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TANAFFOS

Effect of Hyssop (*Hyssopus officinalis*) Syrup on Mild to Moderate Asthma: A Randomized Double-Blind Placebo-Controlled Trial

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Background: Asthma is one of the most common respiratory diseases. *Hyssopus officinalis* L. is a medicinal herb that has shown anti-asthmatic effects and has also been recommended in Persian Medicine literature for its treatment. In this study, the efficacy of hyssop in mild to moderate asthma was investigated considering the patients' phenotype (having productive/non-productive cough).

Materials and Methods: In a randomized triple-blind placebo-controlled trial, 60 mild-to-moderate asthmatic patients were randomized to receive either hyssop syrup (5 ml twice daily containing 6g *Hyssopus officinalis* L. extract) or plain sugar syrup (5 ml twice daily) for 4 weeks as an adjuvant to routine treatment. Outcome measures were the Asthma Control Test (ACT), pulmonary function tests, Expert Panel Report 3 (EPR3), and wheezing severity.

Results: The patients with productive cough in the hyssop group showed significant improvement in forced expiratory volume in 1 second (FEV1), ACT (at the 4th week), peak expiratory flow (PEF), maximal expiratory flow rate 25-75 (MEF25-75%), and wheezing severity. However, those with dry cough got worse regarding these indices.

Conclusion: Hyssop syrup is effective for asthmatic patients with productive cough and a higher BMI, but it is not suitable for those experiencing a dry cough. It highlights the importance of syndrome differentiation in asthmatic patients and a posteriori subgrouping in data analysis. This approach enhances treatment accuracy and response rates while reducing adverse effects. Future trials are guaranteed to approve this categorization in asthma treatment.

Keywords: Asthma; A posteriori; Herbal medicine; *Hyssopus officinalis*; Integrative medicine; Persian medicine; Syndrome differentiation

INTRODUCTION

Asthma is a "complex chronic inflammatory disease" that is clinically diagnosed by the well-known triad symptoms of cough, wheezing, and shortness of breath (1). This condition includes a variability in airway inflammation and airflow obstruction (2). As one of the most common chronic diseases, asthma has an increasing trend with a prevalence rate of 13.4% in Iran (3, 4). It is a heterogeneous disease involving more than 100 genes (5). This heterogeneity reflects the underlying mechanism; it

has been shown that about 50% of asthmatic patients who use two major classes of drugs, i.e., short-term bronchodilators and inhaled corticosteroids, do not respond well because of their genetic background (6). Different genes lead to various clinical symptoms via different mechanisms reflecting individual differences (7). However, the current guidelines have ignored this issue leading to unsuccessful or conflicting results in clinical trials (8).

The Lancet Asthma Commission (LAC) announced that "asthma" is not a single diagnosis; it is a term used to describe a group of clinical symptoms including wheezing, coughing, and shortness of breath which raises the question: "What kind of asthma is this?" (9). A long-term approach to asthma is to categorize patients based on a combination of observed clinical, biological, and physiological features called phenotype (10). Considering these characteristics, the best treatment is to be chosen. However, the defined asthma phenotypes can be applied to a small proportion of asthmatic patients (11).

Persian Medicine (PM) is one of the oldest comprehensive medical systems (same as Chinese medicine and Ayurveda) which has a holistic approach toward human health and diseases (12). In this viewpoint, asthma (which is the closest entity to what is called *Rabv* in PM) has been a well-known disease with various pulmonary and non-pulmonary origins (13, 14). Asthma with pulmonary origin could be generally classified into two main categories with different clinical symptoms and specific treatments: wet (with productive cough) and dry (with non-productive cough) dystemperament of the lung. Various medicinal plants have been introduced in PM literature for the treatment of asthma some of which have shown beneficial effects through clinical trials (15-17). In this regard, one of the most commonly recommended herbs is hyssop (18-20).

Hyssop is a medicinal plant (Labiatae family) scientifically known as *Hyssopus officinalis* L. It is used in a wide variety of foods (including broths and soups), salads, drinks, and spice mixes (21). Avicenna (980–1037 AD), the outstanding Iranian philosopher and physician of medieval Persia (22), has stated in "Canon of Medicine" that the most common application of this plant is treating pulmonary diseases including asthma (23).

On the other hand, different properties of hyssop including antiviral (24), antispasmodic (25, 26), antibacterial (27-30), antioxidant (29, 31), antifungal (32), and immunomodulatory (33) effects have been shown in previous studies. A clinical study by Singhal et al. showed that an herbal compound containing hyssop could improve spirometric indices in asthmatic patients (34).

This study investigated the effect of hyssop on asthmatic patients considering the phenotypic categorization suggested by PM based on productive/non-productive cough.

MATERIALS AND METHODS

Study design

It was a randomized, triple-blind, placebo-controlled clinical trial conducted at Faghihi Hospital and the Persian medicine clinic of Hakim Imad al-Din Shirazi both affiliated with Shiraz University of Medical Sciences (SUMS), Shiraz, Iran. Participants were randomly divided into two groups using block randomization (block size: 4). The coded drug and placebo syrups were prescribed by a pharmacist (blinded to the allocation) keeping the concealment of allocation until the end of the study. In addition to the patients, interventionists, researchers, and the epidemiologist were also blinded regarding the allocations. Over 4 weeks, all the participants took 5 ml of either hyssop or placebo syrup twice daily (in the morning before breakfast and at night before sleep). The duration of intervention in previous herbal studies conducted in asthmatic patients has been similar to the current research (35-37).

There was no recommendation or restriction regarding their nutrition. There was also no alternation in the routine treatments of the patients i.e., inhaled corticosteroid and long-acting β2-agonists, in conformity with the treatment

of mild to moderate persistent asthma in Expert Panel Report 3 (EPR3) (38).

The participants were able to contact the researcher during the study. Moreover, they were visited at the beginning of the study and 4th week. In addition, they were followed up via phone call at the end of week 2, and week 8. Possible adverse events during the study were also asked to be recorded.

Ethical considerations

The local Ethics Committees of the Shiraz University of Medical Science approved the study (ethic code number: IR.SUMS.MED.REC.1396.75). The study was also registered in the Iranian Registry of Clinical Trials (http://www.irci.ir) with the registration code IRCT20110811007297N5. The study was conducted following the CONSORT guideline.

Participants

Patients were enrolled based on these inclusion criteria: 1. Patients older than 18 years of age with a confirmed diagnosis of mild to moderate persistent asthma. 2. Having FEV1% (Forced Expiratory Volume in 1 second) between 60-80% of the predicted value (38). 3. Signed written informed consent. They were assessed by a pulmonologist based on the diagnostic criteria of Global Initiative for Asthma guidelines (GINA). On the other hand, these exclusion criteria were considered: 1. History of allergy to pennyroyal, peppermint, thyme, hyssop, lavender, or savory. 2. Any discontent with the treatment. 3. Inability to express the severity of symptoms. 4. Having complications during the treatment. 5. Consuming theophylline. 6. Diabetes. 7. History of seizure. 8. Pregnant or lactating women. 9. Those who require hospitalization. 10. Disease exacerbation at the beginning of the study. 11. Smokers. 12. Using any complementary and alternative medicine. Participants were asked not to use any other complementary and/or alternative medicines during the study period and follow-up.

Drug preparation

Both drug (hyssop) and placebo (plain sugar) syrups were purchased from Ahura Pharmaceutical Company, Shiraz, *Iran*. *Hyssopus officinalis* L. (hyssop), in the form of dried flowering shrubs, had been purchased from an agricultural engineer who developed the medicinal herbs in Fasa, Iran. The herbs were identified as *Hyssopus officinalis* L. by the Herbarium Center of the School of Pharmacy, Shiraz University of Medical Sciences (Voucher Number: PM984). A traditional procedure was followed in making the herbal medicine: 100g of dried plant was mixed with 1L of boiling water and kept in a closed container for 4 hours. Extraction was done by 7:1 ratio (plant and aqueous solvent parts, respectively). The amount of 0.86 g of extract was added to 5 ml of sugar solution (50%) and 6 g of plant in 5 ml syrup was set as the final concentration. Plain sugar was used to make the placebo. Food coloring was also added to both syrups to make the same appearance and smell. The drug dosage was determined according to the current evidence (21) and PM reference books (39, 40).

Gas chromatography-mass spectrometry (GC-MS)

The Hyssop plant was examined in the Medicinal & National Products Chemistry Research Center using a gas chromatography-mass spectrometry (GC-MS) device (GC set model: Agilent Technologies 7890A, MASS set model: Agilent Technologies 5975C). 100g of the Hyssop plant powder was turned into 0.4 ml of essential oil; 0.2µl of which was diluted with hexane (1:10) and then examined. Identification was performed in reliance on a comparison of the principal components' mass spectrum with reference samples (Adam´s book: Identification of Essential Oil Components) in addition to the assessment and comparison of the retention indices (linked to the retention time of normal alkaline) of each peak in GC-MS chromatogram with reference samples.

Outcome measures

We investigated the efficiency of the treatment using the Asthma Control Test (ACT) questionnaire and pulmonary function tests (PFTs) as the primary outcome measures. The ACT evaluates the asthmatic patients' health condition through 5 questions (four questions regarding the symptoms and one about the patient's self-

assessed level of control). The ACT score ranges between 5 and 25: 20-25 indicating well-controlled asthma, 16-20 is fair control, and 5-15 is an indicator of poor control (41). The Persian-validated translation of this questionnaire was applied in this study (42).

PFTs including the percentage of forced expiratory volume in the first second (FEV1%), maximal expiratory flow rate 25-75 (MEF25-75%), FEV1/FVC, and peak expiratory flow (PEF) were measured using the spirometry set (nSpire Health GmbH; Oberthulba/Germany).

The secondary outcome measure was the wheezing degree. It was categorized as follows (43): 1) Severe: hard breathing when resting, wheezing in expiratory time, generalized rhonchi. 2) Moderate: comfortable breathing when resting, wheezing in expiratory time, generalized rhonchi. 3) Mild: prolonged expiration, outspread rhonchi.

Statistical analysis

According to a similar study on squill oxymel (44) and considering 0.01 as type I error and 0.01 as type II, a minimum sample size of 25 in each group was calculated for FEV1% [Mean differences (SD) in the placebo group was -3.43 (1.73) and in the intervention group was 20.94 (7.68)]. Considering the potential loss to follow-up, 35 patients were considered for each group. At the end of the study, the data were analyzed based on lung temperament categories.

Data were analyzed using SPSS version 22 (SPSS Inc., Chicago IL, USA). The data were described as mean ± standard deviation (SD). Mann-Whitney U test was used to compare baseline characteristics and variable changes between groups. To eliminate the effects of confounding factors, the UNIANOVA test was used. P values < 0.05 were considered as the level of statistical significance.

RESULTS

Study flow

This study was conducted from January 2018 to February 2019. A total number of 102 patients were assessed and 70 were enrolled in the study. Among them, 60 participants completed the study. Considering the study results, a posteriori subgrouping was also applied. The CONSORT flowchart of the study is presented in Figure 1. In the drug group, four patients were excluded for worsened coughing and one for increased dyspnea. In the other group, one was excluded for migration, and four were dissatisfied with the treatment.

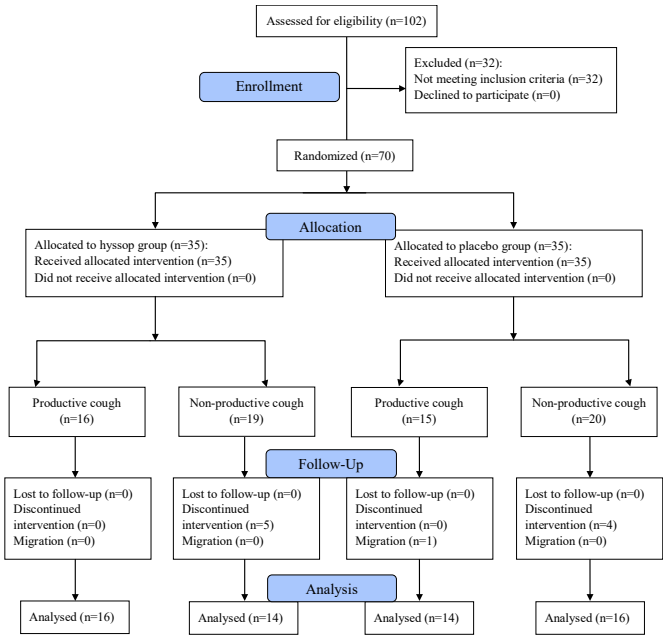

Figure 1. CONSORT flowchart of the study

Drug analysis

According to the results of GC-MS analysis, cis-Pinocamphone, trans-Pinocamphone, and β-Pinene proved to be the three key components of hyssop essential oil (Figure 2). It seems that these components could be responsible for the pharmacological effects of the plant. Table 1 shows the details of the drug analysis.

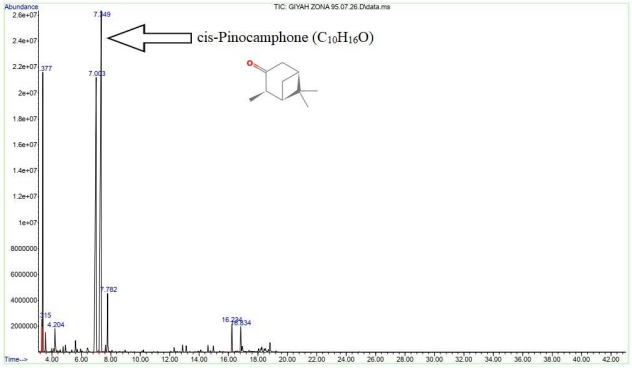

Figure 2. GC/MS fingerprint of hyssop aqueous extract

Baseline characteristics

The baseline characteristics of the study groups are compared in Table 2. There was no significant difference between the patients with productive cough (PC) and non-productive cough (NPC) in drug and placebo groups in terms of the following features: age, gender, body mass index (BMI), FEV1, FEV1/FVC, MEF25-75, EPR3, PEF, and ACT. However, the difference between groups was significant regarding wheezing severity.

Table 1. Hyssop essential oil ingredients

Component	Retention time	Recorded retention indices	Reference retention indices	Total percent
Sabinene	3.315	971.2991	975	1.47
β-Pinene	3.377	975.9819	979	15.24
Limonene	4.304	1026.889	1029	1.69
trans-Pinocamphone	7.003	1162.034	1162	32.76
cis-Pinocamphone	7.349	1177.203	1172	41.60
Myrtenol	7.782	1196.186	1195	3.65
Hedyearyol or Elemol	16.234	1546.9080	1548 or 1549	1.94

Table 2. Comparison of baseline characteristics of the participants with productive cough and non-productive cough in hyssop and placebo groups

Variables	Sub-groups	Hyssop group (n=30)	Placebo group (n=30)	P value
Age (year) (Mean ± SD)	PC	48.38 ± 16.68	61.07 ± 21.29	0.08
	NPC	47.64 ± 15.06	41.50 ± 16.14	0.19
	Total	50.63 ± 20.89	48.03 ± 15.68	0.91
Gender (m/f) (n)	PC	4/12	5/9	0.40
	NPC	7/7	6/10	0.49
	Total	11/19	11/19	1.00
BMI (kg/m2) (Mean ± SD)	PC	29.90 ± 4.73	29.17 ± 2.10	0.73
	NPC	25.10 ± 4.46	25.87 ± 4.32	0.64
	Total	27.66 ± 5.14	27.41 ± 3.80	0.73
FEV1 (%) (Mean ± SD)	PC	67.88 ± 7.51	70.86 ± 7.92	0.29
	NPC	68.29 ± 7.58	69.44 ± 5.37	0.31
	Total	68.07 ± 7.41	70.10 ±6.60	0.13
FEV1/FVC (Mean ± SD)	PC	85.81 ± 13.27	91.93 ± 15.11	0.14
	NPC	93.57 ± 11.38	90.06 ± 12.75	0.37
	Total	89.43 ± 12.83	90.93 ± 13.69	0.57
MEF25-75 (%) (Mean ± SD)	PC	37.44 ± 17.03	45.64 ± 21.86	0.38
	NPC	47.43 ± 11.50	47.81 ± 24.05	0.38
	Total	42.10 ± 15.33	46.80 ± 22.68	0.73
EPR3 (Mean ± SD)	PC	1.69 ± 0.47	1.79 ± 0.42	0.66
	NPC	2.00 ± 0	2.00 ± 0	1.00
	Total	1.90 ± 0.30	1.83 ± 0.37	0.45
PEF (Mean ± SD)	PC	54.69 ± 16.95	54.36 ± 15.66	0.86
	NPC	58.57 ± 17.44	65.63 ± 21.88	0.27
	Total	56.50 ± 16.99	60.37 ± 19.75	0.38
ACT (Mean ± SD)	PC	11.25 ± 2.91	13.14 ± 2.77	0.15
	NPC	17.21 ± 1.76	16.25 ± 3.02	0.47
	Total	14.03 ± 3.26	14.80 ± 3.26	0.49
Wheezing Severity (Mean ± SD)	PC	1.75 ± 0.57	2,43 ± 0.51	0.01
	NPC	2.93 ± 0.26	2.56 ± 0.51	0.09
	Total	2.30 ± 0.75	2.50 ± 0.50	0.38

SD: Standard Deviation; BMI: Body Mass Index; BP: Blood Pressure; FEV1: Forced Expiratory Volume in 1st second; FVC: Forced Vital Capacity; MEF25-75: Maximal Expiratory Flow Rate 25-75; EPR3: Expert Panel Report 3; ACT: Asthma Control Test; PEF: Peak Expiratory Flow; PC: Productive Cough; NPC: Non-Productive Cough.

Primary outcomes

Comparing the patients with productive cough between the two groups showed a significant improvement in FEV1, FEV1/FVC, EPR3, PEF, ACT (at 4th week), and MEF25-75 in those receiving the drug (Table 3).

The same comparison was performed in patients with non-productive cough. It indicated a significant increase in ACT (in the 8th week) and a significant decrease in FEV1, ACT (in the 4th week), and MEF25-75 (Table 3).

Secondary outcome

This study showed a significant improvement in wheezing severity between the study groups in patients with productive cough who received the drug (P=0.001). However, this parameter significantly got worse in patients with non-productive cough receiving the drug (Table 3).

Relationship between BMI and cough

Interestingly, we found that participants with productive cough have higher BMI. To examine this relationship, patients with productive cough (N=30, mean BMI=29.6±3.7) were compared with those with dry cough (N=30, mean BMI=25.5±4.3) using the Mann-Whitney U test. To determine the effect of BMI on cough, the logistic regression model showed that by the increase of one unit of BMI, the odds of having a productive cough (compared to dry cough) will increase 1.29 times (p-value=0.001; 95% CI: 1.102-1.509).

Side effects

Some adverse effects including thirst, dry mouth, and throat irritation were reported in the drug group. Five participants in this group (all with dry cough) seized the intervention due to an increase in coughing and dyspnea.

Table 3. Changes of outcome measures in patients with productive cough and non-productive cough in hyssop and placebo groups

Variables	Sub-groups	Hyssop (Mean ± SD) (N=30)	Placebo (Mean ± SD) (N=30)	P value
FEV1 (%)	PC	20.43 ± 17.18	0.28 ± 3.07	<0.001
	NPC	-8.00 ± 3.30	0.93 ± 1.38	<0.001
P-value		<0.001	0.82	
FEV1/FVC	PC	11.81 ± 15.23	-2.07 ± 12.86	0.03
	NPC	-4.64 ± 10.83	1.25 ± 6.52	0.08
P-value		0.002	0.75	
MEF25-75 (%)	PC	27.18 ± 25.30	2.21 ± 11.74	<0.001
	NPC	-11.35 ± 7.71	1.87 ± 4.88	<0.001
P-value		<0.001	0.82	
ACT (at 4th week)	PC	9.56 ± 4.28	0.50 ± 1.87	<0.001
	NPC	-3.21 ± 2.54	-0.78 ± 1.52	<0.001
P-value		<0.001	0.42	
ACT (at 8th week)	PC	0.31 ± 1.35	-0.78 ± 1.52	0.75
	NPC	2.42 ± 3.03	0.50 ± 1.67	0.01
P-value		0.01	0.13	
EPR3	PC	0.75 ± 0.77	0	0.008
	NPC	-0.21 ± 0.42	0	0.33
P-value		0.001	1.00	
PEF	PC	13.25(20.00	-06.21(15.58	0.006
	NPC	-4.71(18.65	5.31(12.50	0.22
P-value		0.02	0.15	
Wheezing Severity	PC	1.18 ± 0.54	-0.14 ± 0.36	<0.001
	NPC	-0.64 ± 0.49	-0.06 ± 0.25	0.006
P-value		<0.001	0.72	

ACT: Asthma Control Test; SD: Standard Deviation; BP: Blood Pressure; EPR3: Expert Panel Report 3; FEV1: Forced Expiratory Volume in 1st second; FVC: Forced Vital Capacity; MEF25-75: Maximal Expiratory Flow Rate 25-75; PEF: Peak Expiratory Flow; PC: Productive Cough; NPC: Non-Productive Cough.

DISCUSSION

This study was the first clinical trial that investigated the efficacy of *Hyssopus officinalis* L. in patients with mild to moderate asthma considering their syndrome differentiation. Based on the results, daily consumption of 12 g hyssop (6 g twice daily in syrup dosage form) for 4 weeks improved patients with productive cough despite those with dry cough. These contradictory responses could be related to the individual differences that were shown in this study according to productive cough and its positive relation with BMI.

In most asthmatic patients, severity has been reported to be mild to moderate. Meanwhile, heterogeneity has been observed in patients with mild to moderate asthma (45). Such differences could be explained by considering the concept of *Mizaj* (temperament) in Persian medicine. It is one of the basic theories of Persian medicine which addresses individual differences and is determined by standard assessment of specific indices (46, 47).

In this study, wet dystemperament of the lung corresponded with productive cough. Asthmatic patients with productive cough, which had also a higher rate of BMI, significantly improved after using hyssop syrup. However, those with dry cough (having lower BMI at the same time) became worse at most of the assessed indices. Such a dramatic difference in the therapeutic response of asthmatic patients shows the importance of syndrome differentiation and case selection based on Persian medicine for more effective management.

Previously, the Chinese medicine syndrome differentiation algorithm (CMSDA) had been used for the phenotypic classification of asthmatic patients (48). This study identified a novel diagnostic biomarker that revealed the applicability and scientific base of syndrome differentiation. Likewise, the current study suggested new diagnostic indices (i.e., BMI and productive/non-productive cough) for the classification of asthmatic patients according to Persian medicine syndrome differentiation.

A study investigating factors that predict a better response to tiotropium versus salmeterol showed that asthmatic patients with lower resting heart rates (parasympathetic predominance) respond better to anticholinergic drugs (49). Another study has hypothesized that patients with increased cholinergic tone (showing non-respiratory symptoms related to cholinergic predominance before asthma attack including esophageal reflux, stress episodes, abdominal pain, sweating, and heartburn) respond better to anticholinergics compared to others. These symptoms might be considered a new phenotype in asthma management (50).

It is known in the pathophysiology of asthma that acetylcholine binds to muscarinic receptors leading to bronchial spasms, increased mucus secretion, inflammation, and airway remodeling (51). In our study, the significant improvement in patients with productive cough and higher BMI could probably explained by the anticholinergic effects of hyssop through acetylcholine inhibition by β-pinene and cis-pinocamphone (52).

On the other hand, excessive dryness of the airways causes bronchospasm. Accordingly, it is not surprising that clinical symptoms and pulmonary function tests of patients with dry cough worsened in response to hyssop (which has a hot-dry nature) (53). Dryness makes hyperosmolarity in the respiratory surfaces leading to cellular shrinkage and release of inflammatory mediators. This will eventually cause smooth muscle contraction and airway narrowing (54). The improvement in the ACT of patients with dry cough in the hyssop group in the 8th week compared to the 4th week indicates that the drying effect of hyssop is not appropriate for treating this group of patients.

This study's main limitations were the short follow-up duration and the small sample size. It is recommended to conduct trials with larger sample sizes and phenotype-specified interventions for a better assessment of such individualized treatments.

CONCLUSION

This clinical trial showed that hyssop syrup is effective for asthmatic patients with a productive cough and higher BMI, but is not suitable for those experiencing dry cough. This finding emphasizes the necessity of a posteriori subgrouping based on the results obtained. Future studies should further evaluate these results to enhance the phenotypic categorization of asthmatic patients and improve therapeutic responses.

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