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A Clinico- Pharmacological Assessment of a Herbal Preparation for the Treatment of Bronchial Asthma

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Abstract: The discovery of a herbal pharmaceutical preparation can be useful in the treatment of bronchial asthma. The natural agents were isolated from Foeniculum vulgare Miller, *Pimpinella anisum* L., *Thymus vulgaris* L. and *Glycyrrhiza glabra* L. Acute and chronic toxicity were carried out. For efficacy study, rats were allocated into 5 groups. Group 1 exposed to saline aerosol (normal control). Asthma was induced in the remaining groups by ovalbumin (OVA). Group 2 was left untreated (positive control). Group 3 received herbal preparation orally 1 hour before OVA challenge. Groups 4 & 5 received theophylline and salbutamol orally 1 hour before OVA challenge. Ten asthmatic patients were subjected to medical examination, laboratory investigations and spirometric measurements. All tests were performed before and after 4 weeks of therapy. The rats receiving herbal preparation showed no difference in laboratory parameters compared to control and normal histopathological structure and attenuation of OVA-induced changes in lung function tests (tidal volum and peak expiratory volume). Patients showed marked improvement of symptoms and signs. Five cases stopped their conventional bronchodilator therapy, three reduced the dose of inhaled corticosteroids and three cases stopped occasional systemic corticosteroids. The herbal preparation was found to be safe and effective in improving symptomatology, decreasing inflammation and improving pulmonary functions. Moreover, it may replace bronchodilators and decrease the dose of anti-inflammatory drugs.

Key words: Bronchial Asthma · Herbal Preparation · Tidal Volume · Forced Expiratory Volume · Clinical Trials

INTRODUCTION

Asthma is one of the most common chronic diseases in modern society and there is increasing evidence to suggest that its incidence and severity are increasing. Prevalence of asthma in the elderly is same with the other age groups (15%), but the diagnosis rate is quite low. Mortality rate is very higher than the other age groups. Majority of the persons died from asthma are aged 65 and above [1].

Antiasthma herbal medicine intervention could be useful complex interactions between herbal formula constituents produce synergistic effects and reduce possible side effects of some herbs [2].

Alternative treatments for asthma have important potential impacts on health status. Such impacts on health status may be direct, such as beneficial pharmacologic mechanisms or adverse side effects. The impacts of alternative therapies also may be indirect, working through patient-perceived disease control or resulting in the deferral or interruption of prescription medication or other traditional treatment strategies. Because data delineating the patterns and correlates of alternative therapy use in patients with asthma are limited, it is difficult to gauge the potential magnitude of their health impact [3].

Herbal preparations have been cited as the third most popular complementary treatment modality by British asthma sufferers [4].

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It was found that herbal therapy is the most common alternative treatment used [5]. It was reported that the aqueous extract of the dried ripe fruits of *Foeniculum vulgare* Miller, Family Umbelliferae indigenous in the Mediterranean region, raised the mucociliary activity of the ciliary epithelium and is carminative, expectorant and calmative. Also, the essence of the dried ripe fruits of *Pimpinella anisum* L., Family Umbelliferae, frequently cultivated in North Africa, is expectorant, spasmolytic, carminative and antibacterial, the infusion of the seeds is one of the best carminatives for pulmonary conditions, while the dried aerial parts of *Thymus vulgaris* L. Family Labiatae, endemic to North Africa, is used in folk medicine as a bronchial antispasmodic and expectorant. The extract of the roots and rhizomes of *Glycyrrhiza glabra* L. Family Leguminosae is used for hoarseness of voice, cough, respiratory ailments, expectorant. Infusion of the root is used in Folk medicine for cough and bronchitis due to its emollient, depurative and sweating properties. It also possesses an anti-inflammatoy, antiulcer, antimicrobial and antiviral effects [6,7].

In a developing country like Egypt, the moderate cost and relatively few side effects of herbal modalities are a great request, particularly in chronic diseases which necessitate lifelong medical control.

MATERIALS AND METHODS

Preparation of Plant Materials

Plant Materials: Samples of the dried ripe fruits of *Foeniculum vulgare* Miller, Family Umbelliferae and *Pimpinella anisum* L., Family Umbelliferae, the dried aerial parts of *Thymus vulglaris* L. Family Labiatae and the roots and rhizomes of *Glycyrrhiza glabra* L., Family Leguminosae were purchased from Local Market, Cairo, Egypt and authenticated by Dr. Abdelhaleem Abdelmotagaly, Department of flora, the Agricultural Museum, Dokki. Giza, Egypt. The plants were crushed and grinded into suitable size.

Apparatus: Modified Likens and Nikerson apparatus was used for preparation of the volatile constituents. GC/MS: Gas chromatograph coupled with a mass spectrometer GC/MS Finningan Mat SSQ 7000, Digital DEC EL, 70eV for GC/MS analysis of volatiles. UV-visible spectrophotometer. UV-VIS double beam UVD-3500 spectrophotometer, Labomed, Inc. were used for recording UV spectra and measuring the absorbance in UV and visible range. NMR: Varian Inova-500, varianGemmi unity plus 300 NMR and JEOL delta 400 spectrometer apparatus using DMSO, CDCl2-dl, CD, OD-d4 as solvent and TMS as internal standard. The data are expressed in δ-values in ppm and J-values in Hz.

Phytochemical Methods: Five kilograms of the powdered plants under investigation were extracted separately and exhaustively using 70 % aqueous ethanol. These extracts were lypholized and saved for phytochemical examination then dispensing after fractionation.

Phytochemical Screening Tests: The dried powdered plants under investigation were separately subjected to the following preliminary phytochemical screening tests according to the reported procedures [8]. Carbohydrates and/ glycosides, sterols and /triterpenes tannins, flavonoids, coumarins,anthraquinones, alkaloids and/ nitrogenous compounds, saponins and coumarins.

Investigation of Volatile Components

Preparation of Volatile Fraction: Plants under investigation were covered with sufficient water in a round bottom flask and subjected to hydrodistillation in a modified Likens and Nickerson apparatus which allowed the distillation and simultaneous extraction of the volatile components in an organic solvent (n-pentane). The n-pentane layer was collected and cautiously evaporated, dehydrated over anhydrous sodium sulfate and stored in dark tightly closed container at 4°C to be analyzed by GC/MS [9].

GC/MS Analysis of Volatile Constituents: GC/MS analysis of the volatile constituents was carried out on a gas chromatograph directly coupled to mass spectrophotometer (Finnigan SSQ 7000). Identification of the components was performed by comparing the relative retention time and published mass spectra[10].

Investigation of Phenolic Compounds: One hundred grams of the biologically active fractions were chromatographed separately using silica gel column and eluted with chloroform with the proportional increasing of methanol. Purification of the isolated compounds was achieved using Sephadex LH-20 column and elution with methanol. The structures of all compounds were detected by UV-visible spectrophotometer and NMR spectrophotometer. All isolated compounds were structurally elucidated and confirmed through analysis of their UV data with methanol and complex shift reagents and using ^{1}H and ^{13}C NMR. Their identity was further confirmed by comparing their data with those of authentic samples as well as reported literature [11-13].

Preparation of Plant Doses: Equal proportions of the lyophilized bioactive plant ingredients were mixed together up to 100 g for acute lethal toxicity test and pharmacological tests.

Pharmaceutical Preparation for Clinical Trials: The bioactive plant ingredients were mixed under investigation in equal proportions and encapsulated in the suitable pharmaceutical dosage form according to British Pharmaceutical Codex [14] and saved for clinical trials, after receiving the approval of the Ethical Committee of Medical Researches, National Research Centre, Giza, Egypt.

Preclinical Study

Acute Toxicity Test: Mice (8 animals/ group) receiving progressively increasing oral dose levels up to 12g/kg of the herbal preparation show no lethality in the next 24 hours [15], regulatory agencies no longer require the determination of an LD50 value. So the experimental doses used were 1/120 and 1/60 of 12 g/kg (0.1 and 0.2 g/kg).

Long Acting Toxicity Test: Adult male albino rats, weighing 150g were randomly into three groups. Control group was received 0.9% saline, group 1 received herbal preparation (0.1g/kg; po) and group 2 received herbal preparation (0.2g/kg; po) for eight consecutive weeks. Blood samples were collected after four and eight weeks for assessment of serum creatinine, urea and liver function tests (ALT and AST). Finally, liver and kidney tissue were dissected immediately after death, washed thoroughly with saline and fixed in 10% formalin for 24 h at least. All the specimens were washed in tap water for half an hour, dehydrated in ascending grades of alcohol (70% - 90% - 95% - absolute), cleared in xylene and then embedded in paraffin wax. Sections were stained with haematoxylin and eosin for histopathological investigation.

Efficacy Test: Rats were randomly allocated into 5 groups (n=6). Asthma was induced by OVA sensitization followed by OVA challenge. First, animals were sensitized by i.p. injection of 1 mg/kg OVA/100 mg aluminum hydroxide suspended in 1 ml normal saline for 3 consecutive days. Three days after the final injection, the animals were challenged by exposure to 1% OVA for 15 min. Animals were challenged one day/week for 3 successive weeks by aerosolizing OVA solution contained in a specially devised plastic cylindrical chamber (200 ml capacity) introduced in an ultrasonic nebulizer (DEVILBISS ULTRA-NEB 99, 099HD) [16]. Induction of asthma was done in all groups except the first one, in which saline was used instead of OVA to serve as normal control, group 3: received herbal preparation (200mg/kg; po) 1 h before each OVA challenge, group 4 & 5: received theophylline (10mg/kg; po) and salbutamol (0.1mg/kg; po) according to paget table [17] 1 h before each OVA challenge. Group 2 was left un-treated to serve as positive control.

Lung function tests as tidal volume (TV) and respiratory rate (RR) were assessed 24h after the last OVA challenge.

Clinical Study

Patients and Methods: Ten asthmatic patients (2 males and 8 females), defined by American Thoracic Society (ATS) criteria of asthma [18] and defined as those who had small and/or large reversible airway obstruction, were recruited from the Chest Outpatient Clinic and Pulmonary Function Unit in the National Research Centre (NRC). Their age ranged between 21 and 60 years and their weight ranged between 70-90 kg. Any studies involving experimentally induced asthma, patients working in industrial areas as well as pregnant and lactating patients were excluded. All patients gave written informed consent; the study was approved by the ethical committee of the National Research Centre.

All subjects of the study were subjected to medical history taking and medication usage, clinical examination, complete blood count (CBC) erythrocyte sedimentation rate (ESR), C reactive protein (CRP) Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), creatinine (creat), random blood sugar (RBS) and pre- and post-bronchodilator (200mg of salbutamol) spirometric measurements of forced expiratory volume in the first second (FEV1) percent of predicted and forced expiratory flow (FEF) 25/75% of predicted. Normal pulmonary function test (PFT) was considered as FEV1% of predicted > 85%, FEV1/FVC % of predicted > 70%. A significant reduction in FEF25-75% was defined as less than 60% of predicted [19]. Reversibility is determined either by an increase in FEV1 of ≥12 percent from baseline or by an increase ≥10 percent of predicted FEV1 [20] and by an increase in FEF (25 -75) ≥35% [21] after inhalation of a short-acting bronchodilator. Bronchodilators were stopped 24 h before PFT. Corticosteroids were stopped

4 days before PFT. All tests were performed for each case before and after 4 weeks of therapy. The capsules (500 mg each) were taken by the patient 12 hourly for 1 month without stopping their own medications (β agonist, theophylline, inhaled or systemic corticosteroids). The patients were examined weekly during the 4 weeks of the trial.

Statistical Analysis: Data are expressed as mean ± S.E. Data analysis was done using one way analysis of variance (ANOVA) followed by least significant difference (LSD) test for multiple comparisons and T-test [22].

RESULTS

Phytochemical investigation of the bioactive polar fractions by chemical and chromtographic techniques. [paper, thin layer and column chromatography] as well as spectral analysis revealed the presence of volatile oils, fixed oils, carbohydrates and/glycosides, sterols and / triterpenes in all plants under investigation while *Glycyrrhiza glabra* is rich in saponins. The compounds anethole, myrecene and limonene were present in *Foeniculum vulgare*, anethole was also present in high percentage in *Pimpinella anisum* and *Glycyrrhiza glabra* while thymol, linalool, p-cymene and borneol were present in *Thymus vulgaris*. Flavonoids were detected as apigenin, luteolin glycosides in *Pimpinella anisum* and *Thymus vulgaris*, while liquiritegenin and isoliquiritigenin were detected in *Glycyrrhiza glabra*. The hydroxycoumarin umbelliferone was detected in *Foeniculum vulgare* and *Glycyrrhiza glabra*, the latter is rich in triterpenoid saponins specially glycyrrhetic acid, while *Thymus vulgaris is* rich in triterpenes as ursolic and oleanolic acids.

Preclinical Study: Acute toxicity test revealed that the herbal preparation is safe up to 12 g/kg mice body weight which corresponds to 93 g/70kg man body weight for human when the dose of mice was extrapolated to corresponding estimates in human adopting interspecies dosage conversion scheme [17]. This clarified the highest safety of the herbal preparation.

A long acting toxicity test revealed that serum creatinine level showed no significant difference of data collected within the 1st four weeks and the 2nd four weeks for the tested herbal preparation compared to control. Serum urea level revealed significant increase in group 2 receiving (200mg/kg; po) rat body weight by 24.9% from control group (Figure 1). After the total period of eight weeks the serum urea level of this group returned to normal with no significant change from control group.

Liver function tests (ALT and AST) were normal compared to the control group throughout the experiment.

Light microscopic examination of liver tissue section obtained from control group showing normal histopathological structure of hepatic tissue (Figure 2A), group 1and 2 showing kuffer cells (Figure 2B&5C) and group 2 showing sinusoidal leucocytosis and portal infiltration (Figure 2D&2E).

Light microscopic examination of kidney tissue section obtained from control group showing normal histopathological structure of renal parenchyma (Figure 2F), group 1 showing eosinophilic protein cast in the lumen of renal tubules with no histopathological changes (Figure 2G&2H) also group 2 showing no histopathological changes after 4 and 8 weeks (Figure 2I).

Effect of Herbal Preparation, Salbutamol and Theophylline on Lung Function Tests in Rats: Ovalbumin challenge significantly decreased TV to 30.24% as compared with the normal control group. Treatment of rats with and salbutamol normalized OVA-induced reduction in TV while theophylline and herbal preparation increased TV than both the normal and OVA to 655% and 550% respectively, as compared with OVA group. Moreover RR was not affected by OVA (Table 1).

Clinical Study: The study included 2 males and 8 females' asthmatic patients. Blood pressure was normal before & after treatment in all cases. Patients complaining of cough (n= 5), wheezes (n= 4), shortness of breath (n= 7) and nocturnal attacks (n= 2) showed marked improvement and disappearance of symptoms after 4 weeks of treatment. Decreased intensity of breath sounds (n= 4) and rhonchi (n= 4) found on auscultation were also disappeared after 4 weeks of treatment. Five cases stopped their conventional bronchodilator therapy after taking the capsules under study, three cases reduced the dose and two cases remained on the same dose. Three cases reduced the dose of inhaled corticosteroids and three cases stopped taking occasional systemic corticosteroids during the 4 weeks of treatment (Table 2).

There was no statistical significant change in the mean value of Hemoglobin (12.8 and 12.6 g/dL), Total Leucocytic count (9.3 and 6.1 K/uL) and Platelets count

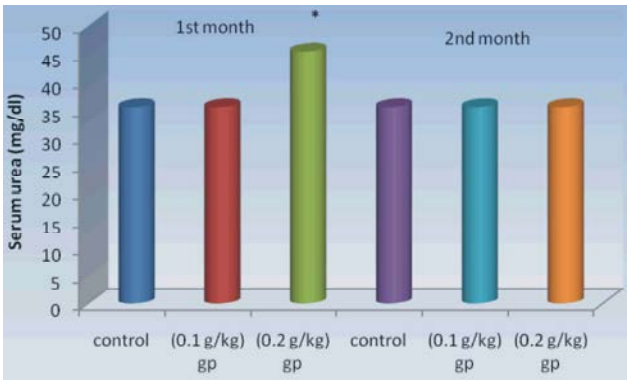

Fig. 1: Effect of herbal preparation on serum urea level
Data were expressed as mean ± SE (n=6).
*Significantly different from normal control at p<0.05

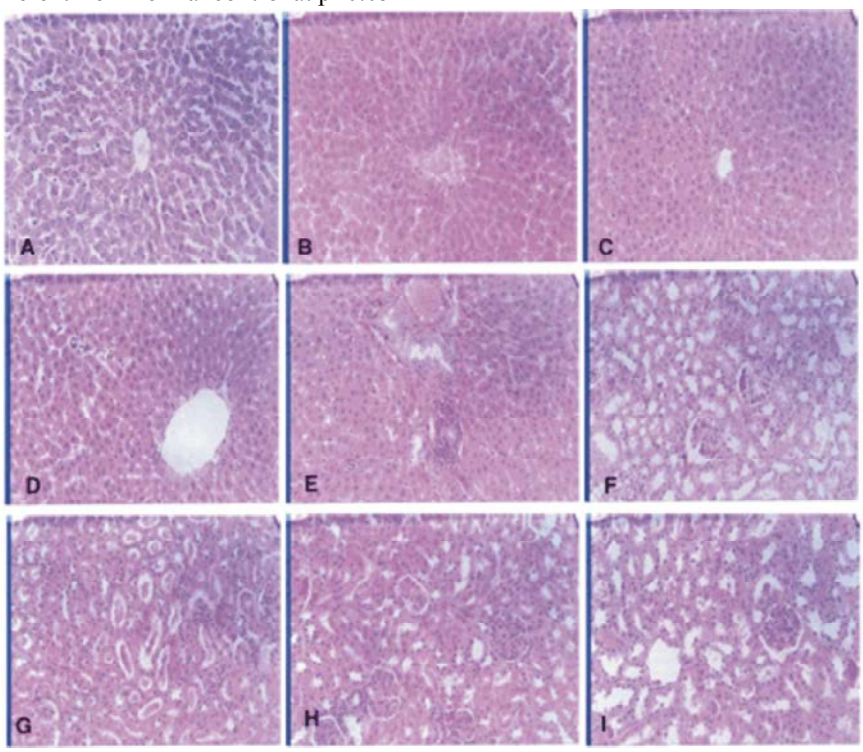

Fig. 2: Photomicrographs of sections of the liver tissue of: (A) Control group showing normal histopathological structure of hepatic; (B) Group 1(100mg/kg) showing kuffer cells; (C) Group 2 (200mg/kg) showing kuffer cells; (D) Group 2 (200mg/kg) showing sinusoidal leucocytosis; (E) Group 2 (200mg/kg) showing sinusoidal portal infiltration. Photomicrographs of sections of the kidney tissue of (F) Control group showing normal histopathological structure of renal parenchyma (G) group 1(100mg/kg) showing eosinophilic protein cast in the lumen of renal tubules; (H) Group 1(100mg/kg) showing no histopathological changes and (I) Group 2(200mg/kg) showing no histopathological changes. (H and E X 200)

Table 1: Effects of herbal preparation, theophylline and salbutamol on lung function parameters in OVA-induced bronchial asthma in rats.

Parameter	Saline	OVA	OVA + Herbal preparation	OVA + Theophylline	OVA + Salbutamol
Tidal volume (TV) ml	0.067±0.002	0.020±0.006*	0.110±0.028@	0.131±0.020*@	0.096±0.005@
Respiratory rate (RR) breath/min	221±22	210±14	180±9	223±14	202±8

Data were expressed as mean ± SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Least Significant Difference test. *Significantly different from normal control at $p<0.05$. @Significantly different from OVA group at $p<0.05$.

Table 2: Clinical data before and after 4 weeks of treatment in studied cases

Total N°=10	Before treatment	After treatment
Cough	N=5	N=0
Wheezes and rhonchi	N=4	N=0
Shortness of breath	N=7	N=0
Decreased intensity of breath sounds	N=4	N=0
Nocturnal attack	N=2	N=0
Bronchodilators	N=10	N=5
Corticosteroids		
- Systemic	N=3	N=0
- inhaled	N=10	N=10
		3 cases reduced dose

Table 3: Statistical comparison between the mean values of the initial and after 4weeks treatment on blood parameters, kidney and liver function in studied cases

		No.	Before treatment Mean ± SD	After treatment Mean ± SD
Hemoglobin (12.6-16.1 g/dL)		9*	12.8+2.26	12.63+2.52
Total Leucocytic count (3.3-8.7 K/uL)		9*	9.3+2.6	6.1+1.6
Platelets count (147-347 K/uL)		9*	295.8+78.63	292.33+74.78
ESR (20 mm/hour)		10	34.4+16.82	25.4+13.33
Random blood sugar (125 mg/dL)	Non Diabetic patients	7	105.25+11.88	96.1+6.91
	Diabetic patients	3	215.5+46.29	262.67+192.79
ALT (7- 55 U/L)		10	20.4+17.92	22+15.65
AST (8 -48 U/L)		10	27.8+12.72	32.8+27.67
Creatinine (0.8 - 1.4 mg/dl)		10	0.9+0.29	0.9+0.43
C reactive protein			Before treatment	After treatment
Negative cases (zero mg/L)			N=5	N=8
Positive cases (>zero mg/L)			N=5	N=2

Data were expressed as mean ± SE (n=10). Statistical analysis was carried out by t- test. * Blood was clotted in the initial sample of one case at $p<0.05$.

295.8 and 292.3) before and after 4 weeks of treatment respectively. The mean value of ESR (34.4 and 25.4 mm/hour) before and after 4 weeks of treatment respectively showed non statistical decrease. Random blood sugar before treatment showed that 3 patients were diabetics; mean value was 215.5 mg/dL and 7patients showed normal finding of random blood sugar; mean value was 105,25 mg/dL. There was no statistical significant difference between the mean value of random blood sugar before and after 4 weeks of treatment in either diabetics and non-diabetics patients; the mean values were respectively 262.67 and 96.1 mg/dL after 4 weeks of treatment (P> 0.05) (Table 3).

There was no statistical significant difference between the mean value of creatinine before and after 4 weeks of treatment; 0.9 and 0.9 mg/dl respectively (P> 0.05) (Table 3).

There was no statistical significant difference between the mean value of ALT (20.4 and 22 U/L) and AST (27.8 and 32.8 U/L) before and after 4 weeks of treatment (P> 0.05) (Table 3).

C- reactive protein CRP were negative (zero mg/L) in 5 cases and positive in 5 (>zero mg/L). After 4 weeks of treatment, 3 patients showed improvement and CRP turned negative (Table 3).

The mean values of the initial reversibility% of FEV1 and FEF25-75 were respectively 12.2% and 48.8% confirming airway reversibility as a diagnostic feature of bronchial asthma (Table 4).

There was a non-significant increase in the mean value of the post bronchodilator FEV1% of predicted (88.4%) after 4weeks of treatment compared to that of the initial post bronchodilator FEV1% of predicted (86.2%) P >0.05. The FEV1reversibility % after 4weeks (6.7%) showed a highly significant decrease compared to the initial FEV1 reversibility % (12.2%) P<0.001 (Table 4).

Figure 3 shows the linear presentation of the values of initial and after 4weeks of treatment post bronchodilator (final) FEV1 % of predicted in studied cases. There was an improvement of FEV1% of predicted after 4 weeks of treatment seen in 7 cases and a reduction in 3 cases.

There was a non-significant increase in the mean value of the after 4weeks treatment post bronchodilator FEF25-75% of predicted (109.7%) compared to that of the initial post bronchodilator FEF25-75% of predicted (90.03%) P > 0.05. The after 4weeks treatment FEF25-75% reversibility % (28.9%) showed a highly significant decrease compared to the initial reversibility % FEF25-75% (48.8%) P < 0.0001 (Table 4).

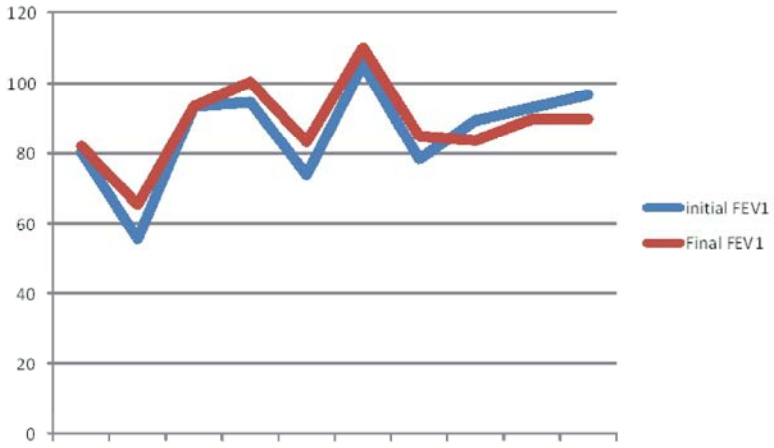

Fig. 3: Initial and after 4weeks post bronchodilator (final) FEV1% of predicted in studied cases

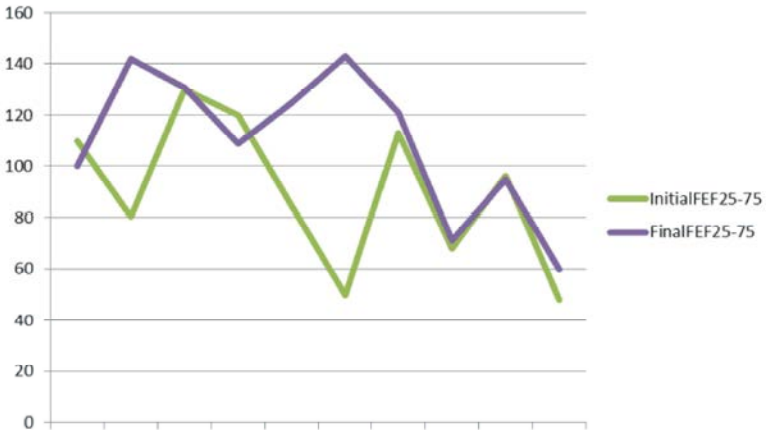

Fig. 4: Initial and after 4weeks post bronchodilator (final) FEF25-75% of predicted in studied cases

Table 4: Statistical comparison between the mean value of initial and after 4 weeks treatment post bronchodilator FEV1% and FEF25-75% of predicted and reversibility in studied Cases

	Initial post bronchodilator of predicated	After 4w. post bronchodilator FEV % of predicated	Initial reversibility%	After 4w. reversibility%
FEV %	86.2 ± 14.3	88.38 ± 11.9	12.185 ± 10.3	6.704 ± 7.9*
FEF25-75%	90.03+28.7	109.65+28.3	48.79+28.6	28.9+33.5**

FEV1 - forced expiratory volume in the 1 s; FEF – forced expiratory flow.

Data were expressed as mean ± SE (n=10). Statistical analysis was carried out by t-test.

*Significantly different from initial reversibility% at $p<0.001$. ** Significantly different from initial reversibility% at $p<0.0001$

Table 5: Statistical comparison between the initial and after 4weeks treatment post bronchodilator FEV1% of predicted and FEF25-75% of predicted in cases with improved and cases with impaired large airway functions

	Cases with improved large airway functions	Cases with impaired large airway functions
Initial post bronchodilator FEV1% of predicted	83.2+16.4	93+3.7
After 4w. post bronchodilator FEV1% of predicted	88.5+14.5*	88+3.5**
Initial post bronchodilator FEF25-75% of predicted	98.03+27.9	70.8+24.4
After 4w. post bronchodilator FEF25-75% of predicted	124.3+15.9**	75.3+17.9

Data were expressed as mean ± SE (n=7 Cases with improved large airway functions; n=3 Cases with impaired large airway functions). Statistical analysis was carried out by t-test. * Significantly different from Initial post bronchodilator FEV1% of predicted at $p<0.001$. ** Significantly different from Initial post bronchodilator FEF25-75% of predicted at $p<0.5$.

Figure 4 shows the linear presentation of the values of initial and after 4weeks treatment post bronchodilator (final) FEF25-75% of predicted in studied cases. There was an improvement of FEF25-75% of predicted after 4 weeks seen in 8 cases and a reduction in 2 cases.

Seven of the studied cases (70%) showed a highly significant increase in the mean value of the after 4weeks post bronchodilator FEV1% of predicted (88.5%) compared to that of the initial one (83.2%) P <0.001. The mean value of the after 4weeks treatment post bronchodilator FEF25-75% of predicted of these 7 cases showed also a significant increase (124.3%) compared to that of the initial one (98.03%) P <0.05 (Table 5).

Only 3cases of the studied group (30%) showed a significant decrease in the mean value of the after 4weeks of treatment post bronchodilator FEV1% of predicted (88%) compared to that of the initial one (93%) P<0.05. The mean value of the after 4weeks post bronchodilator FEF25-75% of predicted of these 3 cases showed a non-significant increase (75.3%) compared to that of the initial one (70.8%) P > 0.05 (Table 5).

DISCUSSION

In a pilot study, a herbal preparation formed from bioactive fractions isolated from *Foeniculum vulgare* (Fennel), *Glycyrrhiza glabra* (Liquorice), *Thymus vulgaris* (thyme), *Pimpinella anisum* (anise) was assessed pharmacologically and clinically as a new safety treatment for bronchial asthma control [23-25].

These herbs were approved for the control of bronchial asthma by three main bodies of scientific researches in phytotherapy; The Egyptian Ministry of Health, The British Herbal Pharmacopeia [26] and The Physician Desk Reference [6] European Commission. These herbs were locally produced and were made available by the Ministry of Health, to the market (Pharmaceutical Companies) for use in different therapeutic modalities.

Our study confirmed the safety of the preparation in human and showed no statistical significant difference, after 4 weeks of the new drug intake, in the mean value of the CBC, RBS even in diabetic patients, liver and renal functions. The ESR and the CRP showed non statistical significant improvement of their values after treatment denoting the presence of an anti-inflammatory role of the drug. This anti-inflammatory effect coincides with the findings in several studies. However with thyme, indigenous to the Mediterranean region, ample evidence suggests that time honored culinary and medicinal plant thyme, prized for its aromatic oil is a generous source of potent antioxidant compounds. It improves the clearance of mucous and helps airway relaxation [27]. Moreover, Raja [28] described the successful use of thyme for bronchial asthma. Thyme is a bronchial antispasmodic, an expectorant and an antibacterial agent. In animal experiments, a spasmolytic effect was demonstrated for its flavone fraction [6] and an expectorant effect on ciliary activity for the terpenoidal content. Farouk *et al.* [29] assessed clinically and by PFT 54 asthmatic patients and demonstrated the superiority of liquorice versus Boswelia for bronchial asthma. In *Glycyrrhiza glabra*, Isoliquiritigenin,an aldose reductase inhibitor exerts antiplatelet effects through inhibition of cyclooxygenase,lipooxygenase and peroxidase activity [30]. The anti-inflammatory effect of glycyrrhizin is enhanced to its anti-thrombin action through inhibition of thrombin induced platelet aggregation [31].

Experimentally, in the present study, OVA challenge (1%) significantly decreased TV, as compared with normal control group, indicating constriction of airway smooth muscle. These changes are in harmony with those of Salama *et al*. [16]. Herbal preparation significantly attenuated OVA-induced decrease in TV more than salbutamol group. Clinically, the preparation was found to improve patients' symptomatology; cough, wheezes, shortness of breath and decreased frequency of night attacks. Moreover, signs also were improved markedly after the drug intake. Five cases stopped their conventional bronchodilator therapy â agonist and/or theophylline and replaced it by the herbal preparation. Three cases reduced the dose of inhaled corticosteroids and three cases stopped taking occasional systemic corticosteroids during the 4 weeks of treatment. This was an evidence of the bronchodilator and anti-inflammatory effects of the studied herbal preparation. Several studies agreed our findings. Potent relaxant effects of Foeniculum vulgare [32] and of Pimpinella anisum [33] on isolated guinea pig tracheal chains were confirmed and the relaxant effect of *Foeniculum vulgare* on isolated guinea pig tracheal chains was documented. Boskabady *et al.* [34] proved the relaxant effect of *thyme vulgaris* on guinea pig tracheal chains and stated that the extract of Thymus is efficient as theophylline. Fennel has an antispasmodic effect. Aqueous fennel extracts raised the mucociliary activity of the ciliary epithelium [6].

Wendy Person *et al.* [35] presented a pilot study investigating the ability of a herbal composite (garlic, white horehound, boneset, aniseed, fennel, liquorice, thyme, hyssops flavonoid constituents) to alleviate clinical signs of respiratory dysfunction in horses with recurrent airway obstruction. It was given

twice /day for 1 month in a cross over manner research. It showed statistical significance in safe reduction of the elevated respiratory rates, through pulmonary function tests and by ventigraph to calculate.

Δ P pl max. CBC, biochemical, mineral analysis and osmolality were also assessed. Tracheal lavage showed increase in the proportion of macrophages.

A15 based plant formula, different however than that of the presently tested herbs has been thoroughly studied in a double blind controlled clinical trials parallel arms and compared with oral salbutamol and theophylline. The formula was found as efficacious as salbutamol alone or even salbutamol plus theophylline [36].

Seventy percent of the total number of cases (7 patients) showed statistical significant improvement in both small and large airway functions after 4 weeks of treatment. Although, the 3 other cases showed statistical significant impairment in large airway functions they showed improvement in small airway functions.

When pulmonary function tests (PFT) are obtained, measuring pulmonary function before and after bronchodilator to determine reversibility is recommended. The degree of airway reversibility correlates with airway inflammation [37]. In addition, those patients who have the greatest degree of reversibility in response to short acting â agonist (SABA) may be at the greatest risk of developing fixed airflow obstruction and have the greatest loss of lung function [38]. The post bronchodilator FEV1 measure can then be used to follow lung growth patterns over time [39].

In this work, a highly significant statistical reduction was demonstrated in both the mean values of reversibility% FEV1 and FEF25-75 after 4weeks of treatment compared to the initial parameters $P < 0.001$, denoting reduced loss of lung function and airway inflammation.

CONCLUSION

The herbal preparation was found to be safe and effective in improving patient symptomatology, decreasing inflammation and improving pulmonary functions. Moreover, it may replace bronchodilators and decrease the dose of anti-inflammatory drugs.

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