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A COMPARISON STUDY OF SYNTHETIC CORTICOSTEROID AND NATURAL PLANT LICORICE CRUD EXTRACT IN REGARD TO PROVOKE ALLERGIC ASTHMA IN RABBITS

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Abstract

In this study about 30 of adult male (New Zealand) white rabbits were used to determine the effect of *Licorice* in reduce the signs and symptoms of inducible allergic asthma in rabbits and to whether extent it can be used as an herbal therapy, instead of oral corticosteroids. Rabbits were randomly divided into several groups. Hence, ovalbumin was used to provoke allergy, and the sensitization challenge was confirmed by the presence of the clinical symptoms of sneezing, wheezing, shortness of breath, chest tightness, elevate changes in the level of total WBCs, Eosinophils, Interleukin-4, Interleukin-5. However, after eight days of allergy induction, *Licorice* rootextract were given in a dose of (160 and 80 mg/kg) as it was administrated orally for eight days. Blood samples were collected on day 16 and 31,for cell count assessment, IL4, IL5serum concentration measurements. Statistical analysis were been done and, the results were showed that ovalbumin could significantly ($p \leq 0.05$) induce allergic response for those of experimental rabbits and it show an increased of total WBCs, Eosinophils, Interleukin-4 and Interleukin-5 levels. While oral treated rabbits with *Licorice* root extract showed a significant decrease ($p \leq 0.05$) in the total WBCs, Eosinophils as well as, for serum levels of Interleukin-4 and Interleukin-5. When analyzed these effects of (the plant extract) in comparable with recorded observable effects with those of prednisolone treated group and healthy control groups respectively

Introduction

Despite the development of the pharmaceutical industry in the post industrial revolution period, Medicinal plants may be defined as any plant that has medicinal use such as garlic, foxglove, opium, Teucrium polium, onion, andrographis, paniculata, sweat pill, Betal net,etc³⁰. It is still a resources among the therapeutic optionsin many countries. Phytotherapeutics are extracted from medicinal plants and their active ingredients may relieve symptoms and even cure

diseases, though they occasionally present adverse effects².Hence, from the present issue, knowledge pointed that, several herbs have ability to cure and control diabetes, additionally they have no adverse effects. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities³². Moreover, during the past few years some of the new bioactive drugs isolated from hypoglycemic plants showed antidiabetic activity with more efficacy than oral hypoglycemicagents used in clinical therapy¹³.Also, there are a wide distribution of other herbs and its constitute compounds, such as; leaves, seeds, stalks, root, sheeth, fruit etc. As, it was present with plant **Licorice**, that seriously approved with multifunctional phytotherapeutic activities.

However, from the experience of traditional data, it was reduced signs and symptoms of allergic condition, particularly for asthma and for the other complicated allergic responses But with non or rare adverse reaction and side effects involve complications³.The immune system has develop to play an axial role in host defence against infection as without a functioning immune system individuals would be predisposed to evolve a variety of infections from viruses, bacteria, fungi, protozoa, and multicellular parasites. The key components of a well-functioning immune system include the ability to generate both natural and adaptive immune responses¹⁰The natural innate immune system comprises cellular elements that are both habitant in tissues (i.e. epithelium, macrophages, mast cells) for a rapid response and circulating leukocytes that are recruited from the blood stream (neutrophils, eosinophils, basophils, mononuclear cells, natural killer (NK) cells, and NK T cells)²¹.

Activation of allergen-specific CD4⁺ Th2 lymphocytes indorses the secretion of numerous cytokines, including IL-4, which induces IgE antibody synthesis, and IL-5, which principals to eosinophil recruitment. The manufacture of these cytokines by Th2 cells is regulated by activation of copy factors, like NFAT proteins (nuclear issue of activated T cells), that effect the copy of genes intricate in allergy and eosinophil role.NFATs are voiced in T, B, mast, and natural killer (NK) cells. NFAT compulsory sites are in the promoter regions of genes encoding IL-4 and IL-5 as well as other cytokines and cell surface receptors²².

Materials nad methods

Laboratory animals:

Thirty male adult rabbits (Breed: New Zealand white) weighing: (2000-2500) grams and 12-16 months old were used in the present work. Animals were left one month for an adaptation before the experiments. The rabbits were housed in the animal house in College of Medicine / University of Babylon in standard cages as 6 rabbits in each cage. Every six

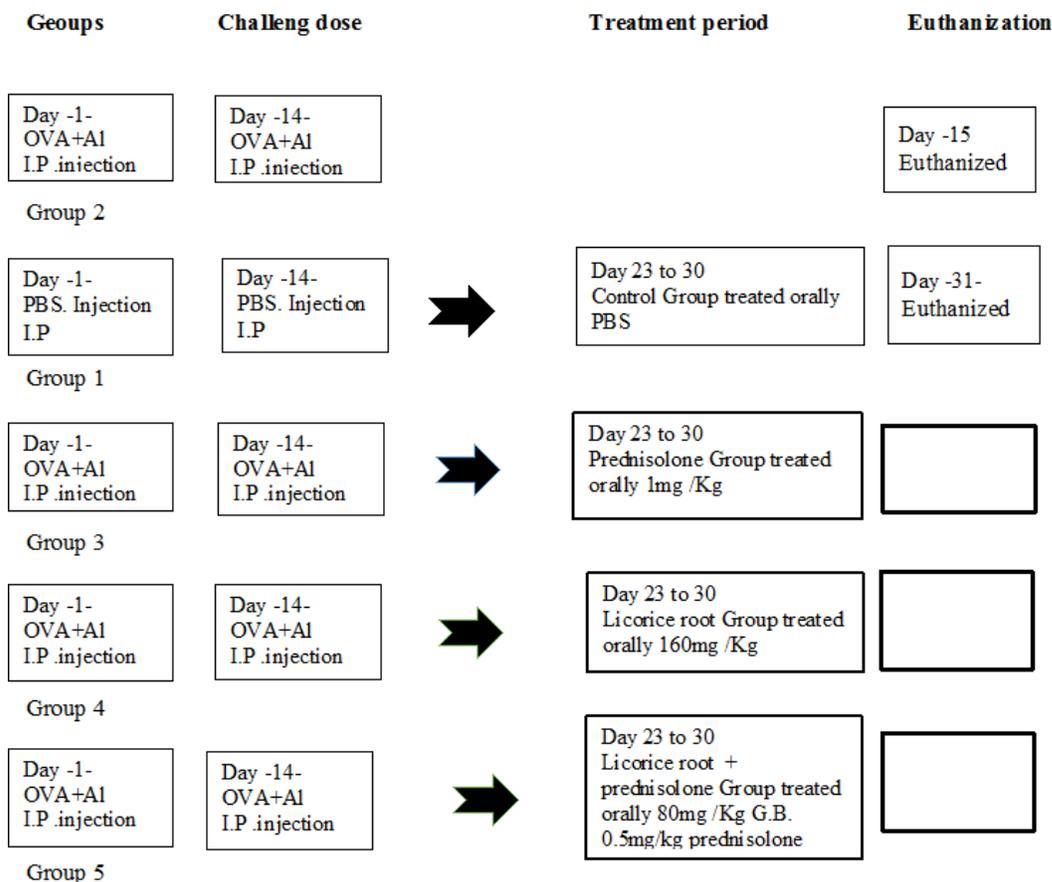
animals were kept in optimized stainless steel cages (1.43 m length, 0.50 m width, and 1.28 m high) and they had been freely feeding on fresh green vegetables, chaw pellets and water. The rabbits were preserved under the same conditions of the temperature (22-25 C°), relative humidity of 75±5% and light regime of 12 hours' light, and 12 hours' darkness. Animals of the study were divided into five groups each group was consists of six male rabbits used for the study design.

Extraction of Licorice Root powder:

The air dried and aerial parts of *Licorice* Root were extracted with ethanol 99.9 %. Using the Soxhlet apparatus 60 minutes for each cycle. The extract was vaporized by using a rotary evaporator. The powdered plant material was collected dried and stored at 5°C in an air tight container without light exposure

Experimental protocol:

The experimental protocols were adopted from Kamaruzaman and his colleagues. Induction of allergic airway inflammation was performed by intraperitoneal (i.p) injection of the Ovalbumin (OVA) 0.1 mg and 10 mg of aluminium hydroxide in 2 ml of phosphate buffer saline (PBS) in day 1 and challenge dose through second sensitization in day 14 of the immunization program as shown within the design study in Figure (1).



Figure(1) Experimental Design.

Statistical Analysis:

Statistical analysis was performed using Statistical Package for Social Science Software (SPSS, version 20). One Way ANOVA test expressed data and mean \pm 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 0.05⁷.

Results

-Impact of prednisolone on total WBCs estimation in the control, asthma motivate and treated rabbits.

The results in table (1.1) showed a significant increase in total WBCs in both of motivate ovalbumin rabbits group in a dose of (ova 0.1ml and 10mg of aluminum hydroxide in 2ml of PBS) and asthma treated (receiving) prednisolone groups (6.380 \pm 1.190 ; 11.880 \pm 1.044) in compare with control group (4.960 \pm 1.289) respectively as it were daily treated with prednisolone dose of (1mg/kg of body weight) along time such period according to schematic program which lasted for 8 days , begin 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.

The present results in figure (1.1) show, that asthma motivate rabbits when treated with 2 different doses of Licorice plant root extract as (160mg/kg) and as a combination dose of (80mg/kg of Licorice plus 0.5 mg/kg of prednisolone to given be together) of body weight were reveal in significant decrease ($P \leq 0.05$) of total WBCs to show (4.840 \pm 1.221 and 4.960 \pm 1.289) respectively in compare with the asthma motivate , prednisolone treated groups and in reflect with control group (4.340 \pm 0.808) in accordance with the schematic program which lasted for 8 days from the days 23 until the days 30 of the experiment .

Table (1.1) Total WBCs estimation (Mean \pm SD) for each of control, asthma motivate and treated rabbits.

Groups	TotalWBCestimation10 ³ cells μ L(Mean \pm SD)
Control Group	4.340 \pm 0.808
Asthma Rabbits (ovalbumin motivate)	6.380 \pm 1.190
Asthma Rabbits treated with 1 mg/kg of prednisolone	11.880 \pm 1.044

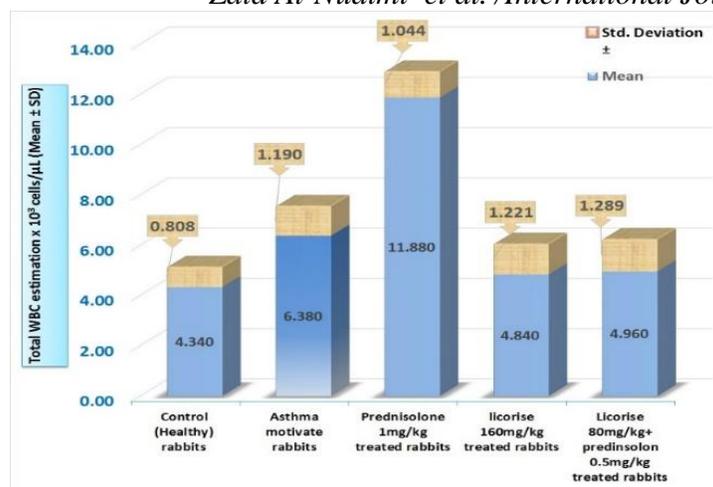


Figure (1.1) A comparison effect of prednisolone and *Licorice root* on the Total WBCs estimation (Mean ± SD) in asthmate, control and treated rabbits.

- Impact of prednisolone on Eosinophils estimation in the control, asthmate, and treated rabbits.

Inflammation cells such as, eosinophils were being estimated after use of same style of immunogen ovalbumin sensitizer, and the immunization program was standard of i/p injection schedule. Result in table (1.2) was observed significant increase ($P \leq 0.05$) of eosinophils in asthmate rabbits (6.20 ± 0.836)% in comparison with eosinophils of control (healthy) rabbits (1 ± 0.707)% as we seen in table (1.2).

Also, that table (1.3) pointed that there is a significant decrease ($P \leq 0.05$) in eosinophils measurement (1.60 ± 0.894)% in compare with asthmate rabbits (6.20 ± 0.836) when asthmatic rabbits treated by prednisolone along for 8 days begin from the day 23 until the day 30 of the experiment.

- Effects of bisector doses of Licorice, prednisolone and medley doses on Eosinophils estimation in asthmate rabbits.

Present result of the two doses of *Licorice root* extraction as in figure(1.2) showed significant decrease ($P \leq 0.05$) in eosinophils measurement in asthmate treated rabbits and control rabbits after receiving 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 80mg/kg + prednisolone 0.5mg/kg) of body weight respectively through the period of asthmate rabbits, asthmate persistence and immunization program comparing with (1 ± 0.707) % control (healthy) rabbits. Hence, along the period of immunization program the result pointed that a combination of treated rabbits were significantly potent to reduce eosinophils and return to the normal value as in figure (1.2).

Table (1.2) Eosinophils estimation (Mean \pm SD) for each of control, asthmamotivate and treated rabbits.

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

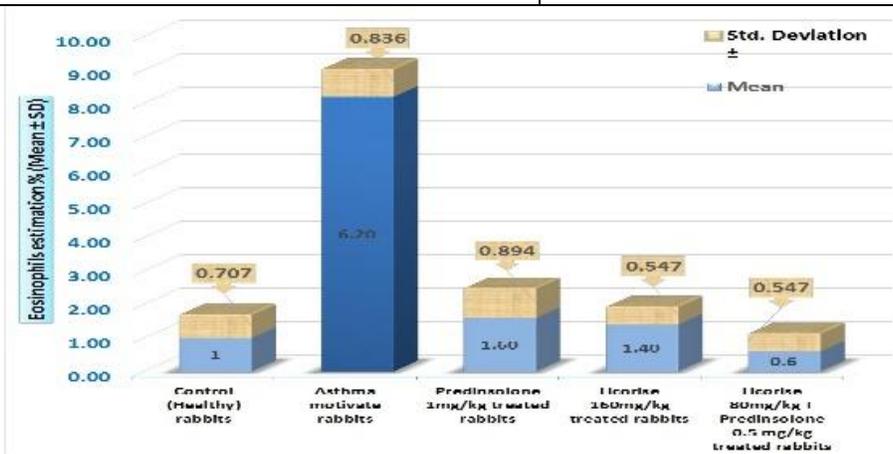


Figure (1.2) A comparison effect prednisolone and *Licorice root* on the Eosinophils estimation (Mean \pm SD) in asthma motivate, control and treated rabbits.

- *Impact given of prednisolone on Interleukin-4 scales in each of control (Healthy) and asthma motivate rabbits.*

The study were show significant increased ($P \leq 0.05$) in Interleukin-4 scales in asthma motivate rabbits with ovalbumin injection (385.87 ± 60.08) pg/ml compared with healthy rabbits (30.44 ± 4.57) pg/ml table (1.3) Whereas, the table show a significant decrease ($P \leq 0.05$) in the scales of Interleukin-4 (32.02 ± 1.84) pg/ml when that asthma motivate rabbits given prednisolone (treated) daily ata dose of 1 mg/kg of body weight at that time of such period. According to the schematic program which lasted for 8 days begin from the day 23 until the day 30 of the experiment.

- *Effects bisector of Licorice, prednisolone and medley doses on the Interleukin-4 scales in asthma motivate rabbits.*

Licorice root showed a cleardecrement in the scale of interleukin-4, but it did not reach to significant value ($P \geq 0.05$) in motivate asthma rabbits (31.45 ± 1.64) pg/ml, through the received a dose of 160 mg/kg body weight of *Licorice root* extract for 8 days. After receiving treatment with a dose of (80 mg/kg) *Licorice root* extract plus prednisolone (0.5mg/kg) of body weight for 8 days there was a significant decrease ($P \leq 0.05$) in interleukin-4 levels (29.34 ± 2.35) pg/ml, which may give precise indication of the effectiveness of *Licorice root* extract in reduction of asthma.

Statistically the values (80+0.5)mg/kg of body weight *licorice* extract compare with prednisolone were significant as shown in Figure (1.3) due to the synergistic effect .

Table (1.3) Interleukin-4 (Mean ± SD) scales in each of control, asthma motivate and treated rabbits.

Groups	IL4 levels pg/mL(Mean ± SD)
Control Group	30.44±4.57
Asthma Rabbits (ovalbumin induced)	385.87±60.08
Asthma Rabbits treated with 1 mg/kg of prednisolone	32.02±1.84

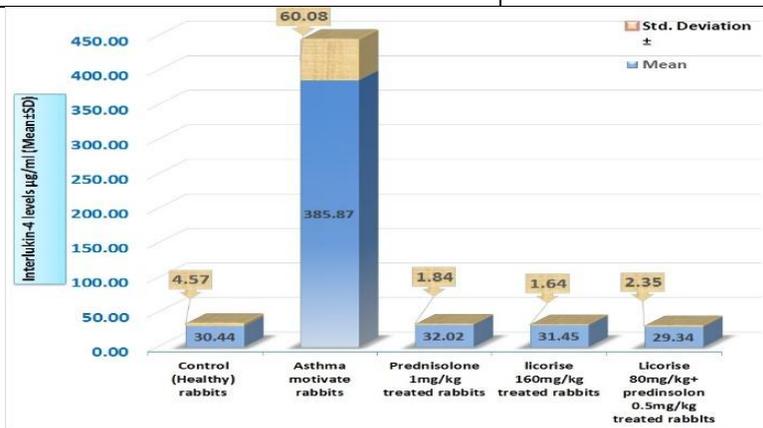


Figure (1.3)A comparison effect of prednisolone and *Licorice* on the scales of Interleukin-4 (Mean ± SD) in asthma motivate, control and treated rabbits.

-Impact given of prednisolone on Interleukin-5 scales in each of control (Healthy) and asthma motivate rabbits.

Study were show significant increase ($P \leq 0.05$) in Interleukin-5 scales in asthma motivate rabbits with ovalbumin injection (245.53 ±38.841) pg/ml compared with healthy rabbits (20.53 ±2.093) pg/ml as shown in table (1.4).Whereas, the table show a significant decrease ($P \leq 0.05$) in the scales of Interleukin-5 (27.12 ±3.439) pg/ml when that asthma motivate rabbits given prednisolone (treated) daily ata dosage of one mg/kg of body weight at that time of such period. According to the schematic program which lasted for 8 days begin from the day 23 until the day 30 of the experiment.

-Effects bisector of Licorice, prednisolone and medley manner on the Interleukin-5 scales in asthma motivate rabbits.

Licorice root showed a cleardecrement in the scales 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. After receiving treatment with a dose of 80 mg/kg plus prednisolone 0.5mg/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 of the effectiveness of *Licorice root* extract in reduction of asthma. Statistically the values (80+0.5)mg/kg of

body weight *licorice* extract compare with prednisolone were significant as shown in Figure (1.4).

Table (1.4) Interleukin-5 (Mean ± SD) scales 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 induced)	245.53±38.841
Asthma Rabbits treated with 1 mg/kg of prednisolone	27.12 ±3.439

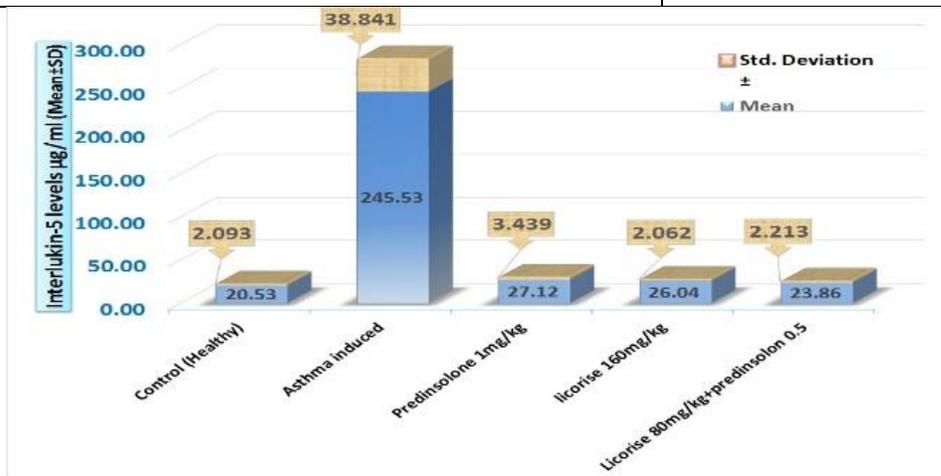


Figure (1.4) A comparison effect of prednisolone and *Licorice* on the scales of Interleukin-5 (Mean ± SD) in asthma motivate, control and treated rabbits

9-Histopathology:

9.1- Lung:

9.1.1- control healthy rabbits (Group1)

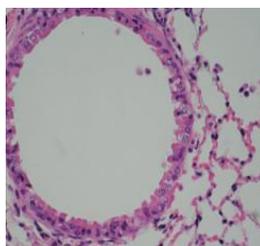


Figure (1.5) x40.

In figure (1.5) show normal structure of bronchioles and alveoli of respiratory tree.

9.1.2- Asthma motivate rabbits (Group 2)

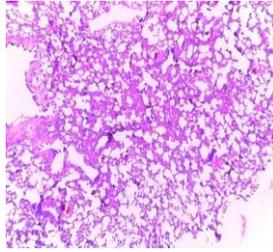


Figure (1.6) 10x10.22.

In figure (1.6) showed:

1. Alveolar wall was thickened.
2. Sub epithelial smooth muscle of bronchus was marked by thickened (infiltration of leukocyte)
3. Inflammatory cells are infiltrated in wall of bronchus.
4. Blood capillary congestion.

9.3-Prednisolone treated rabbits (Group 3)

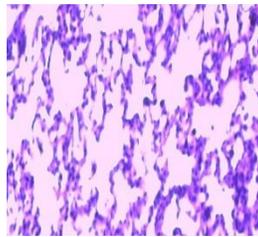
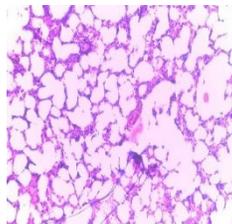


Figure (1.7)10x10.22.In figure (1.7) showed:

1. Alveolar wall thickness was decreased
2. Thick ness of bronchial wall was decreased.

9.4-Licorice treated rabbits (Group 4)



Figure(1.8)10x10.22.In figure (1.8) showed:

1. The alveolar wall thickness is reduced after treated by Licorice.
2. Thickening of bronchial wall become reduced.

9.5- Licorice plus prednisolone treated rabbits (Group5)

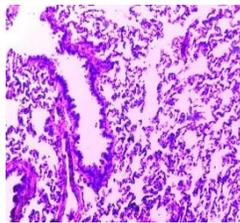


Figure (1.9) 10x10.22.In figure (1.9) Showed lung tissue (bronchial and alveoli) return to normal.

Discussion:

-Effects of oral Licorice intake on the total WBC and eosinophils estimation of treated groups:

Resulted data in figures (1.1) and (1.2) were showing a significant increase of total WBC and eosinophils measurements and expand their role in presenting of asthma signs and symptoms through release of inflammatory mediators of cytokines, chemokines as a reflect to use of OVA and finally to play a major role in distinguishing allergy and asthma characterizations. Meantime, it showed a significant lowering in eosinophils that estimated contribute to the inhibitory effect of flavonoids of *Licorice*, of the present study. Asthma is a chronic respiratory disease, The existing therapy such as corticosteroid is inadequate and has many adverse effects. It needs a better therapeutic molecule preferably of natural origin, which has negligible or no adverse effects. Glycyrrhizin (GRZ), a major constituent of a plant (Licorice root), for its efficacy on asthmatic features. Rabbits were sensitized and challenged with ovalbumin (OVA) to develop the asthmatic features such as allergic asthma and inflammation development .The treated asthmatic group after OVA-sensitization has been evaluate for its protective or reversal effect, respectively on the former asthmatic features. As well as, for other parameters associated with asthma such as interleukin (IL-4, IL-5) and OVA-specific (IGE) were measured by ELISA. It decreased IL-4, IL-5 and eosinophil. Also, it reduced OVA-specific IgE levels in serum. Our results demonstrate that Licorice alleviates asthmatic features in rabbits and it could be useful towards developing a better therapeutic molecule in the future¹. In particular, among the activated cells in the pathogenesis of allergic asthma, (eosinophils) are the most prominent cell type, and an increased number in the airways is correlated with the severity of asthma ; accordingly, selective elimination of eosinophils is often a target for the therapy of various inflammatory diseases including allergic asthma. A previous study demonstrated that recruitment of eosinophils into BALF was clearly observed as expected in the OVA-induced asthma .So the present study, we found elevated leukocyte infiltration, goblet-cell hyperplasia, and mucus hypersecretion, confirming a previous study²⁹. Eosinophilic leukocytes accumulate in high numbers in the lungs of asthmatic patients, and are believed to be important in the pathogenesis of asthma. A potent

eosinophil chemoattractant is produced in the asthmatic lung. This small protein, the chemokine eotaxin, is synthesized by a number of different cell types, and is stimulated by interleukin-4 and interleukin-13, which are produced 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 eotaxin¹¹. However, all the precede improving data were harmonized in close relation with our outcomes of the present findings in this search for *Licorice* effects on the hematological parameter. In a previous study⁴ showed that the flavonoids that played the main role in asthma It is quite possible that GRZ may block eotaxin levels in vivo as well and block infiltration of eosinophils in the lungs. Also, present study demonstrated that GRZ treatment could reduce pathophysiological as well as immunological components of late phase airway response like reduction of airway hyper reactivity reduction of absolute number of eosinophils in the BAL fluid, reduction of eosinophil percentage in the lung and reduction of IL-5 cytokine. Hence, it might be possible that the reduced infiltration of eosinophils into the airway lumen could be due to the effect of GRZ in blocking the activation of NF- κ B in the air ways. In conclusion, GRZ significantly alleviates the asthmatic features such as OVA induced immediate airway constriction, airway hyperreactivity to methacholine, eosinophilia and lung inflammation GRZ, therefore, could be useful towards developing better therapeutic molecule in the future.

-Impact of oral Prednisolone intake on the total WBCs and eosinophils estimation of treated groups:

Figures (1.1) and (1.2) showed the impact of the prednisolone a member of corticosteroids group and had possessed the similar action on hematological parameters. The white blood cell (WBC) count is reflects for the leukocytes distributed in the blood. Leukocytes percentage of normophysiological count were include neutrophils of 60-70%, lymphocytes of 28%, monocytes of 5%, eosinophils of 2-4%, and basophils of 0.5%¹⁴. Neutrophils locate in some compartments; the marginal compartment (those neutrophils attached to the endothelium of the blood vessel) and the circulating compartment (those circulating in the blood vessels along with other cells)²⁸. This singularity is important as neutrophils traveling on the endothelial surface within the lumen of the blood vessel (i.e., in the marginal compartment) are not reverberation in a WBC count. Only the polymorphoneucler freely circulating within the circulatory compartment will be found in the venous sample used for counting. However 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 and thus increase the WBC count. Glucocorticoids are known to do this^{28,20}. As the WBCs gain in our work. Studies of Zubaida³³, Schwartzman and Cidlowski²⁴ had been document the side effect of corticosteroid on a

hematological changes such as increase hemoglobin and red cell content of blood, possibly through retarding erythroid-phagocytosis. Corticosteroids also affect circulating white cells via increased polymorphonuclear 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 contrast, the lymphocytes, eosinophils, monocytes, and basophils decrease in number after administration of 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 in matched with present obtained results, as well to concomitant immunosuppression, also leaves patients susceptible to invasive diseases and easy fungal infections³.

- Effects of oral prednisolone intake on the levels of IL-4 and IL5 in the serum of treated group:

Although treatment with prednisolone, one of the most potent corticosteroids, improved all the immunologic and histopathologic parameters. Study, record no highly significant differences were found in the histopathologic and immunologic parameters between the Licorice, prednisolone and in combination groups. Although not to a statistically significant degree, it was found that rabbits given prednisolone had 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 in the present study may be due to the glucocorticoid-like inhibitory effect of glycyrrhizin in lung cells. Conflicting effects of corticosteroids although corticosteroids are much effective in the clinical management of allergic diseases, some of the cellular and molecular effects of these agents are difficult to fence with their well-established beneficial effects. 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 visible in B lymphocytes treated with hydrocortisone³¹ and our obtained experimental findings for IL 4 and IgE were in complete matching with those using of

corticosteroids, in asthmatic patients after 1 week of treatment with oral doses of prednisolone^{31,19}corticosteroids slope

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 cytokines²³. Corticosteroids might, therefore, be expected to help polarize the immune response towards the proinflammatory Th2 pattern, were it not for the overrun inhibitory effects of these agents on the secretion of IL-4,IL-5, and IL-13.CorticosteroidsAlso, reduction survival of T cells and eosinophils by increment apoptosis, contributing to their suppression of chronic allergic inflammation²⁰.

- Effects of oral Licorice intake on the levels of IL4and IL5 in the serum of treated group:

The present study established 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. Present results in Figures (1.3) and (1.4) show an inhibitory effect of *Licorice root* versus allergic asthma induced by (ovalbumin intraperitoneally) in day 1 and challenge dose in day 14, however, these effects are mainly depend on the concentration of the flavonoids to predestine anti-asthmatic properties of *Licorice root* extraction. In figures (1.3) and (1.4) given data revealed 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 IL-4 levels respectively. While, the combination dose (80+0.5)mg/kg body weight (Licorice+predinsolon) showed supreme significant decrease in their effects toward asthma induced by ovalbumin, this effects may be due to synergistic effect of licorice and prednisolone and to the dependable concentration of the flavonoids in *Licorice* extraction.Chronic inflammation of airways has been identified to play a key role in the pathogenesis of bronchial asthma, which used to be taken as a disease of bronchial smooth muscles⁸ Airway inflammation in asthma is characterized by acute onset airway hyper responsiveness (AHR) and infiltration of inflammatory cells,especially eosinophils²⁵.Eosinophils have been related to the severity of asthma, and several mediators resulting in eosinophil activation may also lead to contraction of airway smooth muscles²⁶The onset of asthma is generally attributed to genetic and environmental factors; however, predominant Th2 cell activity has been identified as part of the core pathogenesis of asthma⁶. Activated Th2 cells will secrete cytokines, such as IL-4 and IL-5.IL-4 has been proved to promote IgE production and T cell differentiation into Th2 cells¹⁶.IL-5, however, induces the differentiation, maturation, and migration of eosinophils to the local tissue of inflammation. Therefore, suppression of Th2 cytokines may have the potential to alleviate chronic airway inflammation

and, subsequently, the symptoms of asthma²⁵. In order to determine the basic structure of *Licoric* extraction previous studies found it consist mainly from the flavonoids, and it has inhibitory effects on IL-4 , IL5 and IgE production, many kinds of flavones, and their related compounds were then screened. Our study evaluated the beneficial effects of Licorice on inflammatory cell and Th2 overexpression in OVA-sensitized rabbit's model of asthma. The levels of inflammatory cell (eosinophil) infiltration, Th2 cytokines (IL-4, IL-5), eosinophil-related chemokine (eotaxin), IgE and AHR were all significantly enhanced in the OVA-sensitized group. However, all the above 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 inflammation³¹. Evidence has been reported that rabbits model which is in lack of IL-5 expression would not develop AHR or eosinophilia though under allergen challenge³². 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-5 and the number of eosinophil and in the lung tissues surrounding the airways as well. 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. IL-4 facilitates the differentiation and proliferation of Th2 cells, the switching of B cells to secrete IgE, and the development of AHR as well³⁴. On the other hand, inhibition of IL-4 by monoclonal antibodies attenuates airway eosinophilia and IgE level in allergic mice therefore, the production of IgE is closely related to the concentration of IL-4. Furthermore, it has been reported that IL-4 deficient mice demonstrated to be spared from asthmatic attack and airway inflammation⁶. Th2 cytokines, especially IL-4 and IL-13, are crucial mediators of asthma, and both are proved to play an important role in up-regulating the eosinophil chemo-attractants of eotaxin. Licorice has been proved to have immune-modulatory effects on allergic diseases. A previous clinical study had revealed that long term therapy with Licorice suppresses total serum levels of IgE , IL-4 and IL5. So in this , we demonstrated that Licorice significantly reduces OVA-induced effects and reduces asthma clinical response, as well as, immunological lowering effects as such²⁰ OVA-specific IgE, IL-4, IL-5 and IL-6 levels in blood of rabbits experimental studying model .

- Effects of oral Licorice intake on the Histology of the lung:

We show in the result of my study, our histopathological findings demonstrate that Licorice decreased the OVA-induced leukocyte. In the asthma group (group2), light microscopic findings revealed irregular respiratory epithelium. Sub

epithelial smooth muscle (Sm) was markedly thickened. By Inflammatory cell infiltration as seen in figure (1.6). Compared with the control group, the asthma group had thickness in the alveolar wall, and sub epithelial smooth muscle of bronchus was thickened, inflammatory cells are infiltrated in wall of bronchus and alveolar wall . These results reveal that the chronic asthma model of rabbits was successfully established in this study as seen in figure (1.5) (1.6) this result is agree with^{34,35}. In the prednisolone treated rabbits group (group 3), alveolar wall thickness was decreased, and the thickness of bronchial wall was decreased as seen in figure (1.7) Prednisolone is accepted as a potent inhibitor of airway inflammation and remodeling Administration of corticosteroids has been shown to inhibit the structural changes Although treatment with prednisolone, one of the most potent corticosteroids, improved all the histopathologic lesion in the present study. This result is agree with^{36,37}. In the Licorice treated rabbits group (group 4), we showed that rabbits receiving Licorice had alleviation of all the evaluated established histopathologic changes of lung in the asthmatic rabbits, including lung parenchymal area appear decreased and bronchial epithelial of respiratory epithelium become decreased so finally the alveolar wall thickness is reduced after treated by Licorice we found that rabbits given prednisolone had decreased smooth muscle thickness compared with those receiving Licorice. The similar effectiveness of prednisolone and Licorice on structural changes in asthmatic airways in the present study may be due to the glucocorticoid-like inhibitory effect of glycyrrhizin in lung cells. Evaluation of lung histopathologic findings demonstrate the thickness of basement membrane, sub epithelial smooth muscle layers, and epithelium of the bronchioles were reduced in the Licorice treated rabbits group compared with the asthma group that were the structural changes were alleviated after Licorice administration and become returned to normal as seen in figure (1.8) This result is agree with^{37,38}. In the combination group Licorice plus prednisolone treated rabbits group (group 5) when comparing the Licorice group (group 4) and prednisolone groups (group 5), no differences were found in the thickness of basement membrane sub epithelial smooth muscle layers, and epithelium of the lung. In this study we use combination in group 5 to reach optimal treatment with low side effect wherefore we see the histological lesion in lung tissue bronchiole and alveoli is returned to normal due to the synergistic effect of the combination that it taken to the rabbits include (80mg/kg) Licorice plus (0.5mg/kg) of prednisolone.

Conclusions:

There is mounting evidence that a general increase in anti-allergic Effects achieved by flavonoids compounds when intake can help diminish allergic symptoms. The extraction of *Licorice root* has a anti-allergic effect against ovalbumin

motivate asthma rabbits. The administration of phenolic extract *Licorice* root produced a significant reduction in, IL4, IL5 and Eosinophils.

References

1. Abbas Azadmehr, Reza Hajiaghaee, Mohammad Ali Zohal, and GhorbanMaliji.(2013) Protective effects of *Scrophularia striata* in Ovalbumin-induced mice asthma model, Published online 2013 Jul 9. doi: 10.1186/2008-2231-21-56.
2. Abeer Fadhil Ibraheem . Hypoglycemic Effect of *Ginkgo biloba* Plant Leaf Extract on Alloxan Induced Diabetes Rats ,a thesis for msc degree in babylon university collage of medicine , 2016.
3. Aleem,H.A.N;naseer,J.H, safaa,H.AL(20016).physiological effect of Teucrium polium extract ameliorates allergic asthma in rabbits. Msc .thesis , collage of medicine . university of Babylon.
4. Arjun Ram a, U. Mabalirajan a, Moumita Das a, Indranil Bhattacharya a, Amit K. Dinda b, Sharad V. Gangal a, Balaram Ghosh(2006)Glycyrrhizin alleviates experimental allergic asthma in mice 30 april
5. B. Ramesh , R. Karuna ,S. Sreenivasa Reddy ,G. Sudhakara and D. Sara lakumari ., Ethanol extract of Commiphora mukul gum resin attenuates streptozotocin-induced alterations in carbohydrate and lipid metabolism in rats .,2013; 12: 556–568.
6. Chang HC, Gong CC, Chan CL, Mak OT. (2013)A nebulized complex traditional Chinese medicine inhibits Histamine and IL-4 production by ovalbumin in guinea pigs and can stabilize mast cells in vitro. BMC Complement Altern Med; 13:174.
7. Daniel WW, editor. 7th ed. New York: John Wiley & Sons; (1999). Biostatistics: a foundation for analysis in the health sciences.
8. Faoud T. Ishmael, MD, PhD(2011)The Inflammatory Response in the Pathogenesis of Asthma,The Journal of the American Osteopathic Association, November 2011, Vol. 111, S11-S17.
9. Fatemeh Moheimani , Alan CY Hsu , Andrew T Reid , Teresa Williams , Anthony Kicic , Stephen M. Stick , Philip M. Hansbro , Peter A.B. Wark , Darryl A. Knight(2016) The genetic and epigenetic landscapes of the epithelium in asthma, springer 22 September.

10. Flores-Villegas AL, Salazar-Schettino PM, Cordoba-Aguilar A, Gutierrez-Cabrera AE, Rojas-Wastavino GE, Bucio-Torres MI, Cabrera-Bravo M, Immune defence mechanisms of triatomines against bacteria, viruses, fungi and parasites , pubmed 105; 523-32.
11. GhadaTabl and Abd El-Hamid Mohamed Elwy (2013) Evaluation of Ginkgo biloba as Alternative Medicine on Ova-Induced Eotaxin and Eosinophilia in Asthmatic Lung.
12. Gillina F. G. Bezemer, SeilSagar, Jeroen van Bergenhenegouwen, Niki A. Georgiou, Johan Garssen, Aletta D. Kraneveld and GertFolkerts (2012) Dual Role of Toll-Like Receptors in Asthma and Chronic Obstructive Pulmonary Disease , April 2012, 64 (2) 337-358.
13. Jiri Mlcek, Tunde Jurikova, SonaSkrovankova and Jiri Sochor (2016) Quercetin and Its Anti-Allergic Immune Response JNSR,V6,no23; p10-3390.
14. Junqueira LC, Carneiro J. Blood cells. In: Basic Histology. 11th ed. Junqueira LC, Caneiro J eds. McGraw-Hill Medical Publishing Division. New York, NY. 2005; 223-237
15. Kamaruzaman, Nurfatimasyikhin, SitiAmrahSulaiman, Gurjeet Kaur, and BadrulYahaya. (2014). "Inhalation of Honey Reduces Airway Inflammation and Histopathological Changes in a Rabbit Model of Ovalbumin-Induced Chronic Asthma." *BMC complementary and alternative medicine* 14(1): 176.
16. Katherine Bao and R. Lee Reinhardt (2015) The differential expression of IL-4 and IL-13 and its impact on type-2 Immunity, *Cytokine* 2015 September; 75(1): 25–37. doi:10.1016/j.cyto.2015.05.008.
17. Lee MY, Shin IS, Lim HS, Shin HK.(2012) A water extract of Samchulkunbi-tang attenuates airway inflammation by inhibiting inos and MMP-9 activities in an ovalbumin-induced murine asthma model. *BMC Complement AlternMed* ; 12:257.
18. Makoto Kudo, Yoshiaki Ishigatsubo,andIchiro Aoki(2013) Pathology of asthma,fmicb.2013.00263.
19. MerimaBublin, Thomas Eiwegger, MD,and HeimoBreiteneder (2014) Do lipids influence the allergic sensitization process?,*J Allergy ClinImmunol.* 2014 Sep; 134(3): 521–529.
20. MohendraRoy,GeonsooJin, DongminSeo, Myung-Hyun Nam and SungkyuSeo (2014) A simple and low-cost device performing blood cell counting based on lens-free shadow imaging technique,j.snb.2014.05.011.

21. NataliyaZlotnikov , Innate immune responses to infection with the Lyme disease pathogen in diet-induced obesity, A thesis submitted in conformity with the requirements for the degree of Master of Science Graduate Department of Dentistry University of Toronto,(2015).
22. RamyaSivangala ,GaddamSumanlatha , Cytokines that Mediate and Regulate Immune Responses , 2015 ; 34: 793–813.
23. Rosalind J. Wright,(2012)Epidemiology of stress and asthma: From constricting communities and fragile families to epigenetics, *Immunol Allergy Clin North Am.* 2011 Feb; 31(1): 19–39.
24. Schwartzman, R.A., Cidlowski,J.A.(1994). Glucocorticoid-induced apoptosis of lymphoid cells. *Int Arch Allergy Immunol*;105:347–54.
25. Sien-Hung Yang, Ting-I Kao, Bor-Luen Chiang , Hsing-Yu Chen Kuang-Hua Chen , Jiun-Liang Chen, (2015)Immune-Modulatory Effects of Bu-Zhong-YiQi-Tang in Ovalbumin-Induced Murine Model of Allergic Asthma.
26. Sien-Hung Yang, Ting-I Kao, Bor-LuenChiang,Hsing-Yu Chen, Kuang-Hua Chen, and Jiun-Liang Chen(2015) Immune-Modulatory Effects of Bu-Zhong-Yi-Qi-Tang in Ovalbumin-Induced Murine Model of Allergic Asthma,Published online 2015 Jun 2. doi: 10.1371/journal.pone.0127636.
27. Tanaka H, Kawada N, Yamada T, Kawada K, Takatsu K, Nagai H.(2008) Allergen-induced airway inflammation and bronchial responsiveness in interleukin-5 receptor alpha chain deficient mice. *ClinExp Allergy*; 30:874–881.
28. Wei Xu, Hai-feng Wu,Shao-gang Ma,Feng Bai, Wen Hu,Yue Jin,andHong Liu(2013) Correlation between Peripheral White Blood Cell Counts and Hyperglycemic Emergencies,*Int J Med Sci.* 2013; 10(6): 758–765.
29. Woo-Young Jeon, Hyeun-Kyoo Shin, In-Sik Shin, Sang Kyum Kim, and Mee-Young Lee (2015) Soshiho-tang water extract inhibits ovalbumin-induced airway inflammation via the regulation of heme oxygenase-1,Published online 2015 Sep 18. doi: 10.1186/s12906-015-0857-3 .
30. Yahaya.n; Amirah.w;saravanan.D; Khamsah,s.(2015)phytochemical screening and prophylactic antibacterial effect of *Andrograhis Paniculata* extract from kemaman Malaysia. *JNSR*,V5,N16,P 67-70.
31. Zeiger RS, Dawson C, Weiss S. (1999).Relationships between duration of asthma and asthma severity among children in the Childhood Asthma Management Program (CAMP). *J Allergy Clin Immunol*;103(3 Pt 1):376–87.

32. Zhijun Wang, Jeffrey Wang and Patrick Chan.Treating Type 2 Diabetes Mellitus with Traditional Chinese and Indian Medicinal Herbs ,Evidence-Based Complementary and Alternative Medicine 2013;2013:343594.
33. Zubaida Umar, Mahmood Rasool,Muhammad Asif, SajjadKarim,ArifMalik,GoharMushtaq, Mohammad A Kamal,andArsalaMansoor (2015) Evaluation of Hemoglobin Concentration in Pregnancy and Correlation with Different Altitude: A Study from Balochistan Plateau of Pakistan,Open Biochem J, v 9,7-14.
34. Taube C, Dakhama A, Gelfand EW. (2004) Insights into the pathogenesis of asthma utilizing murine models. Int Arch Allergy Immunol. ;135:173–86.
35. ArzuBabayigitHocaoglu , OzkanKaraman ,DuyguOlmezErge, , Guven Erbil, , Osman Yilmaz, AlperBagriyanik and NevinUzuner, (2011) Glycyrrhizin and Long-Term Histopathologic Changes in a Murine Model of Asthma .
36. Blyth DI, Wharton TF, Pedrick MS, et al.(2000). Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. *Am J Respir Cell MolBiol.* ;23:241–246.
37. Takei H, Baba Y, Hisatsune A, et al.(2008) Glycyrrhizin inhibits interleukin-8 production and nuclear factor-B activity in lung epithelial cells, but not through glucocorticoid receptors. *J PharmacolSci.*; 106:460–468.