DOI: https://doi.org/10.31351/vol30iss2pp64-70

Study the Anti-Asthmatic Activity of Guggulsterone in Ovalbumin-Induced Asthma In Rat

Zainab H. Ahmed [*,1] and Munaf H Zalzala[**]

[*] Department of Pharmacology and Toxicology, College of Pharmacy, University of Basra, Basra, Iraq.
[**] Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Asthma is a chronic inflammatory respiratory disease associated with the changes of asthmatic airway structural that result from interact remodeling and inflammatory processes lead to obstruction of airway. Guggulsterone (GS) is a bioactive compound and plant steroid present in guggul gum of Commiphora wightii, which has anti-inflammatory and antioxidant activities. This study designed to investigate of anti-inflammatory activity of gugglsterone in improvement of asthma. Forty eight healthy albino male rats divided to six groups, **Group I:** Control group (distal water), **Group II:** Positive control group (distal water) with sensitization, **Group III:** Guggulsterone (25 mg/kg/day) with sensitization, **Group IV:** Guggulsterone (50 mg/kg/day) with sensitization, **Group V:** Prednisolone (4.12 mg/kg/day) with sensitization, **Group VI:** Guggulsterone (50mg/kg/day) without sensitization. Rats were sacrificed and blood samples were collected to prepare of serum samples that used in ELISA kits for measuring of IL-4, IL-5, IL-33, TNF-α, & IgE. In addition, WBC counts in Bronchoalveolar lavage fluid. ALL parameters (IL-4, IL-5, IL-33, TNF-α, & IgE) levels for rats of treated groups with gugglsterone were significant (P<0.05) reduced in compared to sensitized group. Similarly, WBC count for rats treated groups with guggulsterone was significantly (P<0.05) fewer than sensitized group. In conclusion, our results provide a clue that guggulsterone has a potent anti-inflammatory activity that improved OVA-induced asthma and is useful for the preventive of allergic airway disease in rodents.

Keywords: Anti-inflammatory activity, Asthma, Guggulsterone, Ovalbumin.

دراسه فعاليه المضاده للربو للجوجلستيرون لعلاج ربو الجرذان المحفز بالالبومين

زينب هارون أحمد [*،١] ومناف هاشم زلزلة [**]

[*]فرع الدوية والسموم ،كلية الصيدلة، جامعة البصرة ، البصرة ، العراق
[**]فرع الدوية والسموم ، كلية الصيدلة ، جامعة بغداد ، بغداد ، العراق

الخلاصة

الربو هو احد الامراض الالتهابات المزمنه وعند حصوله يكون مصحوب بتغيرات في هيكليه المجاري التنفسيه ناتجه عن عمليات الالتهاب وتغيرات تصمميه تؤدي الى تضيق المجاري التنفسيه، جوجلستيرون احد المركبات النباتيه الاستيروديه الفعاله موجوده في الراتنج الصمغي للشجره شائكه المنتشره في الهند،وهذا المركب يمتلك فعاليات مضاده للالتهاب والاكسده.الغايه من هذه الدراسه لمعرفه فعاليه مضاده للالتهاب للجوجلستيرون في علاج الربو. ٤٨ جرذان البينو الذكور السليمه مقسم الى ستة مجاميع، المجموعه الاولى اعطيت ماء نقي، المجموعه الثانيه اعطيت ماء نقي مع تحسست لتهيج الربوبماده البومين (١٤ يوم)، المجموعه الثالثه اعطيت الجوجلستيرون (٢٥مغم /كغم/يوم) لمده ١٤ يوم مع تحسس ، المجموعه الرابعه اعطيت الجوجلستيرون (٥٠ مغم /كغم/يوم) لمده ١٤ يوم مع تحسس ، المجموعه الخامسه اعطيت بريزيلون (٤,١٢مغم /كغم/يوم) لمده ١٤ يوم مع تحسس.المجموعه السادسه اعطيت الجوجلستيرون (٥٠مغم /كغم/يوم) لمده ١٤ يوم ، وفي اليوم ١٥ تم انهاء حياة الجرذان للحصول على عينات الدم واستخلاص السيروم لقياس عدة متغيرات بجهاز الالايزا، وكذلك حساب كريات الدم البيضاء في سائل الرئوي. واظهرت النتائج نقصان ملحوظ للمتغيرات وكذلك كريات الدم البيضاء عن المجموعه الثانيه ، لذلك نستنتج بان الجوجلستيرون يمتلك فعاليه مضاده للالتهاب قويه و مفيده في معالجه الربو الناتج من التحسس ومنع التهاب المجاري التنفسيه للقوارض.

الكلمات المفتاحية: فعاليه مضاده للالتهاب، الربو، جوجلستيرون، البومين.

Introduction

Asthma is a chronic inflammatory respiratory disease, associated with changes of asthmatic airway structures that result from remodeling and inflammatory processes.(1) In the inflammatory process, common features as exudation, vascular congestion, and inflammatory cell agglomerate in the interstitial tissue. The chronic changes of inflammatory stage develop epithelial-mesenchymal interactions.(2) The fibrosis of subepithelial layer is one of important features of airway remodeling that initiated from simple thickening to prolong fibrosis.(3) 。 The formation of pro-inflammatory cytokines like IL-4, IL-5, can maintain and initiate the features of disease pathophysiology. Whereas IL-4 is crucial for IgE production and allergic sensitization, and IL-5 is important for eosinophil survival in the lungs.(4) IL-33 is a cytokine of the IL-1 family.

[1]Corresponding author E-mail: zha_23980@yahoo.com
Received: 13/10/2020
Accepted: 22/2/2021
Published Online First: 2021-12-09

After tissue injury, IL-33 produces as an alarm signal and stimulates other immune cells to release of IL-5 and 13.

Also IL-33 induces IgE synthesis and B-cell expansion leading to IL4 secretions by innate cells.[5, 6] Furthermore, TNF is a major inflammatory mediator, which has two major forms including TNF-α and -β, The inflammatory mechanisms of TNF-α including TNF-α–stimulated AHR, chemoattractant for eosinophils and neutrophils and up regulation of adhesion molecules lead to migration of inflammatory cells to the respiratory tract.[7] However, the other inflammatory marker is IgE, which is stimulating the produce of transcription of cytokines, vasoactive mediators, and de novo synthesis of leukotrienes and prostaglandins on mast cell. In the lung these mediators are rapidly evolve mucus production, edema of the bronchial mucosa, and constriction of the smooth muscle and lastly induce an inflammatory infiltrate that lead to inflammatory responses of asthma.[8] .

Guggulsterone is a steroid plant (polyphenol) present in guggul gum of Commiphora wightii, which belongs to Burseraceae family and it has two bioactive isomers E- and Z-guggulsterone. Both isomers of guggulsterone have anti-inflammatory activities by inhibit lipopolysaccharide-induced inflammation through inhibiting NF-κB activation and IκB-α degradation.[9] Moreover, guggulsterone drastically curbed TNF-induced promoter bounce of COX-2, which is responsible for degradation of arachidonic acid to thromboxane and prostaglandins that lead to inflammatory responses. On other hand, guggulsterone has antioxidant activity due to present of CH3, H, and O bonds in the steroidal structure that helps in reducing free radicals (singlet oxygen and hydroxyl ions) that lead to inhibit the production of lipid peroxides, therefore gugglsterone is preventing oxidative stress.[10] All these gugglsterone activities may contribute for reducing inflammatory responses associated with asthma.

Materials and Methods

Animals

Forty-eight healthy Albino male rats weighing 150-300 gm, were brought from animal house of the College of Pharmacy / University of Baghdad. Rats were housed under controlled temperatures and photoperiods (12:12-hours light/dark cycle). During the experiment period, these animals were fed commercial pellets. The local Research Ethics Committee in College of Pharmacy, University of Baghdad, approved the research protocol.

Chemicals and kits

Guggulsterone powder was purchased from (Xi'an geekee biotech, China) and was prepared as suspension (5mg/ml). Ovalbumin was purchased from (Chadwll Heath ESSEX, England), Predinsoline (syrup, 15mg/5ml) was taken up from (Pioneer, Iraq). Also IL4, IL5, IL33, TNF & IgE kits were bought from (MyBioSource, USA).

Study design

Rats were randomly divided into six groups (8 rats in each). The dose of guggulsterone (25 & 50 mg/kg)[11, 12] according to the previous studies and predinsoline dose (4.12 mg/kg) depending on a simple practice guide for dose conversion between animals and human.[13] LD_{50} of guggulsterone E and Z was (1600 mg/kg p.o.) in rats.[14] .

Group I: Rats were administrated distal water orally (daily dose) without sensitization for 14 days as control group.

Group II: Rats were administrated distal water orally (daily dose) with sensitization for 14 days as positive control group.

Group III: Rats were administrated (25 mg/kg) guggulsterone orally with sensitization for 14 days as treated group.

Group IV: Rats were administrated (50 mg/kg) guggulsterone orally with sensitization for 14 days as treated group.

Group V: Rats were administrated (4.12 mg/kg) prednisolone orally with sensitization for 14 days as treated group.

Group VI: Rats were administrated (50 mg/kg) guggulsterone orally without sensitization for 14 days as treated group.

Inflammatory sensitization method for OVA-induced asthma of sensitized rats by modified protocol of Manal *et al* (2013), Tong *et al* (2008), and Michael *et al* (1999) studies. The experimental groups were sensitized intraperitoneally (I.P.) with 1 mg OVA adsorbed on 100mg Al(OH)3 gelatinous and dissolved in1ml of PBS on days 1, 2, and 3. Then on the 6th day , the rats were challenged with a 100mg OVA adsorbed on 100mg $AL(OH)_3$ and dissolved in 1ml of PBS. After that, the experimental animals were challenged on the 9th day by glass chamber with volume (20 cm × 20 cm × 20 cm) , connected to a nebulizer with 1% OVA (1gm OVA in 100ml PBS) for 30 minutes daily for 6 days.[15-17]

WBC count and differentiation

After blood collection, the chest was opened, and the trachea with the heart–lung package was excised from the thorax; the left main bronchi were clamped. A cannula was inserted into the trachea in situ, the right lung was lavaged three times with 5ml PBS solution, and BAL fluid was collected and centrifuged (6000 RPM/min for 15 minutes) to separate whole cells as pellets. After the supernatant was removed, the pellet of whole cells was dissolved with 1 ml of normal saline. WBC count was measured and differentiated by Coulter Cellular Analysis System (Brea, USA).

Preparation of serum samples

Blood samples were collected in plane tube and kept in room temperature for 30 minutes. Then, they were centrifuged for 15 min. (1000 x g). After that, serum was removed and stored at (-20ºC or -80ºC). Then, parameters (IL-4, IL-5, IL-33, TNF-α &IgE) were measured by ELISA kits.

Principle of kit

This kit was depended on sandwich (enzyme linked immune-sorbent) assay method. Plate (96 wells) was coated with polyclonal antibody. Polyclonal antibody was connected with the biotin as detection antibodies. The test samples (serum and tissue hemogenate), standards, and biotin connected detection antibody were added to the plate subsequently, and wash buffer used for washing the wells. Then, ABC was added and wash buffer was washed unbound conjugates. TMB substrates were utilized for visualizing enzymatic reaction HRP (blue color). After that stop solution (acidic solution) was added to change the color of reaction (yellow color), that read by a microplate reader (450 nm).

Statistical analysis

Data expressed as means, Standard error mean and percentage. Where, Unpaired Student t-test was used for testing the significant difference between two groups. On other hand, One-way ANOVA analysis was used for testing the significant difference between three or more than groups. Statistically, significant differences were considered for P-value < 0.05.

Results and Discussion

In asthma, there is cellular stimulation and production of inflammatory mediators by degranulation of mast cell and vacuolation of eosinophil. The pro-inflammatory cytokines, interlukines-3, 4, 5, 9, & 13 and GM-CSF (granulocyte–macrophage colony-stimulating factor), which lead to the IgE, eosinophilic and mast cell responses which are produce the distinctives of allergic asthma[18] .

1-The effect of Guggulsterone on WBC counts in Bronchoalveolar lavage fluid (BALF) of OVA-Induced Asthma in Rats

As shown in **figure 1**,WBC count (mean ± Std. Error) in BALF for rats of group II (positive control, induction group) was highly significant elevated ($p<0.001$) compared to group I (control group). Moreover, there was highly significant reduction about 78 % ($p<0.001$) of WBC count in BALF for rats of group III, 73 %($p<0.001$) reduction in group IV, & 51 % ($p<0.001$) reduction in group group V in compared with group II (positive control). Furthermore, there was a significant reduction ($p<0.05$) in WBC count in BALF in rats of group IV (104.3 ± 14.9) compared to group V (192.1 ± 39.6). likewise, there was a significant reduction ($p<0.05$) in WBC count in BALF in rats of group III (86.1 ± 16.9) compared to WBC count in BALF in rats of group V (192.1 ± 39).

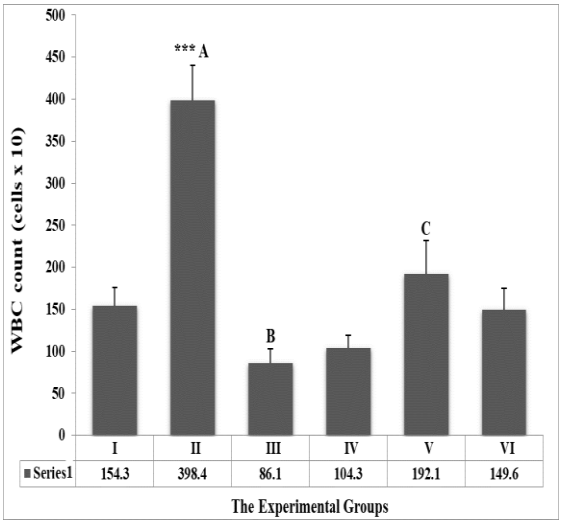

Figure 1. The effect of guggulsterone on WBC counts in bronchoalveolar lavage fluid of OVA-Induced Asthma in Rats.

Values are indicated as means ± Std. Error (n=8) for each group. Group I: Control group, Group II: Positive control group (with sensitization), Group III: Guggulsterone (25 mg/kg) with sensitization, Group IV: Guggulsterone (50 mg/kg) with sensitization, Group V: Predinsoline (4.12 mg/kg) with sensitization, Group VI: guggulsterone (50 mg/kg) without sensitization. *** symbol referred to significant different ($p<0.001$) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different ($p<0.05$). Series 1 referred to means of WBC counts in BALF for each group.

WBC count in BAL was significantly increased in sensitized group (II) compared to control group (I), the previous study, Monteseirín (2009) revealed to significant elevation in WBC especially neutrophils in asthma due to platelet activating factor (PAF) more in asthmatic patients than a healthy group that lead to induce chemoattractive of neutrophils.[19] On other hand, WBC count in BAL fluid for treated groups with guggulsterone and predinsoline have significant reduction because of gugglsterone has anti-inflammatory activity by inhibiting the activation of NFκB and reduce of gene expression of chemokines that lead to reduce of eosinophils and neutrophils migration [20].

2-The effect of guggulsterone on IL4 levels in the serum of OVA-Induced Asthma in Rats:

As shown in **figure 2**, highly significant ($p <0.001$) elevation in serum levels of IL4 was observed in rats of group II compared to group I. Furthermore, in rats of treated groups with (III, IV & V), the serum levels of IL4 were highly significant reduced by 71 % ($P <0.001$), 67% ($P <0.001$) and

39% (P <0.001), respectively compared to group II. However, non-sensitized gugglsterone-treated rats of group VI showed no significant changes in serum levels of IL4.

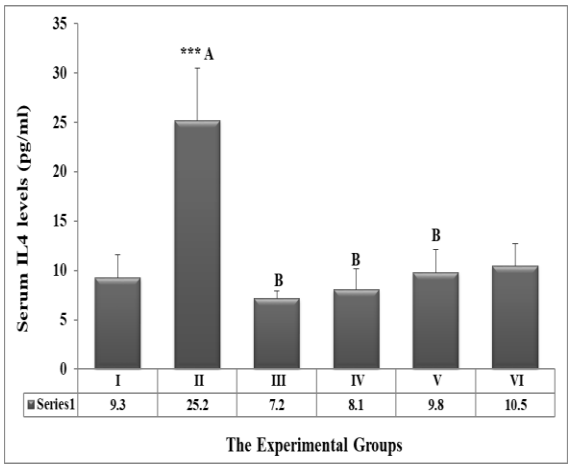

Figure.2. The effect of guggulsterone on IL4 levels in the serum of OVA-induced asthma in rats.

Values are indicated as means ± Std. Error (n=8) for each group. Group I: Control group, Group II: Positive control group (with sensitization), Group III: Guggulsterone (25 mg/kg) with sensitization, Group IV: Guggulsterone (50 mg/kg) with sensitization, Group V: Prednisolone (4.12 mg/kg) with sensitization, Group VI: guggulsterone (50 mg/kg) without sensitization. *** symbol referred to significant different (p<0.001) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different (p<0.05). Series 1 referred to means of WBC counts in BALF for each group.

In current study, IL-4 levels in serum for sensitized group are significantly greater than control group. The other studies like Afshari *et al* (2007) & Gour et al (2015), appeared that in asthma, IL-25, IL-33, thymic stromal lymphopoietin (TSLP) and leukotrienes are activated T helper cell, mast cell and basophil and released IL-4, which has the role in regulating Th2 cell survival and proliferation and IgE synthesis that lead to the initiation of humoral and airway allergic responses associated with high serum IL-4 level.[(8)&(21)] On other hand, guggulsterone-treated groups have significant reduction in IL-4 levels in serum than sensitized group due to gugglusterone has glucocorticiod-mediated effect that lead to inhibit gene expression of proinflammatory mediater including ILs-4, 5, 8 and 13 that lead to reduce inflammatory events associated with asthma[(22)] .

3- The effect of guggulsterone on IL-5 levels in the serum of OVA-induced asthma in rats

As shown in **figure 3**, A highly significant (p <0.001) elevation in serum levels of IL5 was observed in rats of group II compared to group I. Furthermore, treated groups (III & IV), the serum levels of IL5 were highly significant reduced by 34 % (P <0.001), and 54% (P <0.001), respectively compared to group II**.** Furthermore, there was a significant reduction by 47% (p<0.05) in serum IL 5 levels in group III compared with group V.

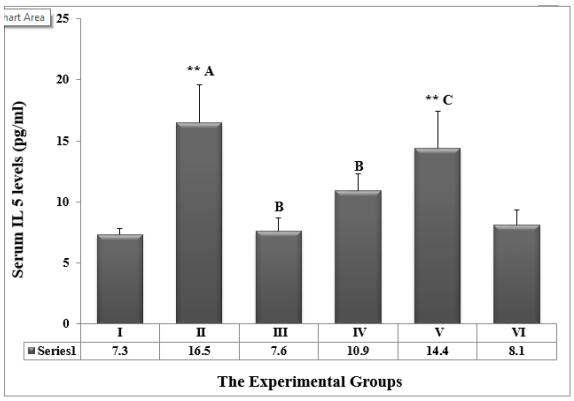

Figure3.The effect of guggulsterone on IL-5 levels in the serum of OVA-induced asthma in rats:

Values are indicated as means ± Std. Error (n=8) for each group. Group I: Control group, Group II: Positive control group (with sensitization), Group III: Guggulsterone (25 mg/kg) with sensitization, Group IV: Guggulsterone (50 mg/kg) with sensitization, Group V: Prednisolone (4.12 mg/kg) with sensitization, Group VI: guggulsterone (50 mg/kg) without sensitization. ** symbol referred to significant different (p<0.001) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different (p<0.05). Series 1 referred to means of WBC counts in BALF for each group.

The other an important inflammatory mediater is IL-5, in serum for rats of sensitized group II is higher than control group I. The role of IL-5 in the pathogenesis of asthma is supported by previous studies, Garcia *et al* (2013), Dunican *et al* (2015),when IL-5 release by mast cells, Th2-lymphocytes and eosinophils and occupy to the specific subunit receptor, IL-5Rα. IL-5/IL-5Rα complex stimulate eosinophil differentiation, proliferation, maturation, and migration to tissue sites with survival, as well as forbidding of eosinophil apoptosis.[(23), (24)] Furthermore, Guggulsterone has anti-inflammatory activity by inhibiting the activation of NFκB and reduce of gene expression of IL-5[(22)] .

4- The effect of gugglsterone on IL 33 levels in the surem of OVA-induced asthma in rats

Figure 4 appeared that highly significant elevation (p <0.001) in serum levels of IL33 was observed in rats of group II compared to group I. Furthermore, in treated groups (III, IV &V), the serum levels of IL33 were highly significant reduced by 36% (P <0.001), 41% (P <0.001) and 49% (P

<0.001), respectively compared to group II. Likewise, there was significant reduction (p<0.05) in IL33 in serum for rats of group III compared to IL33 in serum for rats of group V.

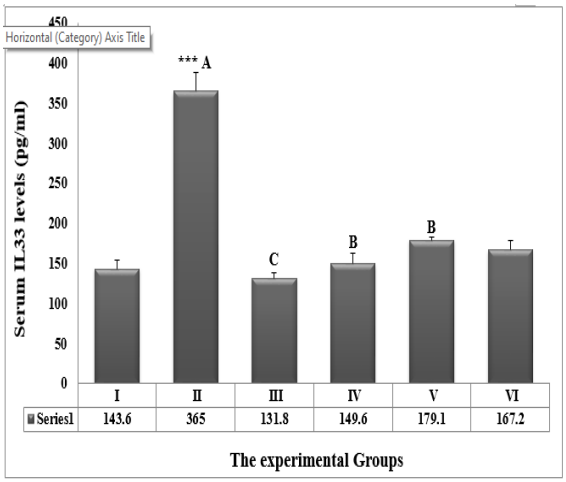

Figure.4. The effect of gugglsterone on IL 33 levels in the surem of OVA-induced asthma in rats.

Values are indicated as (means ± Std. Error (n=8) for each group. Group I: Control group, Group II: Positive control group (with sensitization), Group III: Guggulsterone (25 mg/kg) with sensitization, Group IV: Guggulsterone (50 mg/kg) with sensitization, Group V: Prednisolone (4.12 mg/kg) with sensitization, Group VI: guggulsterone (50 mg/kg) without sensitization. *** symbol referred to significant different (p<0.001) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different (p<0.05). Series 1 referred to means of WBC counts in BALF for each group.

Recently, many studies are suggested that difference in genes encoding IL-33 and IL-1RL1 is present with asthma. IL-33 levels in serum for rats of sensitized group II is significantly more than control group I. Li *et al* (2015) & Momen *et al* (2017) studies, showed that IL-33 levels were higher in asthmatic group than normal group because of IL-33 induces of intracellular molecules by MAP kinase and NF-κB signaling pathway, which is an important role in initiate allergic inflammation in asthma.[25), (26)] On other hand, IL-33 levels in serum for rats treated with guggulsterone has significant reduction in compared with sensitized group due to gugglsterone reduce the activation of transcriptional factors, such as AP-1 and NF-κB, inhibit translation of target genes and/or mRNA stabilization that that lead to inhibit inflammatory responses associated with asthma[(27)] .

5- The effect of gugglsterone on TNFα levels in the serum of OVA-induced asthma in rats

Figure 5 showed that highly significant elevation (p <0.001) in serum levels of TNFα was observed in rats of group II compared to group I. Also, There was highly significant reduction (p<0.001) in TNFα levels in serum for treated groups (III, IV & V), the serum levels of TNFα was highly significant reduced by 43 % (P <0.001), and 39% (P <0.001), & 54% (p <0.001), respectively compared to group II. On other hand, treating non-sensitized animals with gugglsterone showed no significant changes in serum levels of TNFα.

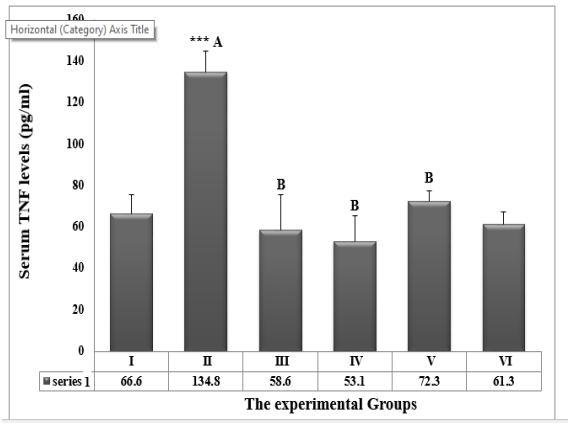

Figure.5. The effect of gugglsterone on TNFα levels in the serum of OVA-induced asthma in rats.

Values are indicated as means ± Std. Error (n=8) for each group. Group I: Control group, Group II: Positive control group (with sensitization), Group III: Guggulsterone (25 mg/kg) with sensitization, Group IV: Guggulsterone (50 mg/kg) with sensitization, Group V: Prednisolone (4.12 mg/kg) with sensitization, Group VI: guggulsterone (50 mg/kg) without sensitization. *** symbol referred to significant different (p<0.001) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different (p<0.05). Series 1 referred to means of WBC counts in BALF for each group.

Moreover, the results of the current study appeared TNF levels in serum are significantly elevated with sensitized group II in compared to normal group I, Nakae *et al* (2007), & Brightling *et al* (2008) revealed that TNF is higher with asthmatic group in compared to normal group due to TNF-α/ TNF-α receptor complex that produces intracellular signaling and phosphorylation of IκBa and stimulation of NF-κB, which is induces the expression of proinflammatory genes like IL-1B, IL-6, IL-8, and TNF-α itself and contributes to the inflammatory airway responses.[(28), (29)] On other hand, guggulsterone with anti-inflammatory steriod activity that inhibit TNF inflammatory pathway through inhibit the activation of pro-inflammatory transcription factors, AP-1 and NFκB that lead to suppress the gene expression of proinflammatory cytokines including TNF-α and reducing cellular and molecular evets associated with asthma[(30)] .

6- The effect of guggulsterone on IgE levels in the serum of OVA-induced asthma in rats:

In figure 6 appeared that highly significant elevation ($p < 0.001$) in serum levels of IgE was appeared in rats of group II compared to group I. **Also IgE levels in serum for rats of treated groups** (IV & V), **were significantly elevated by ($p<0.05$), ($p<0.01$), respectively compared to IgE levels in serum for rats of group I**. Furthermore, in treated groups (III, IV & V), the serum levels of IgE were significantly reduced by 23 % ($P < 0.001$), 33% ($P < 0.001$) & 51% ($p<0.001$), respectively compared to group II. But, treating non-sensitized rats with guggulsterone observed non-significant different in serum levels of IgE.

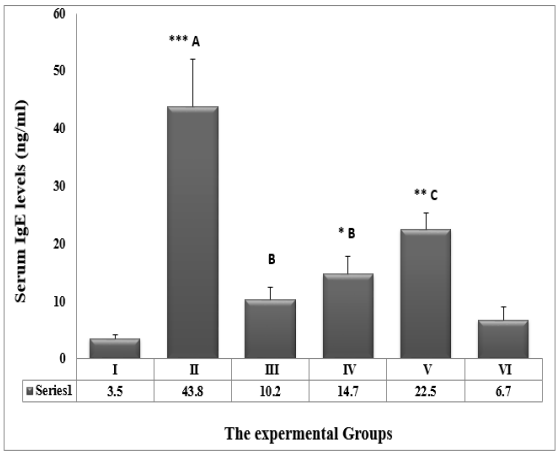

Figure 6. The effect of guggulsterone on IgE levels in the serum of OVA-induced asthma in rats.

Values are indicated as means ± Std. Error (n=8) for each group. Group I: Control group, Group II: Positive control group (with sensitization), Group III: Guggulsterone (25 mg/kg) with sensitization, Group IV: Guggulsterone (50 mg/kg) with sensitization, Group V: Prednisolone (4.12 mg/kg) with sensitization, Group VI: guggulsterone (50 mg/kg) without sensitization. *** symbol referred to significant different ($p<0.001$) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different ($p<0.05$). Series 1 referred to means of WBC counts in BALF for each group.

IgE antibody is immune marker associated with asthma, which is play a an important role in asthmatic pathway. IgE levels in serum of rats of sensitized group II are significantly higher than normal control group I. In the same line, Afshari *et al* (2007) & Froidure *et al* (2016) studies, also showed that higher level of IgE in asthmatic group than control group. IL4 is cytokine that produce switching in B-cells and responsible in elevated IgE synthesis. IgE occupied to the high-affinity of FcεRI (IgE receptor) on mast cells and stimulated the produce of transcription of cytokines, vasoactive mediators, and de novo synthesis of leukotrienes and prostaglandins, which are increasing airway inflammation. But guggulsterone treated groups are significant reduce IgE levels through the ability of guggulsterone for suppression of gene expresion of proiflammatory cytokines specifically IL-4 and reducing the release of IgE from inflammatory cells in the lung.[8] & [22]

Conclusion

Guggulsterone has anti-inflammatory activity on OVA-induced asthma in rats as evident by significant reducing in WBC count in BALF, proinflammatory cytokines (IL-4, IL-5, IL-33 & TNFα) and immune marker IgE antibody in serum. Further studies are required for future work to investigate the beneficial anti-inflammatory activity of guggulsterone for treatment of other inflammatory diseases.

References

1. Fehrenbach H, Wagner C, Wegmann M. Airway remodeling in asthma: what really matters. Cell Tissue Res. 2017;367(3):551-69.
2. Kudo M, Ishigatsubo Y, Aoki I. Pathology of asthma. Frontiers in microbiology. 2013;4:263.
3. Matsumoto H, Niimi A, Takemura M, Ueda T, Minakuchi M, Tabuena R, et al. Relationship of airway wall thickening to an imbalance between matrix metalloproteinase-9 and its inhibitor in asthma. Thorax. 2005;60(4):277-81.
4. Lloyd CM, Hessel EM. Functions of T cells in asthma: more than just T(H)2 cells. Nature reviews Immunology. 2010;10(12):838-48.
5. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005;23(5):479-90.
6. Komai-Koma M, Brombacher F, Pushparaj PN, Arendse B, McSharry C, Alexander J, et al. Interleukin-33 amplifies IgE synthesis and triggers mast cell degranulation via interleukin-4 in naive mice. Allergy. 2012;67(9):1118-26.
7. Brightling C, Berry M, Amrani Y. Targeting TNF-alpha: a novel therapeutic approach for asthma. The Journal of allergy and clinical immunology. 2008;121(1):5-12.
8. Afshari J, Hosseini R, Farahabadi S, Heydarian F, Boskabady MH, Khoshnavaz R, et al. Association of the Expression of IL-4 and IL-13 Genes, IL-4 and IgE Serum Levels with Allergic Asthma. Iranian journal of allergy, asthma, and immunology. 2007;6:67-72.
9. Sairkar P, Sharma A, Shukla N. Estimation of guggulsterone E and Z in the Guggul based commercial formulations using High Performance Thin Layer Chromatography (HPTLC). J Pharm Bioallied Sci. 2016.
10. Shah R, Gulati V, Palombo E. Pharmacological Properties of Guggulsterones, the Major Active Components of Gum Guggul. Phytotherapy research : PTR. 2012;26:1594-605.

11. Sirisha K. Pharmacokinetic and Pharmacodynamic Interactions of Atorvastatin with Guggulsterone in hyperlipidemic rats. International Journal of Pharmaceutical Sciences and Research. 2014;5:4262-8.

12. Gebhard C, Stämpfli SF, Gebhard CE, Akhmedov A, Breitenstein A, Camici GG, et al. Guggulsterone, an anti-inflammatory phytosterol, inhibits tissue factor and arterial thrombosis. Basic research in cardiology. 2009;104(3):285-94.

13. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27-31.

14. Dar M, Masoodi H, Mohi-ud-din R. Guggulipid as an adjuvant therapy for Hyperlipidemia. International Journal of Medicine Research. 2018;3(1):17-22.

15. Algaem MA, Numan IT, Hussain SAJAJoPS. Effects of Valsartan and Telmisartan on the LungTissue Histology in Sensitized Rats. 2013;1(4):56-60.

16. WANG Tong, YIN Kai-sheng, LIU Kou-yin LG-j, LI Yu-hua, Jun-di C. Effect of valsartan on the expression of angiotensin II receptors in the lung of chronic antigen exposure rats (Chin Med J 2008;121(22):2312-2319).

17. Salmon M, Walsh DA, Huang TJ, Barnes PJ, Leonard TB, Hay DW, et al. Involvement of cysteinyl leukotrienes in airway smooth muscle cell DNA synthesis after repeated allergen exposure in sensitized Brown Norway rats. Br J Pharmacol. 1999;127(5):1151-8.

18. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD. Asthma. Nature reviews Disease primers. 2015;1(1):15025.

19. Monteseirín J. Neutrophils and Asthma. Journal of investigational allergology & clinical immunology : official organ of the International Association of Asthmology (INTERASMA) and Sociedad Latinoamericana de Alergia e Inmunología. 2009;19:340-54.

20. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Signal transduction and targeted therapy. 2017;2:17023.

21. Gour N, Wills-Karp M. IL-4 and IL-13 signaling in allergic airway disease. Cytokine. 2015;75(1):68-78.

22. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol. 2009;1(6):a001651-a.

23. Dunican EM, Fahy JV. The Role of Type 2 Inflammation in the Pathogenesis of Asthma Exacerbations. Ann Am Thorac Soc. 2015;12 Suppl 2(Suppl 2):S144-S9.

24. Garcia G, Taille C, Laveneziana P, Bourdin A, Chanez P, Humbert M. Anti-interleukin-5 therapy in severe asthma. European respiratory review : an official journal of the European Respiratory Society. 2013;22(129):251-7.

25. Li R, Yang G, Yang R, Peng X, Li J. Interleukin-33 and receptor ST2 as indicators in patients with asthma: a meta-analysis. Int J Clin Exp Med. 2015;8(9):14935-43.

26. Momen T, Ahanchian H, Reisi M, Shamsdin SA, Shahsanai A, Keivanfar M. Comparison of Interleukin-33 Serum Levels in Asthmatic Patients with a Control Group and Relation with the Severity of the Disease. International journal of preventive medicine. 2017;8:65.

27. Newton R, Shah S, Altonsy MO, Gerber AN. Glucocorticoid and cytokine crosstalk: Feedback, feedforward, and co-regulatory interactions determine repression or resistance. The Journal of biological chemistry. 2017;292(17):7163-72.

28. Nakae S, Ho L, Yu M, Monteforte R, Iikura M, Suto H, et al. Mast cell-derived TNF contributes to airway hyperreactivity, inflammation, and TH2 cytokine production in an asthma model in mice. The Journal of allergy and clinical immunology. 2007;120:48-55.

29. Brightling C, Berry M, Amrani Y. Targeting TNF-alpha: a novel therapeutic approach for asthma. The Journal of allergy and clinical immunology. 2008;121(1):5-10; quiz 1-2.

30. Burris TP, Montrose C, Houck KA, Osborne HE, Bocchinfuso WP, Yaden BC, et al. The hypolipidemic natural product guggulsterone is a promiscuous steroid receptor ligand. Mol Pharmacol. 2005;67(3):948-54.