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Original Research Article

A Study Effects of Licorice Plant and Prednisolone on Some Physiological and Immunological Parameters in Experimental Model

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Abstract

The present study conducted thirty of adult male white rabbits to uncover the impact of Licorice in minimize the signs associate degreed symptoms after induction of allergic bronchial asthma in rabbits and elucidate what extent it will show an beneficial effects herbs therapy, in state use corticosteroids medical care. The rabbits haphazardly divided into 5 groups and albumen sensitizer was used to stimulate allergic reaction beside to use sensitization boost challenge dose that emphasize by the presence of the clinical symptoms of innate reflex, wheezing, chest tightness and shortness of breath. As well as, to changes within the level of total white blood corpuscle count, Eosinophils measurements, Interleukin-5 estimation and body temperature measurements. Meanwhile, eight days after induction allergic reaction. Extraction of Licorice was given in a variety dose of (160 and 80) mg/kg orally was administrated for eight days. Blood collected samples were done on the day sixteen and thirty, for investigate the above previous parameters through collected blood serum samples. statically analysis fore results were showed that the albumen action considerably ($p \leq 0.05$) induce allergic response in those of experimental rabbits associate degreed show an elevation with total white blood corpuscle, Eosinophils and Interleukin-5 levels. Whereas orally treating rabbits with root extract observe a big decrease ($p \leq 0.05$) within the total white blood corpuscle, Eosinophils and with the degree of Interleukin-5. As these effects of (the plant extract) wherever analyzed in comparison with recorded noticeable effects in those of glucocorticoid treated group and those healthy management teams severally.

Key Words: Licorice; *Glycyrrhiza glabra;* flavonoids; IL-5; asthmarabbit's model.

الخلاصة

اجريت الدراسه الحاليه على ٣٠ من الارانب الذكور البيضاء وذلك للكشف عن تاثير نبات عرق السوس (Licorice) في تخفيف حدة العلامات والاعراض المستحثه بواسطة البومين البيض في الربو القصبي التحسسي في الارانب وتوضيح الى اي مدى يمكن الاستفاده من التاثير الناتج عن استخدام العلاج العشبي وذلك بدلا من استخدام العلاج بالكورتيزونات (Corticosteroids). قسمت الارانب بشكل عشوائي الى خمسة مجاميع حيث تم استخدام محسس البومين البيض لتحفيز التفاعلات التحسسيه من خلال تاكيد ظهور العلامات السريريه المشتمله على الصفير وضيق في التنفس وقلته، بلاظافه الى التغيرات في مستويات كل من كرياتالدم البيضاء الكلي ، الخلايا الحامضيه و انترلوكين ٥ (IL–5) وقياس التغيرات في درجة الحراره وتم المعالجه لمدة ثمانية ايام بعد استحثاث التفاعل التحسسي القصبي (الربو). تم اعطاء مستخلص عرق السوس بجرع مختلفه (١٦٠و٨٠) ملغم / كغم من وزن الجسم وبشكل جرع فمويه ولمدة ثمانية ايام متتاليه .جمعت عينات الدم لفصل مصل الدم في يوم ١٦ ويوم ٣٠ من التجربه لغرض التقصي عن المعايير المذكوره اعلاه . التحليل الاحصائي للنتائج بين بان هناك تاثيرات ذات مستوى معنوي لالبومين البيض اثر احداث الحساسيه في مجموعة حيوانات التجربه والتي تزامنت مع زيادة في عدد كريات الدم البيضاء الكلي ، الخلايا الحامضيه وكذلك ارتفاع مستوى انترلوكين ٥ بشكل معنوي. في حين بينت الدراسه ان مجموعة الارانب التي عولجت فمويا بمستخلص نبات عرق السوس قد اظهرت انخفاض معنوي في العدد الكلي لكريات الدم البيضاء ، عدد الخلايا الحامضيه وكذلك انخفاض في مستوى انترلوكين ٥. وقد بين تحليل النتائج ان مستخلص نبات عرق السوس بالمقارنه مع ما سجل من نتائج مؤثره في مجموعه احداث التحسس المعالجه بالكلوكوكورتيكويد في حيوانات التجربه بالاظافه للمقارنه مع مجموعة السيطره .

الكلمات المفتاحية : عرق السوس، غلاسيرايزا كلابارياً، الفلافينات. انترلوكين ٥، موديل الربو الارانب.

Introduction

Asthma is one of the most current chronic diseases, with an estimated 300 million patients afflicted by this disease worldwide. The Global Initiative for Asthma [1] 2004 estimated that more than 10% of the population in Australia, Brazil, Canada, New Zealand, Peru, England, and United States had asthma. The prevalence of asthma is expected to increase, and there may be an additional 100 million peoplewith asthma by 2025 [1]. A 2012 survey revealed that 25.5 million people in the United States alone [2]. Moreover, asthma prevalence has increased from 3.1% in 1980 [3] to 8.3% in 2012 [4, 5]. Asthma is a disease involve a several distinct endotypes manifested by contribution of cellular and molecular biomarkers. The joining of endotypes character with clinical phenotypes character has extremely preceding our recognizing of asthma pathophysiology and the process of novel targeted therapeutics [6]. One of the best-characterized endotypes of asthma is the patient population with eosinophilic airway inflammation, which accounts for approximately 40–60% of patients with severe asthma [7,5]. Several important observations in human initially supported the hypothesis that eosinophils play a critical role in the pathogenesis and severity of asthma. An increased eosinophil count is associated with increased asthma severity, frequency of exacerbations and mortality in patients with asthma [8]. Several studies have demonstrated that sputum eosinophils increase during exacerbations and that increased numbers of eosinophils in the peripheral blood and airways of patients with asthma correlate with disease severity. Perhaps most importantly, persistent, eosinophilic airway inflammation increases the risk of subsequent exacerbations [9]. For example, markers of eosinophilic airway inflammation increase well before the onset of exacerbations induced by corticosteroid withdrawal, and sputum eosinophil number predicts loss of asthma control after corticosteroid reduction or discontinuation [12]. The root of licorice (*Glycyrrhiza glabra*) is one of the most frequently used natural medicines in the world, and has been described as ‘the grandfather of herbs [12]. It has been used medicinally in both Western and Eastern countries for more than 4000 years [13]. It has been traditionally used for respiratory, gastrointestinal, cardiovascular, and skin disorders. The biological and pharmacological activities of *Glycyrrhiza glabra* have been widely studied as its long history and biologically active constituents are still interesting to many research groups [15]. A large number of clinical and experimental studies reported its useful biological properties such as antioxidant, immuno-modulatory, cardioprotective, anti-inflammatory, antiviral and anticacer effects [18]. Recently, *Glycyrrhiza glabra* is reported to have neurological properties such as antidepressant, anxiolytic, and anticonvulsant effects [19]. Chemical analysis of *Glycyrrhiza glabra* root extract showed the existence of triterpenes (glycyrrhizin, glycyrrhetic acid and liquirtic acid), flavonoids (liquirtin and formononetin) and various other substances [20]. The pattern of inflammation in asthma is characteristic of allergic diseases, with similar inflammatory cells seen in the nasal mucosa in rhinitis (Figure 2). An indistinguishable pattern of inflammation is found in intrinsic asthma, perhaps reflecting local rather than systemic IgE production. Acute-on-chronic inflammatory episodes, corresponding to exacerbations of asthma, are usually triggered by upper respiratory tract virus infections or allergen exposure. Although the common pattern of inflammation is eosinophilic, some patients with severe asthma and asthmatics who smoke show a predominantly neutrophilic pattern that is less sensitive to corticosteroids. Several inflammatory cells are involved in asthma [21].

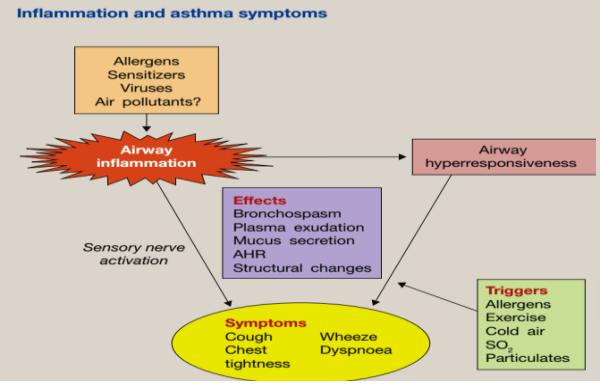

Figure 1: Inflammation in asthmatic airways Results in airway hyper responsiveness (AHR), Inflammation can directly lead to symptoms through , The Activation of Airway sensory nerves.(Barnes, 2011)

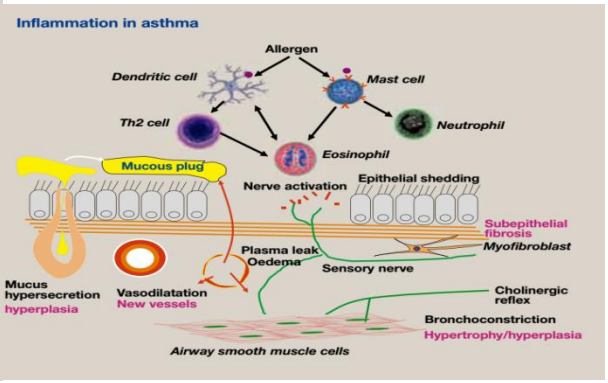

Figure 2: several inflammatory cells interact through the release of inflammatory mediators

Materials nad Methods

Laboratory animals:

The experimental model used of this search were consisted of thirty male adult rabbits with ranging weighing: (2.100-2.600) kg and its age were ranging from(11-16) months. The rabbits were house within the animal house in school of medicine / University of Babylon. Animals were left one month for an adaptation prior the experiments. Animals were kept in optimized stainless steel standard cages as 6 rabbits in each cage. With freely feeding at fresh green vegetables, chaw pellets and free of water. The rabbits were kept under a same circumstances of the temperature range of (22-25°C), relative humidity of 75±5% and light regime of 12 hours' light, and 12 hours' darkness divided into five groups, each group was consists of six male rabbits used according to parts of experimental design.

Experimental Protocol:

The experimental protocols are adopted from (Kamaruzaman *et.al.*,2014). As the Induction of allergic airway inflammation is performed by intraperitoneal (i.p) injection of the Ovalbumin (OVA) 0.1 mg and 10 mg of aluminium hydroxide disolve in 2 ml of phosphate buffer saline (PBS) in day 1 and a booster challenge dose through second sensitization in day 14 of the immunization program shown in the following groups.

Control Group (Group1):

Control group comprised of six rabbits injected (i.p) twice / experiment with phosphate buffer saline (PBS) first injection had been done at the day 1 and the second injection had been done at the day 14 of the experiment and treatment starting from day 23 to 30 orally with 2 ml (PBS), and the blood sample were collected then all animals are sacrificed on day 31 of the experiment.

Asthma Stimulate Group (Group2):

OVA group comprised of six rabbits injected (i.p) twice / experiment with OVA 0.1mg and 10mg of aluminum hydroxide dissolve in 2ml of PBS by immunizationat day 1 of the experiment and booster challenge dose at day 14 of the experiment, and blood sample were collected then all animals are scaron day 15 of the experiment.

Prednisolone 1mg/kg B.W (Group 3):

Prednisolone group comprised of six rabbits injected (i.p) twice / experiment with OVA 0.1mg and 10mg of aluminum hydroxide disolve in 2ml of PBS by immunization at day 1 of the experiment and booster challenge dose at day 14, and then treatment starting from day 23 to 30 orally with 1mg/kg prednisolone (Davis, 1971). And blood sample were collected then all animals are sacrificed on day 31 of the experiment.

Licorice Root 160 m/kg B.W (Group 4):

Licorice group comprised of six rabbits injected (i.p) twice / experiment with OVA

0.1mg and 10mg of aluminum hydroxide disolve in 2 ml of PBS by immunization at day 1 of the experiment and booster challenge dose at day 14, andtreatment starting from day 23 to 30 orally with 160 mg/kg of *Licorice* Root extract, and blood sample were collected then all animals are sacrified on day 31 of the experiment.

Licorice root + prednisolone (80+0.5) mg/kg B.W (Group5):

A combination of ***Licorice*** root plus prednisolone group comprised of six rabbits injected (i.p) twice / experiment with OVA 0.1mg and 10mg of aluminum hydroxide disolve in 2ml of PBS by immunization at day 1 of the experiment and booster challenge dose at day 14, and treatment starting from day 23 to 30 orally with *Licorice* Root 80 mg/kg plus prednisolone 0.5mg/kg together, and blood sample were collected then all animals are sacrified on day 31 of the experiment.

Statistical Analysis:

The data were analyzed by using computer Statistical Package for Social Science Software (SPSS, version 20) program. As well as, to one Way ANOVA test expressed data and mean± SD for a continuous variable. The p-value of less than 0.05 was statistically significant and highly significant for a p-value of less than 0.05 [22].

Results

Impact of Prednisolone on Total WBCs estimation in the Control, Asthma Motivate and Treated Rabbits.

The results in table (1) show a significant increment of total WBCs estimation for both of motivate ovalbumin rabbits group in a dose of (ova 0.1ml and 10mg of aluminum hydroxide in 2 ml of PBS) and for asthma treated (administer) prednisolone groups (6.380±1.190; 11.880±1.044) in comparison with control group (4.340±0.808) respectively as were it daily treated with prednisolone dose of (1mg/kg of body weight) along time such period according to planning program which continue for 8 days, start from the days 23 until the days 30 of the experiment.

Effect of bisector doses of Licorice, Prednisolone and medley doses on Total WBCs estimation in Asthma Motivate Rabbits.

Presented data in Figure (3) observe, that asthma motivate rabbits when treated with 2 different doses of Licorice plant root extract as (160 mg/kg) as a first dose and as a combination of second dose of (80 mg/kg of *Licorice* plus 0.5 mg/kg of prednisolone to given be together) of body weight are appear with a significant decrease ($P \leq 0.05$) of total WBCs measurement of (4.840±1.221 and 4.960±1.289) respectively in comparison with the asthma motivate , prednisolone treated groups and in reflect with control group (4.340 ± 0.808) in consistence with the planning program which continue for 8 days from the days 23 until the days 30 prior the sacrificing day of the experiment.

Table 1: Total WBCs estimation (Mean ± SD) for each of control, asthma motivate and treated rabbits.

Groups	Total WBC estimation x 10^3 cells/µL (Mean ± SD)
Control Group	4.340 ± 0.808
Asthma Rabbits (ovalbumin motivate)	6.380±1.190
Asthma Rabbits treated with 1mg/kg of prednisolone	11.880±1.044

SD: Standerdeviation;**WBC**s: White blood cells

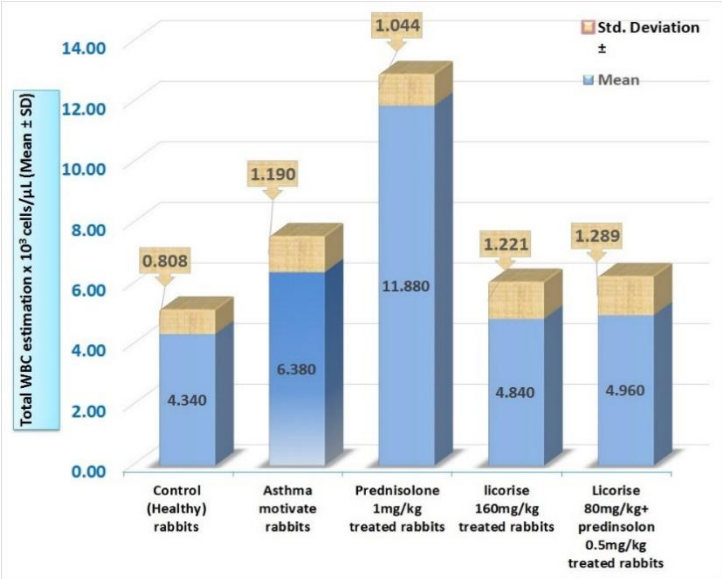

Figure 3: A comparison effect of prednisolone and *Licorice* rooton the Total WBCs Estimation (Mean ± SD) In asthma motivate, control and treated rabbits.

Impact of prednisolone on Eosinophils estimation in the control, Asthma motivate, and Treated Rabbits.

Such inflammation immune cells as, eosinophils wereestimated after use of same agent of immunogen ovalbumin sensitizer, and the immunization program was standard of i/p injection schedule. Results in table (2) is reveal of significant increase ($P \leq 0.05$) of eosinophils in asthma motivate rabbits (6.20±0.836) % in comparison with eosinophils of control (healthy) rabbits (1±0.707) % as we see in Table (1). Also, that table, (2), pointed that there is a significant decrease ($P \leq 0.05$) in eosinophils measurement (1.60±0.894)% in comparison with asthma motivate rabbits (6.20±0.836)% when asthmatic rabbits treated by prednisolone along for 8 days begin from the day 23 until the day 30 prior the sacrificing day of the experiment.

Effects of bisector doses of *Licorice*, Prednisolone and medley doses on Eosinophils estimation in Asthma motivate Rabbits.

Obtaining result of the two doses of *Licoricr root* extraction as in Figure (4) observe significant decrease ($P \leq 0.05$) in eosinophils measurement in asthma motivate treated rabbits and control rabbits after adminster a dose of (160 mg/kg) body weight of *Licorice Root* extract (1.40±0.547) % and (0.6±0.547) for the middle mixed doses of (*Licorice* 80 mg/kg + prednisolone 0.5 mg/kg) of body weight respectively through the period of asthma motivate rabbits, asthma persistence and immunization program comparing with (1±0.707) % control (healthy) rabbits.

Hence, along the period of immunization program the result revealed that a combination of treated rabbits were significantly potent to reduce eosinophils and return to the normal value as in Figure (4).

Table 2: Eosinophils estimation (Mean ± SD) for each of control, Asthma motivate and treated Rabbits

Groups	Eosinophils% estimation(Mean±SD)
Control Group	1±0.707
Asthma Rabbits (ovalbumin motivate)	6.20±0.836
Asthma Rabbits treated with 1mg/kg of prednisolone	1.60±0.894

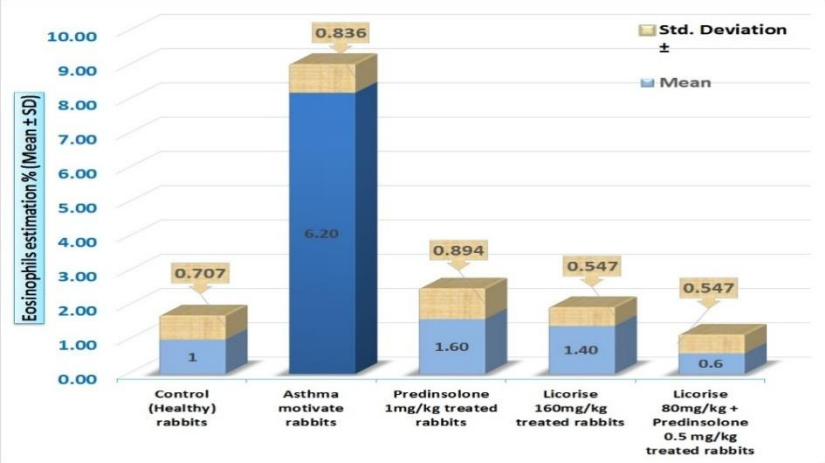

Figure 4: A comparison Effect of prednisolone and *Licorice* root on the Eosinophils estimation (Mean ± SD) in Asthma motivate, Control and Treated rabbits.

Impact of prednisolone on body temperature C° in each of control (healthy); Asthma motivate Rabbits.

Ovalbumin act as an immunogen that expectantly enhance variance in body temperature when used as sensitizer.

Hence, these are proved knowledge, results in Figure (5) exhibit that body temperature is increased significantly ($P \leq 0.05$) in asthma motivate rabbits with ovalbumin injection (39.90 ± 1.216) C° compared with control (37.036 ±0.251) and treated rabbits with 1mg/kg body weight of prednisolone (38.433±1.439) C° and a combination dose of Licorice and prednisolone(38.7.22±1.504) respectively.

Effects of bisector doses Licorice, Prednisolone and medley doses on body temperature °C in control (healthy); Asthma motivate Rabbits.

First dose of ***Licorice*** root extract 160 mg/kg body weight as shown in figure (5) were show significant decrease ($P \leq 0.05$) in body temperature in treated asthma motivate rabbits (38.722 ±1.504) °C which starts after the present of asthma and persist for 8 days after the immunization program of the experiment in comparison with asthma motivate (39.90 ± 1.216) °C rabbits respectively. Meanwhile, Figure (5) also show, significant decrease ($P \leq 0.05$) in body temperature (38.726± 1.446) C° through the treatment with the second bisector dose of 80 mg/kg body weight of ***Licorice*** root extract plus prednisolone 0.5 mg/ kg in comparison with asthma motivate rabbits (39.90 ± 1.216) °C respectively .

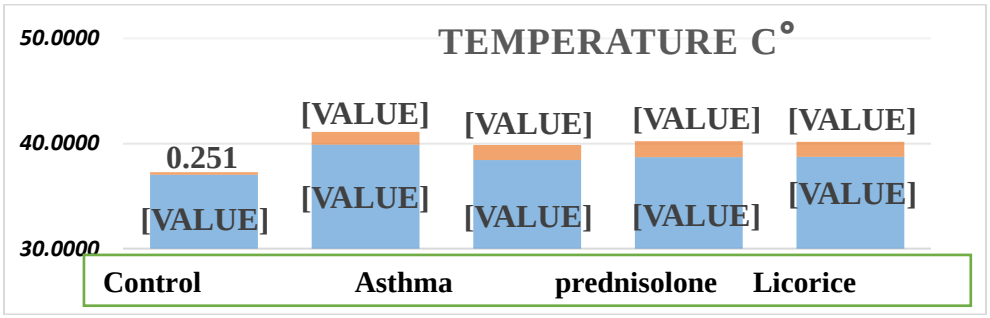

Figure 5: A comparison Effect of prednisolone and *Licorice* root on the body temperature °C in control, asthma motivate and treated Rabbits.

Impact given of Prednisolone on Interleukin-5 Levels in each of control (Healthy) and Asthma motivate Rabbits.

Study shows a significant increase ($P \leq 0.05$) in Interleukin-5 levels in asthma motivating rabbits with ovalbumin injection (245.53±38.841) pg/ml compared with healthy rabbits (20.53±2.093) pg/ml, as shown in Table (3).

Whereas, the table show a significant decrease ($P \leq 0.05$) in the levels of Interleukin-5 (27.12±3.439) pg/ml when that asthma motivate rabbits given prednisolone (administer) daily ata dosage of one mg/kg of body weight at that time of such period. According to the planning program which continue for 8 days begin from the day 23 until the day 30 prior the sacrificing day of the experiment.

Effects bisector of Licorice, Prednisolone and medley manner on the Interleukin-5Levels in Asthma motivate Rabbits.

Objective results data of *Licorice* root shows a cleardecrease in the levels of interleukin-5, to significant value ($P \geq 0.05$) in motivate asthma rabbits (26.04±2.062) pg/ml, through the received a dose of (160 mg/kg) body weight of *Licorice root* extract for 8 days. While, after receiving a combination treatment with a dose of (80 mg/kg) plus prednisolone (0.5 mg/kg) of body weight of *Licorice* extract for 8 days. There was a significant decrease ($P \leq 0.05$) in interleukin-5 levels (23.86±2.213) pg/ml, which may give precise indication for the effectiveness of *Licorice root* extract in reduction of asthma. Beside the statistical provident values of combination dose (80+0.5) mg/kg of body weight *licorice* extract in compare with prednisolone use alonewere significant obvious as shown in Figure (6).

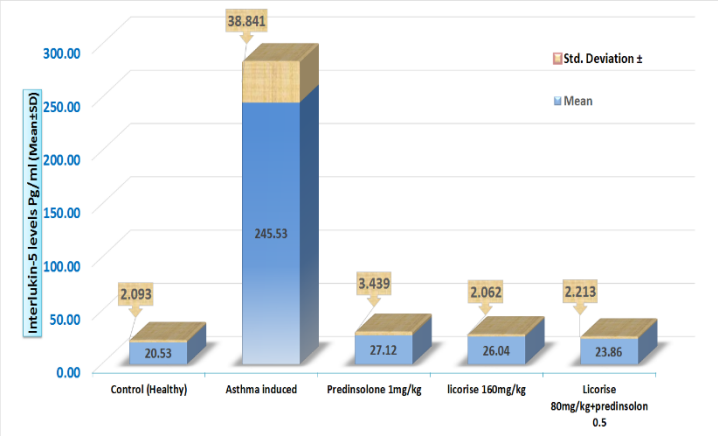

Figure 6: A comparison effect of prednisolone and *Licorice* on the scales of Interleukin-5 (Mean ± SD) in asthma Motivate, control and treated Rabbits.

Table 3: Interleukin-5 (Mean ± SD) levels in each of control, asthma motivate and treated rabbits

Groups	IL5 levels pg/mL(Mean ± SD)
Control Group	20.53 ±2.093
Asthma Rabbits (ovalbumin motivate)	245.53 ±38.841
Asthma Rabbits treated with 1 mg/kg of prednisolone	27.12 ±3.439

Discussion

Effects of oral Licorice intake on the total WBC and Eosinophils estimation of Treated groups:

Presented data in Figures (3) and (4) are pointing a significant increase in total WBC, neutrophils and eosinophils measurements and expand their role in exhibiting of asthma signs and symptoms through releasing of inflammatory mediators of cytokines, chemokines as reflect to use of OVA and going to play a big role in distinguishing allergy and asthma characterizations. Meanwhile, it shows a significant decrease ($p \leq 0.05$) in neutrophils and eosinophils that have estimated contribute to the inhibitory effect of flavonoids of *Licorice*, with the present work.

Asthma is a chronic respiratory disease, and such therapy as corticosteroid is inadequate and has many adverse effects. Therefore it needs better therapeutic agents that to be preferable of natural origin, manifested by modest or no adverse effects. As the Glycyrrhizin (GRZ), is the active constituents of the (Licorice root), which show efficacy on relief asthmatic features. Rabbits sensitized and challenged with (OVA) to induction of asthmatic features, such as allergic asthma and inflammatory response development. Treated asthmatic group after OVA-stimulation has been evaluate for reversal effect, presented on those of asthmatic rabbits. As well as, for other parameters associated with asthma like interleukin (IL-5) and immunoglobulin OVA-specific (IGE) were also detected by ELISA. Results record decrease in IL-5 and eosinophil. Addition to, it reduce level of OVA-specific IgE in serum. So results recorded that Licorice of the asthmatic features in rabbits and it could naturally have high value towards developing a better therapeutic plant agent after time [23].

Most of the activated cells in the pathogenesis of allergic asthma, is eosinophils that present as prominent cell type, and an increased number in the airways is correlated with the severity of asthma ; accordingly, eosinophils is often a target for the therapy of various inflammatory diseases including allergic asthma. A former study illustrated that recruitment of eosinophils into bronchus-associated lymphoid tissue (BALF) was clearly observed as expected in OVA-sensitization asthma. and from here study found elevate leukocyte infiltration, goblet-cell hyperplasia, and mucus hypersecretion, and these finding in a good agreement with previous study of [24].

Eosinophilic cells concentrate in high numbers through the lungs of asthmatic patients, and are consider of importance in the pathogenesis of asthma. A potent eosinophil chemoattractant is produced in the asthmatic lung. These are of a small protein, and called chemokine eotaxin, that produce in a number of different cell types, and is stimulated by interleukin-4 and interleukin-13, which are synthesized by T-helper (TH) 2 lymphocytes. Low molecular weight compounds have been developed that can block the eotaxin receptor C-C chemokine receptor (CCR) 3, and prevent stimulation by eotaxin [25].

However, all the mentioned improved data were coincided in good relation with our outcomes of the present findings this search *Licorice* effects on the hematological parameter and particularly with total WBC and eosinophils.

In a prior study of Arjun *et al*. [26]pointed that flavonoids played the major role in asthma and in quite possible that GRZ may block eotaxin production in vivo as well to blocked infiltration of the eosinophils in the lungs.

In conclusion, GRZ important soften the asthmatic features such as OVA induced immediate airway constriction, airway hyperreactivity, and eosinophilia and lung inflammation.

Impact of oral Prednisolone intake on total WBCs and Eosinophils estimation of Treated groups:

Obtained Figures (3) and (4) demonstrate the impact of the prednisolone a member of corticosteroids group and it similar activities on hematological criteria.

WBC count reflects the leukocytes distribution in blood. Percentage of WBCs normophysiological count include; neutrophils of 60-70%, lymphocytes of 28%, monocytes of 5%, eosinophils of 2-4%, and basophils of 0.5% [27]. Neutrophils located within two compartments; marginal compartment (neutrophils were attached to the endothelium of the blood vessel) and circulating compartment (those circulating in the blood vessels along with other cells) [28]. This distribution is important as neutrophils traveling on the endothelial surface within the lumen of the blood vessel (i.e., in the marginal compartment) are not resonance in a WBC count. Only freely polymorphonuclear (PMN) circulate within the circulatory compartment will be used for counting. Therefore, anything that causes the marginal neutrophils to detach from the endothelial surface of the blood vessel wall will result in a high concentration of neutrophils in the circulating compartment which elevate total WBC count. Glucocorticoids are known to do this [5] Schwartzman and Cidlowski [30] had been document the side effect of corticosteroid on a hematological changing such as increased hemoglobin and red cell content of blood, possibly through retarding erythroid-phagocytosis. Corticosteroids also affect circulating WBCs via increased PMN leukocytes in blood as a result of increased rate of the entrance from marrow and a decreased rate of removal from the vascular compartment. In concomitant with disparity of lymphocytes, eosinophils, monocytes, and basophils decrease in number after treatment with glucocorticoids.

A single dose of cortisol results in a 70% decrease in lymphocytes and a 90% decrease in monocytes, occurring 4 to 6 h after treatment and persisting for about 24 h. Cell numbers then rise 24 to 72 h after treatment. The decrease in lymphocytes, monocytes, and eosinophils is thought to be a consequence of the redistribution of these cells, although certain lymphocytes also undergo glucocorticoid-induced apoptosis. T lymphocytes are more sensitive to glucocorticoid-induced apoptosis than are B lymphocytes, and T-cell subpopulations differ in their glucocorticoid sensitivity. A decrease in basophils occurs by an unknown mechanism.

However, the long-term glucocorticoid treatment leads to increase in neutrophils and decrease in eosinophils, lymphocyte and basophils, that such results were promote the present findings results, as well to concomitant immunosuppression, also leaves patients susceptible to invasive diseases and easy fungal infections [6].

Effects of oral prednisolone intake on the levels ofIL-5 in the serum of treated group:

As, treatment with prednisolones, one of the most potent corticosteroids, known to ameliorate the immunological and pathological affected changing parameters. How ere, study, record no high significant variance are found in the histopathologic and immunologic parameters between the Licorice, prednisolone and in combination groups. Although not to be record a statistical significant value, it is found formerly that rabbits given prednisolone have decreased smooth muscle thickness and fewer goblet cells compared with those receiving glycyrrhizin. The similar effectiveness of prednisolone and glycyrrhizin on long-term structural changes in asthmatic airways. The present study may explanation due to the glucocorticoid-like inhibitory effect of glycyrrhizin in lung cells.[39]

Th2 subtype lymphocytes are seen dominance in atopic individuals, which are secretes IL-4 and IL-5, both of which promote allergic responses. Thus, IL-4, together with the related cytokine IL-13, is

important for isotype switching of B lymphocytes to secrete IgE.

Also, apparently detrimental effect of corticosteroids encompass the IL-4 stimulated production of IgE that is visibl in B lymphocytes treated with hydrocortisone (Zieg *et al*., 1994) and our obtained experimental findings for and IgE were in gose paralledwith those using of corticosteriods, in asthmatic patients after 1 week of treatment with oral doses of prednisolone [5].

So far, corticosteroids downhill the balance towards Th2 cell predominance, may be via suppressing IFN-γ, which normally inhibits Th2 differentiation in response to IL-4, or by suppressing IL-12 production and IL-12 receptor function, which promote expression of Th1 cytokines [34]. Corticosteroids might, therefore, be expected to help polarize the immune response towards the proinflammatory Th2 pattern. Corticosteroids also, reduction survival of T cells and eosinophils by increment apoptosis, contributing to their suppression of chronic allergic inflammation [4].

Effects of oral Licorice intake on levels of IL5 in the serum of treated group:

The present work establishes on the anti-asthmatic characteristics of flavonoids spotted in vitro, it was expected that administration of flavonoids might have beneficial effects on asthma, and indeed, various flavonoids have been shown to suppress airway inflammation and IgE response in asthmatic models. [35].Gained results in Figures (5) observe an inhibitory effect of *Licorice* rootversus allergic asthma induced by (ova intraperitoneally) injecting. However, these effects are mainly depend on the concentration of the flavonoids to prepare anti-asthmatic properties of Licorice extraction. In figures (5) data showed the first treated group 1mg/kg of body wight of prednisolone and second treated group 160mg/kg Licorice of body weight does not have significantly decreased to the IgE and IL-4 levels respectively. While, the combination dose (80+0.5) mg/kg body weight (Licorice+predinsolone) showed maximum significant decrease in their effects toward asthma induced by ova and this is my attribute effects may be due to synergistic effect of licorice and prednisolone and independence concentration of the flavonoids in *Licorice* plant. Chronic inflammation of airways has been identified to play a key role in the pathogenesis of bronchial asthma, which marker as a disease of bronchial smooth muscles [36]. Airway inflammation in asthma is characterized by acute onset airway hyper responsiveness (AHR) and infiltration of inflammable cells, especially eosinophils [37]. Eosinophils have been related to the severity of asthma, and several mediators resulting in eosinophil activation may also lead to contraction of airway smooth muscles [38]. The onset of asthma is generally due to genetic and environmental factors; however, predominant Th2 cell activity has been identified as part of the core pathogenesis of asthma [39]. Activated Th2 cells will secrete cytokines, such as IL-4 and IL-5[40]. IL5 and IL-4 has been proved to promote IgE production and T cell differentiation into Th2 cells [32]. IL-5, however, induces the differentiation, maturation, and migration of eosinophils and localized in the inflamed tissue. [4]. Thus, suppression of Th2 cytokines may have the potential to alleviate chronic airway inflammation and, subsequently, the symptoms of asthma [34]. In order to determine the basic structure of *Licoric* extraction previous studies of [26, 37]found that it consist mainly from the flavonoids, and itssuppressor inhibitory effects on IL-4 , IL5 and IgE production , many kinds of flavones , and their related compounds were then screened. Our search evaluated the beneficial effects of Licorice on inflammatory cell and Th2 overexpression in OVA-sensitized rabbit’s model for asthma. The levels of inflammatory cell (eosinophil) infiltration, Th2 cytokines (IL-5),

eosinophil-related chemokine (eotaxin), IgE and AHR were all significantly enhanced in the OVA-sensitized group. However, all this parameter levels were remarkably reduced in treated group. IL-5 plays a key role in the terminal differentiation, maturation, and survival of eosinophils, and together with the chemokine eotaxin, promotes eosinophil recruitment to the site of inflammation (Kariyawasam and Robinson, 2007; Lee *et al*., 2012). Evidence has been reported that rabbit's model which is in lack of IL-5 expression would not develop AHR or eosinophilia though under allergen threaten [34]. Anti-IL-5 regimens may ameliorate airway allergic inflammation and reduce eosinophils in the blood. In our study, Licorice has been demonstrated to decrease the levels of IL-5and the number of eosinophil and in the lung tissues surrounding the airways also. Although further studies are needed to clarify the detailed mechanism, we suspect that Licorice exerted anti-asthmatic effect through down-regulating Th2 response, especially IL-5 expression, and eosinophilic inflammation was ameliorated subsequently.

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