Preclinical and Post Clinical Studies on Newly Developed Herbal Cough Syrup from *Piper Longum* L. Fruit.

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Abstract

Cough is a defensive mechanism of body preventing any material from entering the airways of lungs and clearing mucus or foreign material from airways. *Piper longum* L. fruit has long been used in traditional medicine for cough, including the Ayurveda system of medicine. Its main constituents are piperine, piper longumine, and piper longuminine. This study has been designed with the objective to explore and confirm the antitussive potential of *Piper longum* L. For this purpose a herbal syrup is prepared containing *Piper longum* L. fruit aqueous extract. Syrup initially evaluated according to pharmacopeia standards for its stability under extreme humidity, temperature and light followed by clinical trials on patients after having informed consent.

Keywords:

Cough, Fruit of *Piper longum* L., Herbal Cough Syrup, Clinical trials.

1. INTRODUCTION

A cough itself is not a disease but can be a common symptom of different upper and lower respiratory tract diseases. Sometimes cough response occurred even if no lung disease exists. A cough can happen when something irritates the nerve endings, called cough receptors. Inhaling particles, vapours, smoke, fumes, dust, or cold air may irritate these receptors resulting in cough (Irwin *et al*., 1998).

A respiratory infection typically associated with coughing and vomiting is called pertussis (whooping cough). There are three types of cough time periods, acute (lasts less than 3 weeks), subacute (lasts 3 to 8 weeks) and chronic (lasts more than 8 weeks and does not let up). An acute cough is most often caused by the common cold which slowly starts to improve by the third to fifth day. Cough from the common cold usually is not a serious threat to health and usually does not last longer than 21 days. A subacute cough most commonly happens after a respiratory infection

(often from a virus). Other common reasons for a subacute cough are whooping cough (pertussis) and flare ups of conditions such as asthma, chronic bronchitis, sinusitis, or bronchiectasis. A chronic cough can happen to someone for many reasons. Often, it is a combination of reasons. Chronic cough can happen from upper airway conditions such as inflammation (swelling) of the membranes inside the nose (rhinitis) and sinuses (sinusitis) because of allergies or infections or from poorly controlled asthma. Cigarette smoking can cause chronic bronchitis and result in a chronic cough, Gastroesophageal reflux disease (when contents from the stomach backup) (Irwin *et al*., 1998).

Plants have been the source of medicines since thousands of years. Traditional herbs and herbal cough products have been considered to be mild, nontoxic, non-sedative and even harmless. Cough Syrup, a liquid dosage form, usually given to individuals who can't swallow solid dosage form or children because of ease of administration. Different herbal syrups are available in Pakistan (such as Ezicof, Linkus, Banafsha Shehtoot, Toot Siah, Surficol, Coferb, Suduri, Prospan, Cofloz, etc). Mostly they contain the ingredients like: Glycyrrhiza glabra, Hyssopus officinalis, Piper longum, Zingiber officinale, Hedera helix, Thymus vulgaris Primula veris and Althea officinalis, etc. Species of the genus Piper are among the important medicinal plants used in various systems of medicines. *Piper longum L. (Piperaceae)*, commonly known as "long pepper", is widely distributed in the tropical and subtropical regions of the world, throughout the subcontinent, Middle Eastern countries and the Americas. It is also growing wild in Malaysia, Singapore, Bhutan, and Myanmar. *Piper longum (Piperaceae)* has effects on relaxation of muscles tension, inhibitory activities on prostaglandin and leukotrienes, COX-I inhibitory effect and thus exhibit anti-inflammatory activity. Studies have also revealed its antiasthmatic and anticonvulsant properties (Athavale *et al*., 1978, Zaveri M *et al*., 2010, Nagatome Y *et al*., 2005). In this study, an effective herbal cough syrup is formulated from *Piper longum*. and evaluated on standard pharmaceutical parameters;

Table 1: Formulation of Cough Syrup

S. No.	Ingredients	Source	Weights	UoM*	Proportion%
01	*Powder (active drug fruits)*	Herbal store	250	Mg	25
02	*Methyl paraben*	Herbal store	2	Mg	0.2
03	*Sucrose*	Herbal store	667	G	66.7
04	Purified water	Donated by OBS Pakistan	Qs 1000ml		

2. MATERIALS AND METHODS

2.1. Preclinical study

This study has been designed with the objective to explore and confirm the antitussive activities which are claimed for *Piper longum L.*

2.2. Method of Preparation:

Aqueous extract of herbs and parabens solution added to sugar syrup in stainless steel pressure vessel, heated with occasional stirring, until boiled. Volume was checked through dip stick (already marked and validated) and made up to 1000 ml (Table 1). Solubility was checked by observing the clarity of solution visually. The final herbal syrup was then subjected for evaluation.

Stability Studies

For stability studies (Table 2-5) and clinical trials (Table 6).

Table. 2: Light stability test of cough syrup

Strength	Sample Quantity	Sunlight					Fluorescent tube light				UV light				Infra-red lamp light			
		Months	0	1	3	6	0	1	3	6	0	1	3	6	0	1	3	6
250mg	100ml		-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	+
500mg	100ml		-	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+
1000mg	100ml		-	-	+	+	-	-	-	+	-	-	+	+	-	-	+	+
Control	10ml		-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+

(-) No change (+) Degradation

All samples were chromatographed on silica gel fluorescence (254 nm) plates in a suitable solvent system (ethyl acetate-MeOH-H2O, 7:1:0.1) to monitor the stability of our cough syrup containing main ingredient (250, 500mg and 1g along other minor ingredients. Control is without main ingredient but contain all other minor component.

Table 3: Thermal Stability Test of Cough Syrup

Strength	Sample Quantity	Ambient					45°C				80°C			
		Months	0	1	3	6	0	1	3	6	0	1	3	6
250mg	10ml		-	-	-	-	-	-	-	-	-	+	+ +	+ + +
500mg	10ml		-	-	-	-	-	-	+	++	+	++	+ +	+ + + + +
1000mg	10ml		-	-	-	-	-	+	++	+++	-	-	+	+ + + + +
Control	10ml		-	-	-	-	-	-	-	-	-	-	+ +	+ + +

All samples were chromatographed on silica gel fluorescence (254 nm) plates in a suitable

solvent system (ethyl acetate-McOH-H2O, 7:1:0.1) to monitor the stability of our cough syrup. Confirmatory test for stability was carried out on silica gel (254 nm fluorescent) plates. The silica gel plate was developed in a suitable solvent system (i.e., ethyl acetate-MeOH-H_2O, 7:1:0.l) and after the development of plate it was observed under ultraviolet light of wavelength 254 and 366 nm.

Humidity stability test at different temperatures

In this experiment a group of three samples and a control was used. Each experiment was carried out at least three times (n=3). The average data is given below.

Table 4: Humidity Testing

Strength	Sample Quantity	30% Humidity				50% Humidity				70% Humidity				90% Humidity			
		1	6	12	24	1	6	12	24	1	6	12	24	1	6	12	24
250mg	10ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
500mg	10ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
1000mg	10ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Control	10ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(-) No change (+) Degradation

Table 5: Stability under Room Temperature

Strength	Sample Quantity	Room temperature from 25 to 35 Degree Centigrade			
		Months	0	1	3	6
250mg	100ml		-	-	-	-
500mg	100ml		-	-	-	-
1000mg	100ml		-	-	-	-
Control	100ml		-	-	-	-

(-) No change (+) Degradation

2.3. Clinical Study

2.3.1. Inclusion criteria

The clinical studies on the prepared syrup were carried out after informed consent from the patients. Patients having symptoms like sore throat, cough produce by seasonal variation, bronchial infection, asthma condition, dry and wet cough, nasal congestion, runny nose, sneezing, upper respiratory tract allergy, cough, and influenzas were included in the study; with no

severe or chronic diseases presently or previously.

2.3.2. Exclusion criteria

Patients diagnosed with sleep apnea or any other chronic respiratory disease; or having any acute or chronic condition that would limit the ability of the patient to participate in the study, along with those who refused to give informed consent, were excluded from the study.

Observation

Clinical studies were carried out on, at least 127 patients of different ages. They were divided into four groups: Group-l (3-7 years), Group-Il (8-15 years), Group-V (16-60 years) and Group-IV (above 60 years), and in each group consists of normal, heart, T.B, diabetes, asthma, respiratory infection and malaria patients were considered for treatment (NikunjVora et al., 2012, A. Potdar et al., 2006) (Table 6).

Table.6: Dosage regimen

Strength: each bottle (60 ml) contains 150 mg drug.		
	Age group	**Dosage**
Children	Under 3 years	Under Physician or pharmacist's supervision
	3-7 years	1/4 or 1/2 teaspoon to be taken 3 times daily
	8-15 years	(Age 1/2 or 1 teaspoon to be taken 3 or 4 times daily (after meal and before bed).
Adults	16-60 years	2 teaspoonful to be taken 3 or 4 times daily
Special Age Group	Above 60 years	On physician/ pharmacist recommendation

3. RESULTS AND DISCUSSIONS

Results are presented in Table 7 and 8.

Patient Groups	**Group-I (3 to 7 years)**		**Group-II (8 to 15 years)**		**Group-III (16 to 60 years)**		**Group-IV (Above 60 years)**		**Efficiency**
	Patient treated	**Patient cured**	**Patient treated**	**Patient cured**	**Patient treated**	**Patient cured**	**Patient treated**	**Patient cured**	
Normal	14	14	10	10	15	11	3	3	90.47%
Cardiovascular	0	0	0	0	10	8	4	3	78.57%
T.B.	0	0	0	0	5	5	2	2	100%
Diabetes	0	0	0	0	7	6	2	2	88.88%

Asthma	0	0	5	5	7	7	2	2	100%
Respiratory infection	12	12	2	2	1	1	0	0	100%
Malaria	12	12	6	6	8	8	0	0	100%
Total in %	38	38	23	23	53	46	13	12	
	100%		**100%**		**86.79%**		**92.30%**		

Table 8: Percentage of cured patient

Patient Groups	Efficiency	Results (Cured following conditions)
Normal	90.47%	Cough and bronchitis
Cardiovascular	78.57%	Cough and bronchitis
T.B.	100%	Cough
Diabetes	88.88%	Cough
Asthma	100%	Asthmatic cough
Respiratory infection	100%	Asthma
Malaria	100%	Cough and bronchitis

Plants play an important role in the discovery of new therapeutic agents (WHO., 2003 and 2007). According to an evaluation of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs (HMPC., 2005), the reason behind is high cost of prescription medicine and their side effects. Traditional herbs and herbal cough products have been considered to be mild, nontoxic, non-sedative and even harmless. Cough Syrup, a liquid dosage form, usually given to individuals who can't swallow solid dosage form or children because of ease of administration (Chang AB *et al*., 2008). Keeping all these factors in consideration ; we we developed a herbal cough syrup from *Piper longum* with pharmaceutical standard. *Piper longum* is often used as an effective drug for the treatment of asthma and chronic bronchitis in traditional, Grecko-Arab, Ayurvedic system of medicine (Trivedi MN *et al.*, 2011 and Pullela SV *et al.*, 2011). Most of the pharmacological properties of P. nigrum fruits are attributed to a piperidine alkaloid, piperine, which is present in the fruits in amounts of 1.7-7.4%; which act as CNS depressant, antipyretic, analgesic, anti-inflammatory, antioxidant and possess hepatoprotective activities (Somesh M., 2015). Before clinical evaluation, we tested the stability of herbal cough syrup on different parameters;

it was found that Physical appearance remained same throughout the study. Slight variation in PH from 5.03 to 5.17 occurred but remained within standard throughout the study. Quantitative and qualitative estimation results fall within ±2% variation from 1st sample; suggesting that the product remain stable throughout the study. It is quite interesting to note that when treated with herbal cough syrup; patients gave a positive and quick response for it. In total about 127 patients, and proved it as an ideal drug for cough, asthma, respiratory infections, and tuberculosis. The effectiveness of our drug was compared with allopathic, herbal and homoeopathic drugs. The parameters on which this medicine was compared are, bronchodilator, mucolytic, cough, corticosteroid, decongestant and antitussive agents. Among conventional drugs, only ephedrine was found to be effective for only two parameters i.e. bronchodilator and decongestant.

Most of the homoeopathic medicines showed single effect. This poly herbal formulation is found highly effective because of its bronchodilator, mucolytic, cough (wet and dry), decongestant and antitussive effects and no reported side effects. It has short duration efficacy up to 3 days only. Easy to prepare, economical, pleasant taste long shelf life and the safest drug for all age groups. During this research work, the medicine prepared from *Piper longum L.* showed excellent and extraordinary results which are differently found from the existing medicines of all other systems. It is efficacious in patients having previous or present chronic diseases such as diabetes, cardiovascular, asthma, T.B etc. Stable in high and low temperature, easy formulation, during taking dosage sensation/feeling of curing of cough and sore throat. On the above findings it is recommended for cold, cough (dry and wet), congestion, bronchitis, asthma, respiratory infection etc. Due to its significance efficacy it has been patented and reserved the rights of uses.

4. CONCLUSION

In present study, we developed an effective herbal cough syrup, which is found stable, effective and safe as per international guidelines and standards. And could be easily and efficiently used for the relief of cough in place of any prepared cough syrup based on conventional method.

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