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Efficacy and safety of liquorice extract in asthmatic patients

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Abstract

Liquorice is one of the commonly used herbs in the field of medicine due to its pharmacological activities. Among these activities, the anti-inflammatory and antiallergic activities that support its use in asthma. This study included 80 asthmatic patients who were classified into two groups, group 1 (Placebo group) maintained on inhaled corticosteroids (ICs in moderate to high doses) and long-acting beta agonist (LABA) and received starch capsule (500 mg starch) twice daily as placebo and group 2 (active treatment group) maintained on ICs (in moderate to high doses) and LABA and received liquorice extract capsule (500 mg equivalent to 100mg glycyrrhizin) taken twice daily. The efficacy of liquorice was measured by estimation of pulmonary function and blood eosinophils %. On the other hand, safety was assessed by blood pressure measurement and determination of serum potassium level. The results revealed that addition of liquorice capsules to ICs and LABA resulted in a non-significant improvement in blood eosinophils (P-value 0.754). However, it resulted in a highly significant improvement in Forced Vital Capacity (FVC) % and Forced Expiratory Volume1 (FEV1) % when compared to group 1 (P-value 0.031.and 0.040 respectively). Regarding liquorice safety, neither Blood pressure (systolic and Diastolic) (SBP and DBP), nor serum K level showed any significant change in patients received liquorice capsules. Conclusion: liquorice in the used dose significantly improved FVC % and FEV1 % but did not affect blood eosinophils. There was no significant effect on blood pressure and serum K level, so, this dose is safe with no observed side effects.

Key words
Asthma, liquorice, glycyrrhizin, pulmonary function

1. Introduction

Asthma is a chronic inflammatory disease of the airway [1] that is characterized by cough, wheeze ,chest tightness and breath difficulties [2]. Eosinophil is the main inflammatory cells involved in the development of asthma and usually found in high % in the blood and sputum of uncontrolled asthmatic patients [3], so, eosinophil is important for evaluation of asthmatic patients and determination of the degree of control of asthma [4]. Inhaled corticosteroids are known to decrease the count of blood eosinophils by inhibiting their survival [5] and, thus, blood eosinophils can be considered an important indicator for the steroidal therapy efficiency [6]. Pulmonary function is another indicative parameter in asthma diagnosis and control and it includes Forced Vital Capacity (FVC) that is the volume of air forcefully exhaled from the lungs after taking a very deep breath, and Forced Expiratory volume in one second (FEV_1) that is the volume of air that a person can forcefully exhale in one second after full inspiration. Both FVC and FEV_1 help to determine the severity of the disease as their values decreased in asthma. FVC % and FEV % for a given individual are compared to reference values(predicted values) that is based on healthy individuals with normal lung function and depends on sex, age and height [7]. Higher values of FVC and FEV_1 indicate a better control of asthma. Asthma treatment guidelines for patients above 12 years old include five steps [8], the first step is short acting beta agonist (SABA) as needed, the second step include the use of low dose ICs and SABA as needed. The third step includes the use of low dose ICs/LABA combination and SABA as needed, the fourth step includes the use of medium or high dose ICs/LABA combination and SABA as needed. The last step includes the addition of low dose of oral corticosteroids or anti-IgE or both of them. Liquorice root contains triterpenoid saponins, mostly glycyrrhizin(G) ,that is a mixture of potassium and calcium salts of glycyrrhizic acid (also known as glycyrrhizinic acid and a glycoside of glycyrrhetinic acid) which is considered to be the main biologically active component of liquorice [9]. The antiinflammatory effect of G is attributed, mainly, to β-glycyrrhetinic acid (the main metabolite of G) and its effect is mediated through the inhibition of glucocorticoids metabolism by inhibition of 11β-hydroxysteroid dehydrogenase (11-β-HSDH), the enzyme responsible for the conversion of cortisol to inactive cortisone, thus, potentiate cortisol effects [10]. Glycyrrhizin, also, inhibits prostaglandin E2 (PGE2) production [11] and has the ability to suppress the expression of pro-inflammatory genes [12] and ,as a result, reduces the inflammatory cytokines production like IL-4, IL-6 and IL-8 [13]. Glycyrrhizin, also, exhibits anti-allergic activity and this effect is due to its ability to relieve Immunoglobulin-E (IgE)-induced allergic diseases such as

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dermatitis and asthma. In addition, Glycyrrhetinic acid and its derivatives have antitussive activity and the oral administration of aqueous liquorice extract decreased the number of citric acid-induced cough efforts in guinea pigs more effectively than codeine [14]. These pharmacological activities of liquorice encourage its use in asthma. However, Liquorice has important side effects that must be taken into consideration when liquorice is used. The most important side effects are hypertension and hypokalemia and these effects are due to G content of liquorice that accumulates cortisol resulting in activation of renal mineralocorticoid receptors leading to a state of apparent mineralocorticoid excess syndrome [15] that is characterized by sodium-water retention, hypertension ,metabolic alkalosis and hypokalemia and the latter occur as a result of the increase in potassium excretion due to cortisol accumulation [16]. So, it is important to adjust the dose of liquorice to avoid these side effects. It was found that the safe dose of G is 217 mg/person/day, [17] at which, no observed side effects were detected . Another study showed that high doses of liquorice (equivalent to 270 and 540 G) increased both SBP and DBP significantly [18]. A case report revealed that chronic administration of 225mg daily of G resulted in hypertension and hypokalemia [19] .The objective of this study is to evaluate the effectiveness and safety of liquorice extract in asthmatic patients.

2. Patients and method

2.1. Study design

The study is a randomized trial in which eighty patients of chronic stable moderate bronchial asthma and maintained on moderate to high doses ICs/LABA combination, selected randomly from outpatient clinic of the Cardiothoracic university hospital, Minia University and the sample size was according to the number of patients attending the clinic with the inclusion criteria and with our exclusion criteria. Patients were classified into two groups: Group 1 maintained on the ICs fluticasone or budesonide in a moderate to high doses ICs plus LABA salmeterol or formoterol and received 500 mg starch capsule twice daily as placebo. Group 2 maintained on the same asthma treatment as group 1 in addition to 500 mg aqueous liquorice extract capsule (equivalent to 100mg G) twice daily [the extract was purchased from Changsha Zhongren Biotechnology Company, Hunan, China (Mainland) and it is 20 % G] One and the study lasted for 4 weeks. The followings were excluded from the study: hypertensive patients, patients in acute asthma exacerbation, patients with liver or kidney diseases, patients with heart diseases especially those taking digoxin, pregnant and lactating mothers, women taking oral contraceptives, patients taking drugs that cause hypokalemia as thiazide diuretics , Children < 12 years and elderly patients. Patients had been subjected to:

2.1.1. Full medical history

Including name, age, sex, smoking, history of present illness with special emphasis on symptoms of bronchial asthma as cough, wheeze and dyspnea, past history and family history of hypertension and BP measurement using mercury sphygmomanometers.

2.1.2. Blood eosinophilic % determination

One milliliter of venous blood was collected on ethylene diamine tetra acetic acid tubes for complete blood count (CBC). Automated cell counter celltac ME-7300K, Nihon Kohden Corporation, Japan, was used for CBC. Blood smears were stained with leishman stain to confirm eosinophil count. Normal values of eosinophils % in the blood is up to 5 %.

2.1.3. Serum K level determination

One milliliter of venous blood was collected in plain tube and left to clot, then, centrifuged and the serum was separated and used to measure K level on the ion selective electrode ST-200 Sensacore Medical instrumentation PVT LTD, India. Normal serum potassium level is (3.5-5) mEq/l.

2.1.4. Pulmonary function test

Pulmonary function tests (FVC % and FEV_1 %) were performed using Spirostik that is a USB- spirometer that can be connected to lab top and comply with the latest guidelines from American Thoracic Society(ATS) and European Respiratory Society (ERS) and provide automatic interpretation. Patients were advised to take a very deep breath, then make a forceful expiration. Normal values for FVC % and FEV_1 % are equal to or more than 80 % of the predicted value. Decline in their values indicates improper control of asthma and problems in the air flow.

2.2. Ethics approval

The (ethics committee in El Minia University Hospital) reviewed and approved the research protocol (Registration No.80-11/2018). This ethics committee followed the International Conference on Harmonisation - Good Clinical Practice (ICH-GCP) guidelines. The trial was explained to each subject and his or her consent was obtained.

2.3. Statistical methods

Data were collected, revised, verified, and coded for statistical analysis that was done using IBM SPSS statistical package version 20[20]. Independent qualitative data were done using the Chi-squared test. Comparison between two studied groups was done using Mann-Whitney U test. Paired comparison was done using Wilcoxon signed-rank test. Kolmogorov-Smirnov for normality test was used to differentiate between parametric data and non-parametric data. Numeric data were displayed as median and interquartile range (IQR). For all tests, the results are significant if the P-value is less than 0.05, highly significant if P is less than 0.01 and very highly significant if P is less than 0.001.

3. Results

A total of 80 patients diagnosed with moderate bronchial asthma and under treatment with ICs in moderate to high doses and LABA were selected. Regarding the demographic data (age, sex, and smoking), the median (IQR) for age in group 1 was 26.5 (25-34), while in group 2 was 30(23.25-40). It was found that 24 (60 %) of patients were males and 16 (40 %) of patients were females in group 1 while 16 (40 %) of patients were males and 24 (60 %) of patients were females in group2. Regarding smoking, it was found 20(50 %) of patients were smokers and 20(50 %) of patients were non-smokers in group 1 while 12 (30 %) of patients were smokers and 28(70 %) of patients were non-smokers in group 2. There was no statistically significant difference in the median age (P-value =0.247), sex (P-value=0.055) and smoking (P-value= 0.068) of patients in both groups as shown in **(Table 1)**.

Regarding patients data before being enrolled in the study, it was found that blood eosinophils % was 2.5(2-4) in group 1 and 2 (2-3) in group 2 (P-value = 0.415). Serum K level was found to be 4 (3.8-4) mEq/l in group 1 and 3.9 (3.8-4.3) mEq/l in group 2 (P-value= 0.598). In addition, SBP was found to be 120(100-130) mmHg in group 1 and 120 (100-120) mmHg in group 2 (P- value= 0.223) while DBP was 85 (70-90) in group 1 and 80 (70-80) mmHg in group 2 (P-value= 0.057). Regarding pulmonary function test, it was found that FVC % was 80 (64-86) in group 1 and 90 (68-95) in group 2 (P-value= 0.253). FEV1 % was 84 (74-91) in group 1 and 89 (78-90) in group 2 (P -value= 0.062), so, there was no statistically significant difference in all measured data between the two groups before starting the study as shown in **(Table 2)**.

Regarding the efficacy of liquorice extract, the results of improvement in blood eosinophilic % in both groups showed that there was a non-significant improvement in blood eosinophilic % in group 2 when compared to the improvement in blood eosinophils % in group 1 (P-value= 0.754), While there was a significant improvement in FVC % and FEV_1 % in group 2 when compared to group 1 (P-value =0.031 and 0.040 for FVC % and FEV_1 % respectively) as seen in **(Table 3)**.

Regarding the safety of liquorice, it was found that SBP changed from 120 (100-120) to 120 (110-120) mmHg (P-value = 0.236) and DBP changed from 80 (70-80) to 80 (70-80) mmHg (P-value= 0.113) in group 2, so, both SBP and DBP non-significantly changed before the study and at the end of the study in group 2 as shown in **(Figure 1)**.

While serum K level in group 2 changed from 3.9 (3.8-4.3) to 4 (3.6-4.17) mEq/l (P-value= 0.105). So, serum K level was not significantly changed before the study and at the end of the study as shown in **(Figure 2)**.

4. Discussion

The incidence of asthmatic disorders is increasing around the world as about 300 million persons have asthma worldwide and the incidence is still increasing and only 50 % of asthmatic patients achieve adequate control by the current therapies [21] and most of the prescribed drugs have many side effects and, as a result, care is given to new drugs especially from natural origin. In view of this, we selected liquorice to evaluate its effectiveness in asthma due to the anti-inflammatory anti-allergic activities of its main component G. In addition, the study estimated the incidence of side effects of liquorice in the selected dose.

Regarding liquorice efficacy, the use of 500mg of aqueous liquorice extract capsule (equivalent to 100 mg G) twice daily in addition to ICs and LABA resulted in no additional effect on blood eosinophils % when compared to group 1(P-value= 0.754). The lack of effect of liquorice extract on blood eosinophils may be due to the dose used in our study and the reduction in blood eosinophils may require higher doses. So, the improvement in eosinophils % is related only to the effect of corticosteroids that suppresses the blood eosinophils [22]

In addition, Comparing pulmonary function improvement in both groups revealed that there was a significantly higher improvement in group 2 when compared to group1 (P-value= 0.031 and 0.040 for FVC and FEV_1 % respectively). These results come in agreement with a randomized study of liquorice on asthmatic patients that revealed a significant elevation of FEV_1 % and FVC % after 3 weeks of treatment with liquorice [23].

Regarding liquorice safety , the use of 500 mg of aqueous liquorice extract (equivalent to 100 mg G) capsule twice daily in addition to ICs and LABA (group 2) resulted in a non-significant increase in both systolic and diastolic blood pressure (P value= 0.236 and 0.113 respectively). These results are in agreement with a study on the safe dose of G, taken as dried aqueous liquorice root extract, and this study demonstrated no adverse effect with a dose up to 217 mg/person/day [17]. In addition, a case report of a 47-years-old woman, who took 225 mg of G daily for treatment biliary cholangitis was found to have a blood pressure about 230/110 mmHg without any past history of being hypertensive [19] and this may be due to the long duration of treatment with G that was too long(three years) when compared to the duration of treatment in our study(4 weeks). In addition, the reported case used pure glycyrrhizin that has greater side effects than liquorice extract used in our study. Another case report of a 78-years-old male patient who received G 280 mg/day and after two months, he had a blood pressure of 160/100 mmHg [24] and this can be explained by the higher dose of G and the longer duration of treatment than the present study.

Regarding serum K level, the use of 500 mg liquorice extract in addition to ICs and LABA resulted in a non-significant change in serum K level (P-value= 0.105). These results come in agreement with a study on 15 healthy volunteers who received pure licorice extract equivalent to 250 mg glycyrrhizin daily for 2 months and this study showed no significant change in serum K level [25], in addition to the study on the safe dose of G taken as aqueous extract and the study reported that a dose of 217 mg glycyrrhizin/person daily, (taken as aqueous extract), resulted in no hypokalaemia [17]. So, it can be said that the low dose of liquorice used in our study is the cause of absence of hypokalemia that is a common side effect of liquorice. In conclusion, aqueous liquorice extract capsules significantly improved the pulmonary function in the asthmatic patients at a

Table 1: Demographic data of patients in both groups.

Data		Group1 (n=40)	Group 2 (n=40)	P-value
Age (years)	Median(IQR)	26.5(25-34)	30(23.25-40)	0.247
Sex: n (%)	Males	24(60 %)	16(40 %)	0.055
	Females	16(40 %)	24(60 %)	
Smoking: n (%)	Yes	20(50 %)	12(30 %)	0.068
	No	20(50 %)	28(70 %)	

Table 2: Blood eosinophilic %, serum K, blood pressure measurement and pulmonary function in patient groups before being enrolled in the study.

Data	Group 1 (n=40) Median(IQR)	Group 2 (n=40) Median(IQR)	P-value
Blood eosinophil %	2.5(2-4)	2(2-3)	0.415
Serum K	4(3.8-4)	3.9(3.8-4.3)	0.598
SBP	120(100-130)	120(100-120)	0.223
DBP	85(70-90)	80(70-80)	0.057
FVC%	80(64-86)	90(68-95)	0.253
FEV_1%	84(74-91)	89(78-90)	0.062

Table 3: Improvement in blood eosinophils % and pulmonary function in patient groups after the end of the study.

Data	Group 1 (n=40) Median(IQR)	Group 2 (n=40) Median(IQR)	P-value
Blood eosinophil %	0.5(0-2)	0(0-1.75)	0.754
FVC%	0.5(0-5)	8(0-18)	0.031
FEV_1%	2(0-4)	13(0-20)	0.040

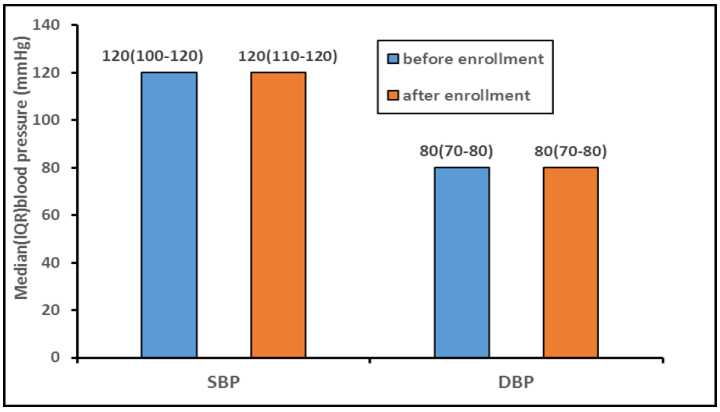

Figure 1: Blood pressure before and after patient enrollment in the study in group 2.

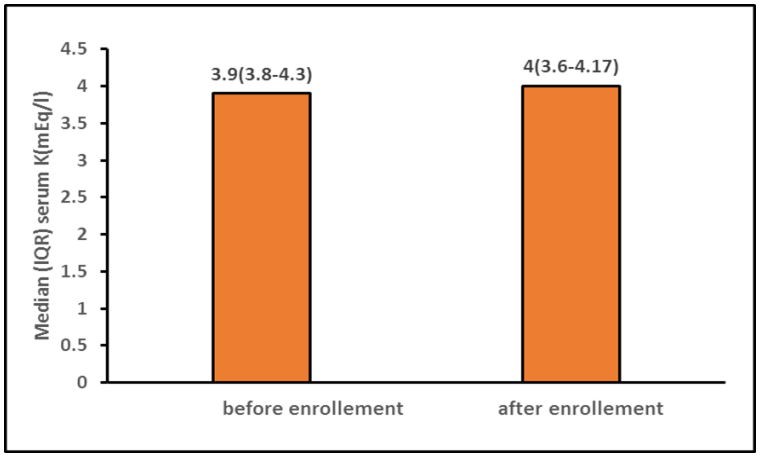

Figure 2: Serum K level before and after patient enrollment in the study in group 2.

dose equivalent to 200 mg glycyrrhizin daily despite the non-significant effect on blood eosinophils % that may need a higher dose. The dose used in the present study induced no significant elevation in the blood pressure and no significant reduction in serum K level.

Declarations of interest: none

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