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Effect of Loaded Glycyrrhizic Acid on PLGA Nano-particle on Treatment of Allergic Asthma

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ABSTRACT

Asthma is considered a complex disease of the respiratory system that is characterized by bronchoconstriction, airway inflammation, cough, dyspnea, and wheezing. Allergic reactions are the main reason behind asthma which is known as an important health problem with a high rate of morbidity and mortality in patients with respiratory diseases. Liquorice, the root of *Glycyrrhiza*, is primarily effective for asthma which is widely used in herbal medicine. In the present study, we designed nano-particles that carry Glycyrrhizic acid as the effective component of Liquorice.

After Poly (D, L-lactide-co-glycolic acid) PLGA nanoparticle preparation and Glycyrrhizic acid loading, the morphology of the nanoparticle, the electric charge distribution, and drug-releasing ability were studied. Then the effect of Glycyrrhizic acid-PLGA on the animal model of allergic asthma was investigated.

Glycyrrhizic acid-nanoparticle had a mean±SD size of 350±50 nm. about 67% of the effective component was released after 10 h. The interleukin (IL)-4, IL-5, IL-13, and IL-25 levels and the Muc5ac mRNA expression were decreased in the Glycyrrhizic acid-PLGA treated group. In addition, a significant decline was observed in goblet cell hyperplasia, mucus hyper-secretion, and eosinophilic inflammation around bronchi and vessels of the nano-drug treated group, compared with the asthmatic group.

We found that Glycyrrhizic acid-PLGA nanoparticle had an anti-asthma effect which may be used as a new drug to cure asthma. It can prevent bronchial obstruction, breathlessness, and asthma attacks.

Keywords: Allergy and immunology; Asthma; Medicinal plants; Nanoparticles; Traditional medicine

INTRODUCTION

Asthma is a complex and multifactorial disease of the respiratory system with an increased worldwide prevalence. It afflicts more than 350 million individuals globally and is the major cause of morbidity and

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mortality in patients with respiratory diseases. It is characterized by bronchoconstriction, airway inflammation, cough, dyspnea, and wheezing. The main reason for asthma occurrence is allergic reactions that are found at all ages. However, allergic asthma occurs more frequently in children.[1,2] Environmental allergens and genetic predisposition are the dominant triggers in subjects with asthma exacerbation. In atopic patients, immune system response leads to Th2 cytokines secretion and increased levels of total IgE. Produced IgEs bind to their receptors on the mast cells' surface and after secondary collision with an allergen, allergic mediators will be released from activated cells and atopic symptoms will appear. If these reactions are in the airways, allergic asthma will appear.[3,4]

In fact, in all countries, a heavy annual economic burden is imposed on the national budget for providing healthcare services for patients with allergies and asthma. However, there is no complete cure for asthma. Some herbal agents can modulate innate and adaptive immune system responses and allergic mechanisms. So, traditional herbal medicine has significant effects on the immune system that may be an important treatment in the development of an anti-asthma drug. Herbal medicine is a valuable modality for the treatment of asthma, but some herbs may be toxic and cannot be used in inhalation form.[4,5]

Traditional Chinese medicine (TCM) is one of the ancient medical practices for centuries in the prevention and treatment of diseases.[6] Liquorice is the root of *Glycyrrhiza* and is widely used in herbal medicine. It has a sweet-tasting compound with various documented therapeutic applications in TCM. Liquorice is primarily effective for asthma.[7] Several studies have reported that Glycyrrhizic acid as an anti-inflammatory factor can reduce asthma symptoms. It is believed to have anti-asthmatic and anti-allergic effects that may be used as a novel herbal drug to control allergic asthma.[8-10] Liquorice root and its extract had been used in traditional medicine, especially in China and Iran to treat respiratory diseases. To increase the efficacy and overcome the side effects, we used nano-particles; carrying effective components of *Glycyrrhiza glabra (liquorice*) root; Glycyrrhizic acid. The nano-approach of using medicinal plants is a notable way for the administration of traditional medicinal plants. Inhalation of Glycyrrhizic acid in pure form can irritate the airway and may harm the airway epithelium.[7,9]

Biodegradable nanoparticles improve the solubility, permeability, absorption, and bioavailability of the drug. Poly lactic-co-glycolic acid (PLGA) is an FDA-approved nanoparticle for drug delivery systems. PLGA is the most popular biodegradable polymer because of its long clinical experience, favorable degradation, and possibilities for sustained drug delivery. In particular, PLGA is extensively used for the development of controlled delivery of small molecules and drugs. PLGA degradation can be employed for drug release at desirable doses.[11-13] So, in this study, we used herbal medicine and nano-technology by applying PLGA as a delivery vehicle to produce herbal nano-drug for the treatment and control of allergic asthma.

MATERIALS AND METHODS

Herb Component and Nanoparticle Preparation

Glycyrrhizic acid was purchased [Formula: C42H62O16·NH3, Molecular Weight: 839.96, assay: ≥95.0% (NT); Sigma Aldrich, USA]. PLGA nanoparticles were prepared according to a previous study and loaded with Glycyrrhizic acid.[14] In brief; poly-D, L-lactic, poly Capron, poly-D, and Lglycolic were added to sorbitan monostearate and mineral oil (Sigma-Aldrich, USA). Then, acetone (Merck Millipore, Germany) and poloxamer 188 (Sigma-Aldrich, USA) were added to the mixture. Afterward, the double emulsion method was used in a hydrophilic environment. Glycyrrhizic acid was added to a solution under sonication (100 W). The emulsion was added to 2% gelatin with the sonication for secondary emulsification. The resulting suspension was centrifuged at 12,000 rpm for 10 min to remove un-loaded Glycyrrhizic acid.

The morphology of the nanoparticle was assessed by scanning electron microscopy and the average size and the electric charge distribution of nanoparticles were determined by Zeta Plus Particle Size Analyzer. All methods have been approved by the ethical committee of the animal house of ix.med.vet.dep, 2019 (No. IX.MED.VET.DEP.REC.2019.250021.10).

Drug Loading and Releasing

Drug loading and releasing were determined by the bicinchoninic acid assay and spectroscopy. For this purpose, standard concentrations of the Glycyrrhizic

acid were prepared and the standard curve was plotted. A batch of nanoparticle-Glycyrrhizic acid (nano-drug) was solved in dichloromethane, and mixed with water. Glycyrrhizic acid was separated from nanoparticles. The mixture was centrifuged and the solution was used to measure the Glycyrrhizic acid amount trapped in the batch was calculated. Also, Glycyrrhizic acid release was evaluated. Ten series of microtubes were provided and examined. After saluting and separating the nano-drug, the supernatant liquid absorption was evaluated by a spectrophotometer. The released Glycyrrhizic acid concentration was determined according to the standard curve.

Asthma Animal Model and Treatment Schedule

Female BALB/c mice were purchased and acclimatized under standard laboratory conditions. All Ethical protocols were applied. A total of 40 mice were divided into 4 groups [n=10 in each group, 5 mice for pathological analysis and 5 mice for bronchoalveolar lavage fluid (BALf) sampling]. Allergic asthma was induced in 3 groups; using ovalbumin (OVA, Sigma-Aldrich, USA), according to a previous study.[15] One group was considered as the negative control (healthy) group that was sensitized and challenged with phosphate-buffered saline (PBS). Briefly, the BALB/c mice were sensitized via intraperitoneal injection of 20 μg of OVA with 50 μL alum adjuvant on day 1 and repeated on 14. Then, the mice were challenged via 1% OVA solution (inhalation form for 30 min per day) on days 24, 26, 28, and 30. Two of the three asthmatic groups were also treated with nanoparticle-Glycyrrhizic acid (10 mg/ml of in aerosolized solution for 30 min per day) and budesonide on days 25, 27, and 29 (inhalation for 30 min per day). Then, the mice were euthanatized by CO2, and then, blood, BALf, and lung tissue were collected on day 31 and were stored at -70°C for analysis.

Cytokines Levels Study

Interleukin (IL)-4, IL-5, IL-13, and IL-25 levels were measured in BALf; using Bio-Plex Pro™ Mouse Cytokine, Chemokine, and Growth Factor Assays (Bio-Rad, Nederland) as described before.[15]

Quantitative Real-time PCR

From BAL cells, the total RNA was isolated and reverse transcribed to the first-strand cDNA. Quantitative PCR analysis was also performed; using a Rotor-Gene SYBR Green PCR Kit and a Rotor-Gene Q thermal cycler (Qiagen, Hilden, Germany). Primers for the target gene (*Muc5ac*) of the mucus gene expression and *GAPDH* (endogenous control) are mentioned; GAPDH Sense sequence (5′−3′): TGTTCCTACCCCCAATGTGT, Antisense: GGTCCTCAGTGTAGCCCAAG, Muc5acSense: CAGGACTCTCTGAAATCGTACCA, Antisense: AAGGCTCGTACCACAGGGA.

Histological Study

The lungs tissues were isolated and fixed and after preparation, the left side of the lungs was stained with H&E and PAS. The inflammation study, goblet cell hyperplasia, and ratio of mucus production were determined in 10 randomly selected microscopy fields on histological sections. The scoring measurement has been described in a study by Athari et al.[15]

Statistical Analysis

Data were expressed as means±SD. The SPSS version 19 was used for the statistical analysis. Correlation analysis was carried out by Pearson's method. Results were tested with a one-way ANOVA followed by the Newman-Keuls test and considered statistically significant if $p<0.05$. PLGA-Glycyrrhizic acid releasing time was analyzed by paired t-tests and graphs were plotted on Graph Pad Prism software.

RESULTS

Herb Component and Nanoparticle Preparation

The obtained image of nanoparticles morphology (Figure 1) shows the nano-drug has suitable physical conditions (mean size, 350±50 nm). Zeta potential curves (Figure 1) indicated that Glycyrrhizic acid was loaded to the PLGA.

Drug Releasing

The percentage of Glycyrrhizic acid was determined; using spectroscopy. Glycyrrhizic acid was released from the PLGA nanoparticle at different times. The release in the first hour was 28%; while 67% release was observed after 10 h (Figure 1).

Cytokines Levels Study

The levels of IL-4 (87.11±4.05 versus 94.25±5.37 pg/mL, $p<0.05$), IL-5 (40.0±10.8 versus 83.33±7.36 pg/mL), IL-13 (69.25±1.36 versus 129.98±2.5 pg/mL),

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and IL-25 (48.98±2.01 versus 59.78±7.96 pg/mL) were decreased in the Glycyrrhizic acid-PLGA group compared with asthma animals (p<0.05). However, IL-4 and IL-25 did not have a significant difference in the Glycyrrhizic acid-PLGA group compared with asthma animals (Figure 2).

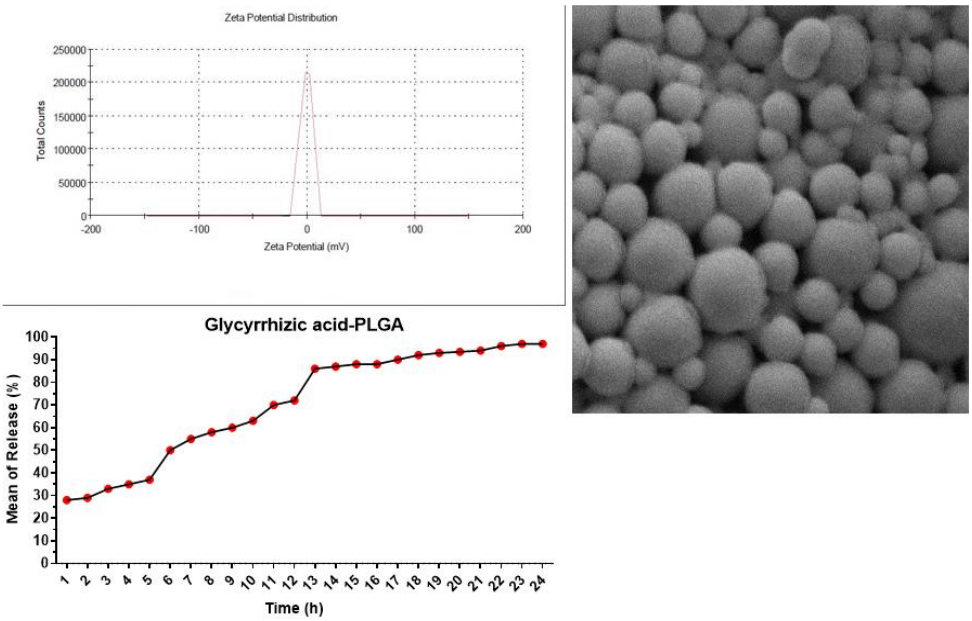

Figure 1. Characteristics of nanoparticles and release curve. The morphological study of the nano-drug was assessed by scanning electron microscopy (SEM; ×20,000). Zeta potential curve shows the distribution of its electric charge. Poly lactic-co-glycolic acid (PLGA)-Glycyrrhizic acid releasing in various periods (24 h) was evaluated. This was presented as the mean of 3 replicates representative of 3 independent experiments.

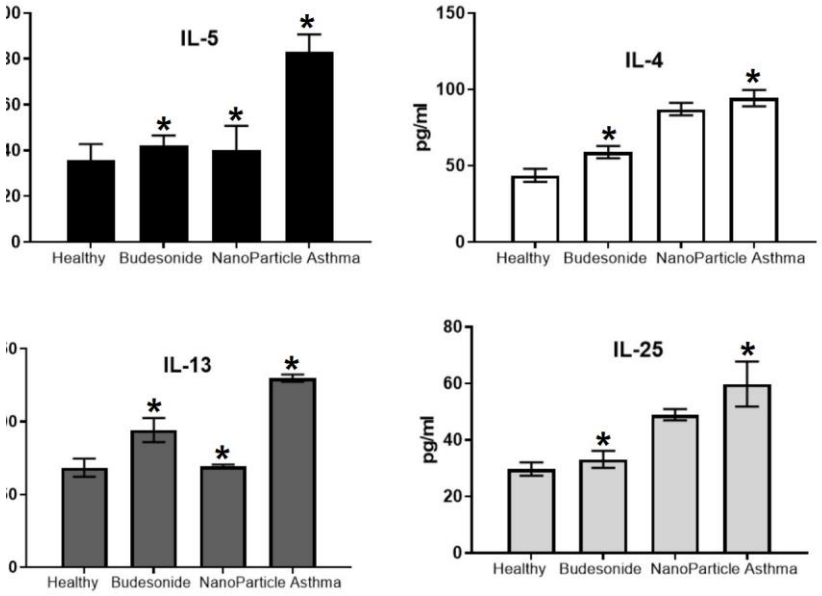

Figure 2. Cytokines levels. The levels of interleukin (IL)-4, IL-5, IL-13, and IL-25 in bronchoalveolar lavage fluid (BALf) were measured by enzyme-linked immunosorbent assay (ELISA) method in the healthy group, asthma group, and asthma groups that were treated by budesonide and glycyrrhizic acid nano-drug. *p<0.05

Quantitative Real-time PCR

In the Glycyrrhizic acid-PLGA group, the mRNA expression of Muc5a (8.05±0.50) was decreased significantly (p<0.05) compared to the positive control group (12.00±1.00) (Figure 3).

Histological Study

Goblet cell hyperplasia (3.5±0.2 fold) and mucus hyper-secretion (3.4±0.3 fold) were significantly increased in the asthmatic mice, compared with healthy mice (mucus secretion: 0.5±0.2, goblet cell: 0.5±0.3). Mucus hypersecretion in the airway of nano-drug treated group (1.5±0.5 fold) and goblet cell hyperplasia in bronchi of nano-drug treated group (2±0.5 fold) (Figure 4) was decreased in comparison with the asthmatic group (p<0.05). According to histological analyses, the infiltrated eosinophils around bronchi and vessels were significantly decreased in the airway of the nano-drug treated group (peribronchial inflammation: 1.7±0.4, perivascular inflammation: 1.5±0.3) (Figure 4), compared with the asthmatic group (peribronchial inflammation: 3.6±0.2, perivascular inflammation: 3.8±0.1) (p<0.05). Treatment with budesonide decreased mucus hypersecretion (2.5±1 fold) and goblet cell hyperplasia (2.7±0.5 fold), but the decrease was not significant (p>0.05) compared to the asthma group. Treatment with budesonide could significantly decrease (p<0.05) the peribronchial inflammation (1.5±0.6) and perivascular inflammation (1.8±0.2) compared to the untreated asthma group (Figure 4).

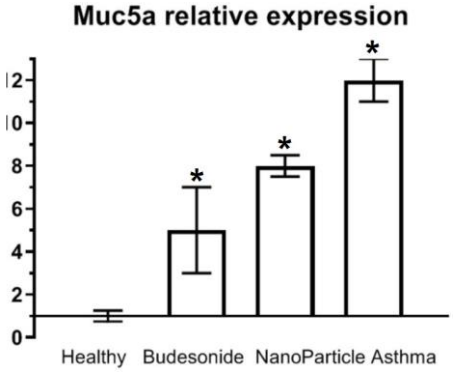

Figure 3. Real-time PCR. Effect of nano-drug (PLGA-Glycyrrhizic acid) on the mRNA expression of mucin in bronchoalveolar lavage (BAL) cells. The expression of the *Muc5ac* gene was determined by real-time PCR. *p<0.05

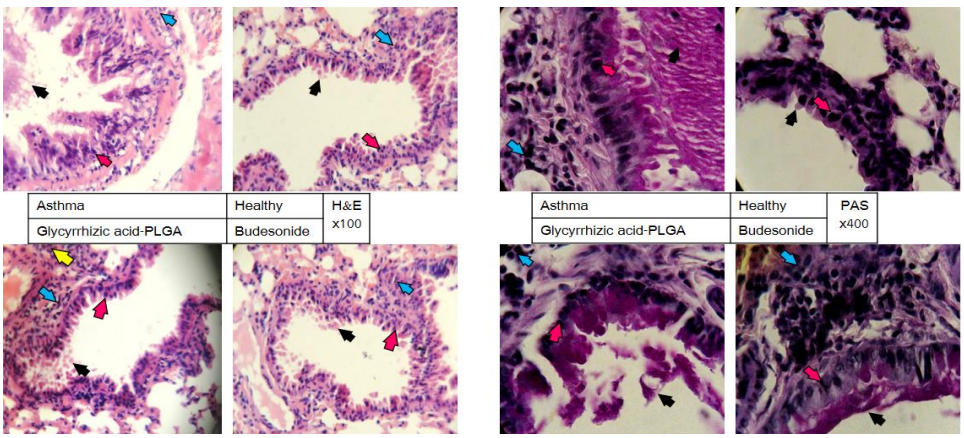

Figure 4. Histopathology: Lung tissues (left side) were stained with hematoxylin and eosin (H&E) and Periodic Acid-Schiff (PAS) staining. Afterward, the inflammation on the perivascular and peribronchiolar, hyperplasia of the goblet cell, and hyper-secretion of the mucus was evaluated. The perivascular inflammation is shown with yellow arrows, peribronchiolar inflammation is shown with blue arrows, mucus secretion is shown with black arrows, and goblet cells are shown with red arrows.

DISCUSSION

Liquorice; the root of the *Glycyrrhiza* plant, has been used as a food additive and in traditional medicine for centuries. In this study, we provide nanoparticles as carriers of the affective component of Liquorice to control allergic asthma. We designed an herbal nano-drug to determine the effect of this nano-drug on allergic asthma in the animal model.

Morphology of PLGA as FDA approved nanoparticle is suitable as an inhalation drug and could be used in the treatment of asthma. Liquorice has been used as an herbal drug in traditional medicine. Releasing the drug (Glycyrrhizic acid) from the carrier is important, especially in vital diseases. More than half of the drug is released in less than 10 hours and it is a notable factor in the design of anti-asthma drugs. Asthma attacks are begun and inflammation may simultaneously be restricted in one day. Therefore, the initial hours of an asthma attack are critical for the release of the drug. Other drugs are released in the next hours and could continuously control asthma attacks and act as a maintenance dose. In this study, Glycyrrhizic acid-PLGA treatment reduced type 2 cytokines levels (IL-4, IL-5, and IL-13). IL-25 as upper-hand of these cytokines in the BALf was also reduced; revealing that produced nano-drug could inhibit immune response shifting to the Th2 response with the regulation of Th2 cytokines over-secretion. Unlike IL-13 level, the reduction of IL-4 was not significant. Similarly, IL-4 was more significantly decreased by nano-drug compared with budesonide.

Glycyrrhetinic acid as a bioactive component of *Glycyrrhiza* possesses anti-inflammatory properties and immunoregulatory functions in allergic diseases. Liquorice component suppresses airway hyperresponsiveness, infiltration of inflammatory cells, and decreases T helper 2 cytokines levels. In the lungs, Liquorice attenuates the expression of *IL-4*, *IL-15*, and *IL-13* genes; while it up-regulates *peroxisome proliferator-activated receptor gamma (PPARγ)* gene expression. Moreover, it exerts immunomodulatory effects by suppressing the type2 cytokines production through forkhead box *p3 (Foxp3)* up-regulation, and downregulation *of GATA-binding protein 3 (GATA-3)*, *signal transducer and activator of transcription (STAT6)*, and *retinoic acid-related orphan receptor γt (RORγt)* gene expression. Therefore, the anti-asthmatic activity may occur by suppression of IL-4, IL-5, IL-13, and OVA-specific IgE production and inhibition of *ROR-γt*, *STAT6*, and *GATA-3* pathways. However, the *Foxp3* transcription pathway is up-regulated. Also, Liquorice has a protective effect against oxidative stress and glycyrrhetinic acid can inhibit *NOX4*, *p67phox*, *p47phox*, *p22phox* (NADPH related genes) expression. So, it could be a therapeutic component for the treatment of allergic asthma.[16,17] Also, we found that Liquorice component can decrease *Muc5a* mRNA expression and can reduce mucus hypersecretion and airway obstruction. This decrease is parallel with IL-13 level secretion because *Muc5a* gene expression is the main source of mucus and IL-13 secretion.

Asthma exaggerates lower airway response to environmental factors exposure. In asthma exacerbations, inflammation of the airway is the main result of the immune response and mucus production; leading to airflow obstruction and increased airway hyperresponsiveness.[18,19] Transient receptor potential cation channel subfamily V member 1 (TRPV1) and transient receptor potential cation channel subfamily A member 1 (TRPA1) as cation channels are expressed on the airways and have key roles in inflammatory signaling pathways; resulting in inflammation and cough in asthma. Liquorice components involve both TRPV1 and TRPA1 and prevent tissue damage and inflammation; while activating the NF-κB signaling pathway in the lung tissue.[20] In our study, we observed that Glycyrrhizic acid nano-drug decreased the infiltrated eosinophils around the airway and vessels, controlled peribronchial inflammation, and decreased perivascular inflammation compared with the asthmatic non-treated group. Glycyrrhizic acid nano-drug prevents airway obstruction by controlling airway inflammation as well as reducing goblet cell hyperplasia and mucus hypersecretion. Decreasing inflammatory cell infiltration (especially eosinophil in asthma) and mucus hypersecretion are the main achievement in asthma treatment; being observed in this study. Therefore, this nano-herbal medicine significantly improved lung function and asthma symptoms and decreased the dose of Glycyrrhizic acid consumption, with no serious adverse effects. Therefore, this traditional nano-herbal medicine could pave the road in asthma treatment after being validated in human patients.

Liquorice root has a long history of use in traditional medicines.[21] Studies presented that Liquorice and its components such as Glycyrrhizic acid have anti-inflammatory and antioxidant effects and these activities are beneficial in controlling and treatment of allergic asthma.[22,23] Also, previous studies showed that Liquorice extract has the potentials to improve antioxidant capacity and radical scavenging activity as well as decrease the reactive oxygen species. This increases glutathione peroxidase and catalase activities and decreases superoxide anion, hydrogen peroxide, and thiobarbituric acid reactive substance levels.[24,25] We found that our produced Glycyrrhizic acid-PLGA nanoparticle has anti-inflammatory and anti-asthma effects which could be used as a new drug to cure asthma. It could control eosinophil infiltration and attack to the airway and with the control of Th2 cytokines and mucus production, it could prevent bronchial obstruction, breathlessness, and asthma pathological effects on the lung tissue. However, there were several limitations in the present study. First, we only tested nano-drug on BALB/C mice. Second, we did not study other asthma and allergy-related factors. Third, we chose acute airway inflammation and did not study the chronic form for a long time. However, it seems to have an acute effect, so it may be effective in the chronic form as well. Fourth, we only detected Th2 cytokines concentrations in BALf and did not check Th1 cytokines. Although evidence elsewhere shows a determinative relationship between airway inflammation and Th2 cytokines, we may neglect the potential roles of other inflammatory markers. All these limitations should be addressed in future investigations. Produced Glycyrrhizic acid-PLGA nanoparticle with anti-asthma effects could be used as a new treatment to control asthma. It can control inflammatory responses in airways, the Th2 cytokines and mucus production, also prevent bronchial obstruction, and breathlessness.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Athari SS, Athari SM. The importance of eosinophil, platelet and dendritic cell in asthma. Asian Pac J Trop Dis. 2014;4(S1):41-7.
2. Athari SS. Targeting cell signaling in allergic asthma. Signal Transduct Target Ther.2019;4(5):45-9.
3. MehrabiNasab E, Athari SM, Motlagh B, Athari SS. Effects of oral administration of Ocimumbasilicum on goblet cell hyperplasia and upstream cytokine gene expression in allergic asthma. Rev Fr Allergol. 2020;60(2):64-8.
4. Athari SM, MehrabiNasab E, Athari SS. Study effect of Ocimumbasilicum seeds on mucus production and cytokine gene expression in allergic asthma mice model. Rev Fr Allergol. 2018;58(7):489-93.
5. Ch Ma, Ma Z, Liao EL, Liu J, Fu Q, Ma S. Immunoregulatory effects of Glycyrrhizic acid exerts anti-asthmatic effects via modulation of Th1/Th2 cytokines and enhancement of CD4+CD25+Foxp3+ regulatory T cells in ovalbumin-sensitized mice. J Ethnopharmacol. 2013;148(3):755–62.
6. Li XM. Traditional Chinese herbal remedies for asthma and food allergy. J Allergy Clin Immunol. 2007;120(1):25–31.
7. Wang X, Zhang H, Chen L, Shan L, Fan G, Gao X. Liquorice, aunique “guide drug” of traditional Chinese medicine: A review of its role in drug interactions. J Ethnopharmacol. 2013;150(3):781-90.
8. Ram A, Mabalirajan U, Das M, Bhattacharya I, Dinda AK, Gangal SV, et al. Glycyrrhizin alleviates experimental allergic asthma in mice. Int Immunopharmacol. 2006;6(9):1468-77.
9. Fouladi S, Masjedi M, GanjalikhaniHakemi M, Eskandari N. The Review of in Vitro and in Vivo Studies over the Glycyrrhizic Acid as Natural Remedy Option for Treatment of Allergic Asthma. Iran J Allergy Asthma Immunol. 2019;18(1):1-11 .
10. Kakegawa H, Matsumoto H, Satoh T. Inhibitory effects of some natural products on the activation of hyaluronidase and their anti-allergic actions. Chem Pharm Bull. 1992;40(6):1439-42.
11. Alshetaili A. Gefitinib loaded PLGA and chitosan coated PLGA nanoparticles with magnified cytotoxicity against A549 lung cancer cell lines. Saudi J Biol Sci. 2021; In Press.

12. Mir M, Ahmed N, Rehman AU. Recent applications of PLGA based nanostructures in drug delivery. Colloids Surf B Biointerfaces. 2017;159(2):217-31.
13. Ding D, Zhu Q. Recent advances of PLGA micro/nanoparticles for the delivery of biomacromolecular therapeutics. Mater Sci Eng. 2018;92(4):1041-60.
14. Athari SS, Mortaz E, Pourpak Z, Moin M, Moazzeni SM. VIP-loaded PLGA as an anti-asthma nanodrug candidate. Comp Clin Pathol. 2016;25(8):791–6.
15. Athari SS, Pourpak Z, Folkerts G, Garssen JO, Moin M, Adcock IM, et al. Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. Eur J Pharmacol. 2016;15(791):811-20.
16. Kim SH, Hong JH, Lee JE, Lee YC. 18β-Glycyrrhetinic acid, the major bioactive component of Glycyrrhizae Radix, attenuates airway inflammation by modulating Th2 cytokines, GATA-3, STAT6, and Foxp3 transcription factors in an asthmatic mouse model. Environ ToxicolPharmacol. 2017;52(11):99-113.
17. Ploeger B, Mensinga T, Sips A, Seinen W, Meulenbelt J, DeJongh J. The pharmacokinetics of glycyrrhizic acid evaluated by physiologically based pharmacokinetic modeling. Drug Metab Rev. 2001;33(2):125–47.
18. Zhang HP, Wang L, Wang Z, Xu XR, Zhou XM, GLiu, et al. Chinese herbal medicine formula for acute asthma: A multi-center, randomized, double-blind, proof-of-concept trial. Respir Med. 2018;140(12):42–9.
19. N Yang N, S Patil S, J Zhuge J, MCWen, Bolleddula J, Doddaga S, et al. Glycyrrhizauralensis flavonoids present in antiasthma formula, ASHMI™, inhibit memory Th2 responses in vitro and in vivo. Phytother Res. 2013;27(9):1381–91.
20. Liu Z, Wang P, Lu S, Guo R, Gao W, Tong H, et al. Liquiritin, a novel inhibitor of TRPV1 and TRPA1, protects against LPS-induced acute lung injury. Cell Calcium. 2020;88(12):102198.
21. Isbrucker RA, Burdock GA. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. RegulToxicolPharmacol. 2006;46(3):167–92.
22. Fu Y, Chen J, Li YJ, Zheng YF, Li P. Antioxidant and anti-inflammatory activities of six flavonoids separated from licorice. Food Chem. 2013;141(2):1063–71.
23. Haraguchi H, Ishikawa H, Mizutani K, Tamura Y, Kinoshita T. Antioxidative and superoxide scavenging activities of retrochalcones in Glycyrrhiza inflata. Bioorg Med Chem. 1998;6(3):339–47.
24. Zhang Y, Luo H, Liu K, Jia H, Chen Y, Wang Z. Antioxidant effects of liquorice (Glycyrrhiza uralensis) extract during aging of longissimus thoracis muscle in Tan sheep. Meat Sci. 2015;105(7):38-45.
25. Zhang YW, Luo HL, Chang YF, Jiao LJ, Liu K. Effects of liquorice extract on the activity and gene expression level of antioxidant enzymes in longissimus dorsi muscle of Tan lamb. Small Rumin Res. 2017;154(5):23-8.