR technique [Figure 7]. The mRNA expression of TXNIP protein was upregulated in the OVA-induced spleen mononuclear cells. Interestingly, kirenol (20 and 30 μM)-supplemented cells significantly downregulated the mRNA expression of TXNIP ($P < 0.05$). The results obtained for 30 μM of kirenol and DEX were found to be similar [Figure 7].

DISCUSSION

AR is a type-1 inflammatory reaction, and its pathological progression accompanies with the penetration of inflammatory cells and the liberation of inflammatory mediators. The signs and symptoms of AR are recurrent nasal rubbings, sneezing, and other unpleasant symptoms.[27,28] It has been established that the upper airway epithelial cells play an important role in the immune response of an individual to the external allergic stimuli and the mitochondrial ROS regulates the allergic inflammation in the upper airway via mediation of different inflammatory proteins. Currently, the therapeutic drugs for AR mainly focus on the specific symptoms.[29] At present, immune modulator therapies serve novel ways to treat the AR and attain few achievements, but still, some major problems exist.[30] Moreover, anti-IgE antibodies have also demonstrated potency against AR in clinical studies with few drawbacks such as high cost and intravenous therapy.[31]

OVA has been extensively utilized to provoke/induce AR in experimental animal models.[32] In this study, we established the AR model by injecting (i. p.) OVA to the mice and the AR was confirmed via increased levels of OVA-specific IgE; histamine; pro-inflammatory regulators such as IL-4, IL-5, IL-6, IL-33, and TNF-α and increased frequency of nasal rubbing and sneezing. The pro-inflammatory cytokines recruit inflammatory cells in the airway to further increase the processes of AR development and inhibit the AR ameliorating chemokine secretion. In the OVA-induced AR in mice, OVA acts as an antigen, and it can bind to the antigen-presenting cells and trigger the activation of B cells to secrete the OVA-specific IgE, which, in turn, induces the mast cells to secrete inflammatory mediators and to speed up the AR development.[33]

During the early stages of AR development, the augmented OVA-specific IgE status in the serum and elevated pro-inflammatory regulators such as prostaglandins, histamine, cytokines, and leukotrienes stimulate the nasal area which results in nasal itching, rubbing, sneezing, and discomfort.[28] Hence, decreasing the level of OVA-specific IgE in the serum could potentially ameliorate the AR. During the late stage, the activated mast cells and eosinophils in a nasal epithelium further secrete the pro-inflammatory mediators such as TNF-α and ILs, LTC-4, chemokines, and cyclooxygenase-2 which maintain the allergic

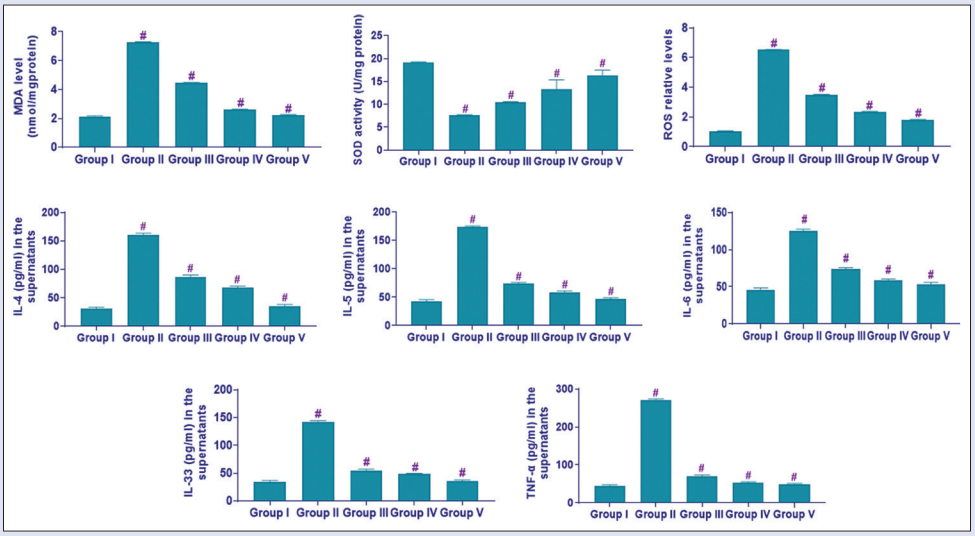

Figure 6: Effect of kirenol on the oxidative stress and inflammatory markers in the ovalbumin-challenged spleen mononuclear cells. The 20 and 30μM of kirenol supplemented spleen mononuclear cells possessed the marked reduction in the malondialdehyde and intracellular reactive oxygen species and improved the superoxide dismutase activity and also suppressed the inflammatory markers i.e., interleukin-4, interleukin-5, interleukin-6, interleukin-33 and tumor necrosis factor-α. Data were portrayed as mean ± standard deviation of triplicate measurements. The significance was determined via analysis of variance successively Tukey's *post hoc* study; note: $^{\#}P < 0.05$ compared with control and $^{\#}P < 0.05$ compared with ovalbumin-group

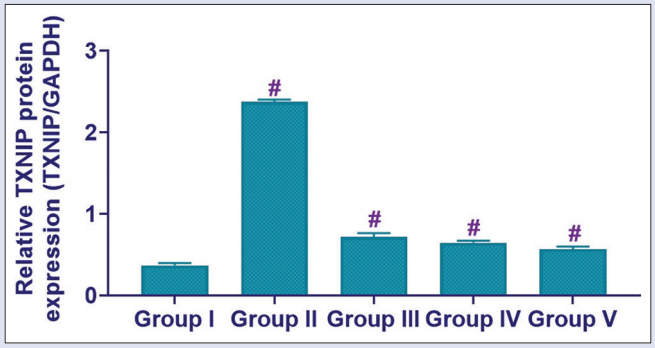

Figure 7: Effect of kirenol on the expression of thioredoxin-interacting protein in the ovalbumin-challenged spleen mononuclear cells. The 20 and 30μM of kirenol supplemented cells exhibited the appreciable down-regulation in the mRNA expression of thioredoxin-interacting protein. Data were portrayed as mean ± standard deviation of triplicate measurements. The significance was determined via analysis of variance successively Tukey's *post hoc* study; note: $^{\#}P < 0.05$ compared with control and $^{\#}P < 0.05$ compared with ovalbumin-group

reaction.[28] The stimulation of mast cells via IgE leads to the penetration of dendritic cells, which often results in the allergic response with clinical signs such as nasal rubbing and sneezing.[28] In this study, we obtained similar results [Figure 1]. IgE increases the production and secretion of prostaglandins and leukotrienes, which, in turn, speeds up the arrival of eosinophils, improves microvascular leakage, and decreases edema and mucous secretion. Furthermore, mast cells play an important role in instant allergic reactions via discharging the cytokines and histamine through degranulation.[34] Our results also proved that kirenol decreased the level of IgE, PGD2, and LTC4 in AR mice [Figure 3].

AR is distinguished via protuberant invading of inflammatory cells like lymphocytes and eosinophils.[17] Nearly 40% of the patients with asthma often experience AR, and patients with AR more likely to develop bronchial asthma and their chances of developing bronchial asthma is three times more than that of non-AR patients.[35] Previous reports show that ILs are responsible for the expression of numerous signaling cascades in airway inflammatory ailments.[16] For example, IL-4 acts as a growth factor for mast cells.[36] IL-5 is responsible for the penetration of eosinophils in the airway which leads to late-stage allergic reactions.[37] In this study, kirenol decreased the levels of pro-inflammatory mediators in both NLF and spleen mononuclear cells of AR mice [Figures 4 and 6]. These results demonstrate the anti-inflammatory potential of kirenol against the OVA-induced AR in mice.

Previous literature also indicates that AR is regulated via oxidative stress which occurs in response to inflammatory reactions and exposure to an environmental allergen.[38] In this study, we found that MDA and ROS were drastically increased and the activity of the SOD, antioxidant enzyme, was reduced in the OVA-induced spleen mononuclear cells [Figure 6]. Surprisingly, kirenol markedly reduced the levels of MDA and ROS and increased the activity of SOD. TXNIP is a crucial mediator of redox homeostasis. The triggering of TXNIP might increase the generation of ROS which, in turn, increases the oxidative stress.[39] In this study, we found that the kirenol significantly downregulated the mRNA expression of TXNIP [Figure 7]. In summary, our findings showed that kirenol ameliorated the OVA-induced AR in mice.

CONCLUSION

Our findings demonstrated the therapeutic actions of kirenol against the OVA-induced AR in mice. Kirenol appreciably reduced the OVA-induced nasal symptoms, inflammation, and oxidative stress through its anti-inflammatory and antioxidant properties. Kirenol effectively ameliorated the OVA-induced inflammatory condition in the AR mice. These outcomes confirmed the curative role of kirenol against the OVA-induced AR in mice, and it can be a promising agent to treat AR in future. However, further studies on the mechanistic role of kirenol are still needed in future to find the therapeutic benefits of kirenol against AR.

Acknowledgements

2017 Shaanxi Province "special support plan", "regional development talents" project, and 2015cxtd-10.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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