An open label, randomized, multicenter, comparative, three arm Phase II clinical study to evaluate the safety and efficacy of mixture of five species of ocimum in subjects with acute common cold

Pallavi Shelke, Sudhir Pawar, Anirudh Tripathi*, Aasin Maurya**, Manisha Srivasthav, Payal Laad, Sanjay Agrawal, Mangesh Palsodkar** and Khushbu Kabra**

Department of Preventive and Social Medicine, Lokmanya Tilak Municipal Medical College and Hospital, Mumbai-400022, Maharashtra, India
*Life Veda Treatment and Research Centre, 7/6 Century Quarters, P.B. Road, Worli, Mumbai-400030, Maharashtra, India
**Integrity Healthcare Services, 301/302, Opulence Building, Santacruz East, Mumbai-400 055, Maharashtra, India

Abstract

Basil and its various species are known to have an effect on common cold and possess immunomodulatory properties. This paper describes a clinical trial of a herbal formulation, Panchtulasi drops in acute common cold; the formulation consists of essential oils of five species of ocimum, namely; *Ocimum sanctum* Linn., *Ocimum basilicum* Linn.*, Ocimum gratissimum* Linn.*, Ocimum citriodorum* Linn. and *Ocimum canum* Linn.

This was an open label, three arm Phase II study to determine the safety and efficacy of Panchatulasi drops in patients with acute common cold. The primary endpoint was reduction in symptoms of cold and number of days of illness. This was assessed using SF-8 and WURSS21 questionnaires. The secondary endpoint was changes in serum interleukin-8 (IL-8) and neutrophil count. The study duration was 8 days with telephonic follow-up on 10th day. A total of 60 patients were randomized to treatment, placebo and a control (standard medication) in a radio of 2:1:1.

The symptom severity, quality of life and number of days of illness measured by WURSS-21 questionnaire was significantly reduced in the treatment arm between baseline and day 4 ($p<0.05$). The physical component score and overall health score of the SF-8 questionnaire also improved considerably in the treatment arm compared to placebo and control arms between baseline and day 4. However, mental component score did not improve significantly until day 6. Concentration of IL-8 did not show any statistically significant change in any of the study arms due to high variability. Neutrophil count, on the other hand, decreased in the treatment arm ($p=0.0153$) compared to placebo and control arms where it increased. Panchatulasi drops is an effective herbal remedy for common cold and can be used as an alternative to current treatments. There was a significant reduction in days of illness, severity of symptoms, and improvement in quality of life compared to placebo and control groups.

Key words: *Ocimum sanctum* Linn., basil, common cold, interleukin-8, herbal medicine

1. Introduction

The "common cold" is an acute, catarrhal syndrome and is the most common infection in humans, with children having 3-8 colds per year and adults suffering from about 2-5 colds per year (Myint, 1996). According to the estimates, 80% of nasal infections are caused by viruses and the rhinovirus accounts for 30-50% of all colds (Heikkinen and Järvinen, 2003). Although the infection is self-limiting and usually resolves within 7 days, it can sometimes last as long as 2-3 weeks, and is associated with an enormous economic burden (Nicholson *et al.*, 1997; Fendrick *et al.*, 2003).

List of abbreviations

ATCC	American type culture collection
ANCOVA	Analysis of covariance
BUN	Blood urea nitrogen
IL-8	Interleukin 8
IP	Investigational product
ITT	Intention-to-treat
NET	Neutrophil extracellular traps
PP	Per-protocol analysis
SGPT	Serum glutamic pyruvic transaminase
SGOT	Serum glutamic oxaloacetic transaminase
SF	Short form
SAS	Statistical analysis software
TNF-α	Tumor necrosis factor-alpha
VAS	Visual analog scale
WURSS	Wisconsin upper respiratory symptom survey

Author for correspondence: Dr. Aasin Maurya
Integrity Healthcare Services, 301/302, Opulence Building, Santacruz East, Mumbai- 400 055, Maharashtra, India
E-mail: amaurya@ihsindia.com
Tel.: +91-9821471425

DOI:

Over half of patients seen for common cold are prescribed with antimicrobials or antihistamines. However, in addition to side effects like immunosuppression and sedation, clinical trials have shown that these do not alter the course of the cold or reduce symptoms significantly (Doyle *et al*., 1988; Rosenstein *et al*., 1998; De Sutter *et al*., 2015). Alternative treatments like vitamin C and zinc lozenges have also not shown consistently significant results in clinical trials. The limitations of current treatments could be due to their symptomatic nature, and a lack of understanding of the underlying mechanisms of the common cold (Turner, 1997). Newer efforts that target the pathogenesis of the symptoms are, therefore, likely to be more successful.

The symptoms of the common cold or other upper respiratory tract infections (URTIs) are triggered in response to viral infection, and more importantly to the immune response to infection. The main cell involved in monitoring invasion of the body by pathogens is the macrophage. The surface of the macrophage displays receptors that combine with components of bacterial and viral pathogens and trigger the release of cytokines (Eccles, 2005). There is evidence that these 'early' cytokines are involved in causing many clinical signs of common cold. Among these proinflammatory cytokines are interferon-a (IFN-a), tumour necrosis factor-a (TNF-a), interleukin-1 (IL-1) a and b, interleukin-6 (IL-6), interleukin-8 (IL-8) and monocyte-attracting chemokines, which induce symptoms such as fever, sore throat and nasal congestion through their stimulating effects on neutrophils and macrophages (Röseler *et al*., 1995; Netea *et al*., 2000; Leon, 2002). The selective recruitment and activation of neutrophils seems linked to increased concentrations of IL-8, which has been shown to play a causative role in acute inflammation (Harada *et al*., 1994). Previous studies have also shown a correlation between IL-8 and severity of symptoms of cold, and this particular cytokine was constitutively present in studies of rhinovirus infection (Turner *et al*., 1998; Gern *et al*., 2002).

This study investigated the effect of an herbal formulation consisting of essential oils from 5 species of basil. Basil has been used for thousands of years in Ayurveda (traditional Indian medicine) for its diverse healing properties. Basil has been found to have strong expectorant properties, and is used in catarrh, cough, bronchitis and other ailments of the respiratory system (Nagaraju and Manoharachary, 2016). Previously conducted studies on basil have established its immunomodulatory properties, in addition to anti-microbial, antitussive, anti-inflammatory, antipyretic and analgesic properties (Aziba *et al*., 1999; Chiang *et al*., 2005; Jeba *et al*., 2011; Mondal *et al*., 2011; Mahajan *et al*., 2013).

Extensive preclinical studies on Panchatulasi demonstrated immunomodulatory, anti-inflammatory and antibacterial properties. Cytokine inhibition was determined in mouse splenocytes from male BALB/c mice aged 6-7 weeks (22 - 26 g), using ELISA (R&D Systems kit). The results were comparable to anti-inflammatory compounds like dexamethasone and paclitaxel (Singh *et al*., 2016; unpublished data).

In vitro minimum inhibitory concentration (MIC) of the Panchatulasi oil different concentrations was performed using micro broth dilution method and showed MIC <100 µg/ml against gram positive and gram negative bacterial ATCC 49619 (American Type Culture Collection*)* and ATCC 700904 *Streptococcus pneumoniae* strains, suggesting good antibacterial properties (Singh *et al*., 2016; unpublished data)

The production of proinflammatory metabolites by lipoxygenase enzyme induces secondary effects such as secretion of mucus glycoprotein by the airway and augmentation of the early response to allergen challenge (Polito and Proud, 1998), and has been implicated in several diseases including rhinitis, asthma and chronic bronchitis (Knapp, 1990; Batt, 1992). Lipoxygenase inhibition of Panchatulasi was, therefore, compared with DMSO and indomethacin (Singh *et al*., 2016; unpublished data).

Lipoxygenase inhibition and inhibition of prostaglandin and leukotriene production (products of lipoxygenase and cyclooxygenase activity) has been shown to dampen the inflammatory response to acute infection with a respiratory virus. The above result, therefore, suggests that Panchatulasi is likely to be a successful remedy for cold infections.

The pharmacological activities of the *Ocimum* species could be attributed to the presence of chemical constituents: eugenol has antidepressant, antimicrobial, anti-inflammatory and antistress properties (Kurian *et al*., 2006). Linalool, present in Panchatulasi drops has anxiolytic and anticonvulsive activities (de Almeida *et al*., 2009). Whereas, ß-caryophyllene possess antiviral effect (Astani *et al*., 2011). Considering the established safety of Ayurvedic medicines, and preclinical studies which showed immunomodulatory and antibacterial properties, Panchatulasi could be an effective alternative to conventional treatments for cold.

Study hypothesis

Panchatulasi could be an effective alternative to conventional treatments for the common cold, and will reduce the number of days of illness and show significant relief from common cold symptoms within ≤ 6 days in at least 70% of the population.

2. Material and Methods

2.1 Patients

Patients were recruited from outpatient clinic from Department of Preventive and Social Medicine (P.S.M.), Lokmanya Tilak Municipal General Hospital and Lokmanya Tilak Municipal Medical College' Urban Health Centre, Dharavi, Mumbai and Life Veda Treatment and Research Centre, Worli. Patients were enrolled after completing an informed consent process as per the Indian Council of Medical Research Ethical Guidelines for Biomedical Research and the principles enunciated in the Declaration of Helsinki (World Medical Association, 2013).

60 patients were enrolled in the study. The inclusion criteria were: (1) male or female between 18-60 years of age, (2) willing to give written informed consent and follow up as per the protocol, (3) had an acute common cold within 36-48 h. (from past 1-2 d.), (4) had at least the first four symptoms of common cold: sneezing, nasal obstruction, nasal discharge, sore throat, chilliness, headache, malaise and cough and (5) had a score of ≥ 6, as mentioned in the modified Jackson's score table (Jackson *et al*., 1958).

Patients were excluded if they had: (1) bacterial infection which may or may not be acute and cannot be treated with Panchatulasi drops, (2) complications of common cold - high fever, significantly swollen glands, severe sinus pain, and a cough that produces mucus, (3) history of allergic rhinitis or asthma and reported itchy eyes, sneezing, cough or shortness of breath, (4) increased neutrophil

count and lymphocyte in the blood due to chronic bronchitis, (5) taken home remedies or other treatments which may interfere with the study, (6) tuberculosis, chronic bronchitis, COPD, sinusitis or lower RTI infection, (7) medical conditions like known history of HIV infection, malignancy, cardiovascular disease, hypertension, renal pulmonary or hepatic abnormalities, neurologic or psychiatric disease, multiple sclerosis, pneumonia and rheumatoid arthritis, (8) taken the following medication within 7 d. from the onset of common cold, antiplatelets like aspirin, immunosuppressive drug, corticosteroids, antidepressants, anticholinergic, antispasmodics, sedatives and hypnotics, antihistaminics like cetirizine, anti-coagulants or NSAIDS-aspirin, diclofenac, ibuprofen, (9) showed active dependency on alcohol or other drugs, (10) taken salicylates and propionic derivatives, celecoxib, dextromethorphan, meperidine, naproxen, anti-inflammatory, antibiotics, URTI-antibiotics-dextromethorphan, codeine, antiallergics, antitussive, herbal, Ayurvedic or Homeopathic medicine, (11) used prohibited medication like macrolide derivatives, ofloxocin and ornidazole, derivatives of penicillin, (12) suffer from hypersensitivity, (13) were pregnant, child bearing or breast feeding women and (14) had a major surgical procedure in the previous six months.

2.2 Study design

This was an open label comparative three arm study, with block randomization generated through software assuming maximum of 5 study sites and screening of around 200 for recruitment of 60 patients. A 2:1:1 randomization was done where 30 subjects received study drug, 15 subjects received placebo, and the other 15 subjects received a standard treatment.

Patients with a score ≥ 6 on Jacksons scale were screened for other selection criteria. Patients satisfying the selection criteria were requested for baseline safety blood investigations, *i.e.,* complete hemogram, liver profile (SGPT, SGOT and total bilirubin), renal profile (BUN, serum creatinine) and interleukin-8 from blood/serum. At the first visit, patients were assigned an ID and dispensed with the investigational product (IP) depending on randomization. Follow up visits were scheduled on day 4, 6 and 8, with a telephonic follow up on day 10.

2.3 Study objective

The primary endpoints were reduction in symptom severity and number of days of illness. The secondary endpoints were plasma levels of IL-8 and neutrophil count.

2.4 Assessments

On the first visit, patients were screened using the Jackson scale. Patients who were enrolled were given a patient diary, and were provided with the study medication.WURSS21questionnaires (used with permission from Wisconsin Alumni Research Foundation) and SF-8 scores were recorded on each of the follow-up visits to assess the severity of common cold symptoms. A linguistic validation was carried out for Hindi and Marathi (local language) translations of the WURSS-21 questionnaire. This was done with 12 patients who were asked to fill the forms, and were debriefed by the Principal Investigator to assess the clarity and acceptability of the questionnaire. The linguistic validation of questionnaire in Hindi and Marathi was submitted to the Staff and Research Society Ethics Committee. IL-8 count from blood/serum and other blood investigations were performed on day 1 and day 8 of the study

(Diaclone ELISA kit). Physician and Patient's Global Assessment score and VAS score were recorded on day 8 to assess overall patient health. Physical examination and vital signs were recorded on each visit.

2.5 Study medication

The oil from the leaves of different species of basil were extracted with petroleum ether from a distillate, after distillation with steam. The oil extracts were mixed in fixed ratios, along with solubilizers (Table 1). The mixture was stirred using magnetic stirrer in aseptic conditions and transferred to containers. Standardization was ensured by identification of chemical markers using gas chromatography with flame ionization detection (GC-FID). Refer to the appendices for chromatograms of the individual oils as well as the formulation.

Table 1: Composition of Panchatulasi formulation

Sr. No.	Ingredient	Quantity present
1	*Ocimum sanctum*	6.0 ml
2	*Ocimum gratissimum*	0.5 ml
3	*Ocimum canum*	0.5 ml
4	*Ocimum citrodorum*	0.5 ml
5	*Ocimum canum*	0.5 ml
6	Solubilizer	2.0 ml

The placebo was a mixture of flavors of *Ocimum sanctum* and clove oil mixed with distilled water and titrated to match the taste and aroma of the treatment. The dosage for both was 5 drops in warm water to be taken 4 times a day. Patients in the control group received acetaminophen 600 mg-1200 mg in divided doses.

2.6 Rescue medication

Antipyretic drugs (acetaminophen), nasal decongestants containing pseudoephidrine, combination containing paracetamol and pseudoephedrine and product containing normal saline for treating decongestion were used as rescue medication.

2.7 Statistical analysis

Estimated difference in % mean change in severity of symptoms of common cold was 75% for Panchatulasi versus placebo and 50% for control vs placebo. A sample size of 60 patients provided 80% power to detect the above. All data were evaluated using SAS 9.3. Changes in IL-8 and neutrophil count were analyzed using paired t-tests. WURSS-21 and SF-8 scores were analysed using Friedman's Nonparametric tests for multiple comparisons (Cochran-Mantel-Haenszel Statistics Based on Rank Scores) and Wilcoxon two sample t test to obtain p values for reduction in scores within the same group over time.

3. Results and Discussion

Of the 60 patients enrolled, 55 patients completed the study, of which 54 patients were considered in PP analysis and 1 patient was considered in ITT analysis. One patient dropped out due to low haemoglobin, but continued to take the study drug along with concomitant medications (Figure 1 and Table 2).

Patients screened and enrolled (N) = 60

Patients enrolled in study drug arm (N) = 29

Patients enrolled in Placebo arm (N) = 15

Patients enrolled in Control arm (N) = 16

Patients completing the study (N) = 27

Patients completing the study (N) = 14

Patients completing the study (N) = 14

Patients lost to Follow-up (N) = 2

Patients lost to Follow-up (N) = 1

Patients lost to Follow-up (N) = 1

No. of Dropouts (N) = 0

No. of Dropouts (N) = 0

No. of Dropouts (N) = 1

Patients included in ITT (N) = 1

Patients included in ITT (N) = 0

Patients included in ITT (N) = 0

No. of Adverse events (N) = 2

No. of Adverse events (N) = 0

No. of Adverse events (N) = 0

No of SAE (N) = 0

No of SAE (N) = 0

No of SAE (N) = 0

Figure 1: Flowchart of combined patient enrolment for each of the two study sites. In both sites, all patients who completed the study were analysed by per protocol (PP) analysis, except one patient in ' LTMMC & LTMGH Sion Hospital' who refused to give blood on the last visit and was, therefore, considered under intention to treat (ITT) analysis.

Table 2: Demographic information including gender, age and Jackson Scale score for patients in each treatment arms

	Panchatulasi N = 29	Placebo N = 15	Control N = 16
Gender, n(%)			
Male	27 (93.10%)	13 (86.67%)	12 (75.00%)
Female	2 (6.90%)	2 (13.33%)	4 (25.00%)
Age (Years)			
n	29	15	16
Mean	38.5	37.1	35.4
Std. Dev.	13.20	11.82	12.77
Median	36.0	37.0	34.5
(Min, Max)	(19, 59)	(20, 58)	(18, 58)
Jackson Scale			
n	29	15	16
Mean	7.8	7.7	7.9
Std. Dev.	1.17	0.88	1.18
Median	8.0	8.0	8.0
(Min, Max)	(6, 11)	(7, 10)	(6, 10)

There were no serious adverse events noted in any of the study groups. The only adverse event was diarrhoea which occurred in one patient in the treatment group. Another patient in the treatment group reported headache, however, this is a symptom of common cold and was not considered an adverse event.

WURSS-21 scores were similar at baseline in all three groups. When comparing scores from baseline to day 4, question 1 (overall illness) showed more significant reduction in Panchatulasi compared to placebo and control. Question 21 (how is your cold compared to yesterday) also showed greater and more significant reduction in the Panchatulasi arm (p=0.0105), compared to placebo (p=0.527) and control (p=0.428). This suggests fewer days of illness in the Panchatulasi group, as by day 4 patients were already feeling better compared to other groups. Analysis of question 2-12 (Severity of illness) suggested greater and faster relief in symptoms in the Panchatulasi arm, with the symptom score decreasing by 8.57±0.59 from baseline to day 4 (p<0.0001), compared to 4.07±0.79 (p>0.05) in PL arm and 4.94±0.1 (p=0.0161) in C arm. Similarly for questions 12-20 (quality of life), reduction in scores was not significant in placebo and control arms compared to Panchatulasi arm (Table 3).

Table 3: Table showing WURSS-21 questionnaire results for the three treatment arms, along with p values calculated using the Wilcoxon Two Sample Test

WURSS-21 Questionnaire results	PL (Mean, ±SD)	p value	PT (Mean, ±SD)	p value	C (Mean, ±SD)	p value
Overall illness (Q1)						
Baseline score	4.6±0.63		4.53±0.73		4.72±0.73	
Mean reduction from baseline to day 4	0.73±0.6	0.0486	1.14±0.16	<.0001	0.97±0.02	0.002
Mean reduction from baseline to day 6	1.23±0.44	0.001	1.6±-0.27	<.0001	1.28±0.16	0.001
Mean reduction from baseline to day 8	1.8±-0.42	<.0001	2.24±0.32	<.0001	1.84±0.33	0.0002
Symptom severity (2-11)						
Mean at baseline	23.97±5.85		25.91±5.85		24.16±4.38	
Mean reduction from baseline to day 4	4.07±0.79	0.1253	8.57±0.59	<.0001	4.94±0.1	0.0161
Mean reduction from baseline to day 6	8.53±1.33	0.0007	12.88±0.42	<.0001	6.72±1.4	0.0028
Mean reduction from baseline to day 8	12.5±0	0.0002	17.66±1.19	<.0001	11.72±0.54	0.0001
QoL (12-20)						
Mean at baseline	21.6±5.13		19.84±5.83		19.72±5.63	
Mean reduction from baseline to day 4	4.27±1.35	0.0533	4.12±1.09	0.0071	3.31±0.89	0.1191
Mean reduction from baseline to day 6	7.6±1.85	0.0046	7.16±0.51	<.0001	5.63±0.96	0.0112
Mean reduction from baseline to day 8	9.4±1.19	0.0009	12.67±0.7	<.0001	10.34±1.09	0.0003
Sickness compared to yesterday (Q21)						
Mean at baseline	4.1±0.39		4.09±0.54		4.16±0.57	
Mean reduction from baseline to day 4	0.23±0.84	0.527	0.43±0.06	0.0105	0.16±0.25	0.428
Mean reduction from baseline to day 6	0.8±0.71	0.0072	0.93±0.19	<.0001	0.56±0.09	0.0243
Mean reduction from baseline to day 8	1.43±0.68	0.0004	1.59±0.64	<.0001	1.06±0.29	0.0005

Analysis of SF-8 results showed the overall health and physical health improvement from baseline to day 4 was statistically significant in the Panchatulasi arm, but not in placebo and control arms. Mental health reduction in score on day 4, however, was more significant in the placebo group (p=0.0991), compared to Panchatulasi (p=0.1218) and control (p=0.3235), although none of these are statistically significant. From baseline to day 6, the improvement in overall health, mental health and physical health were more significant in Pachatulasi (p<0.0001), compared to placebo (p>0.01) and control (p>0.05). By day 8, SF-8 total score decreased by 49% in Panchatulasi, compared to 25% in placebo and 32% in control. (Figure 2). Interestingly, panchatulasi shows better relief compared to the control acetaminophen, an antipyretic and analgesic drug that has only minor anti-inflammatory properties. This provides support for similar properties in Panchatulasi, and could suggest that presence of additional anti-inflammatory and antibacterial properties of basil may contribute to more effective action compared to acetaminophen (Figure 2).
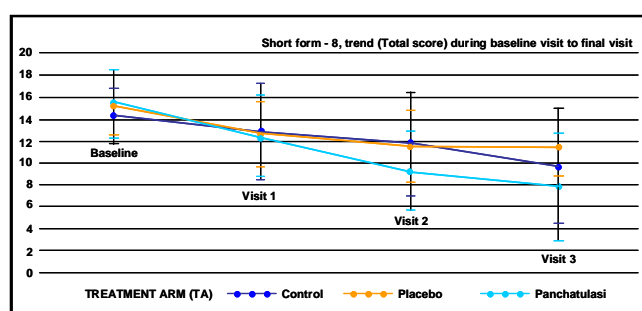

Figure 4: Improvement in the total score of short form 8 from baseline to visit 3 for control, placebo and Panchatulasi.

VAS scores was used to assess the relief of symptoms from common cold from day 1 to day 8. The average mean of the score was assessed by using ANCOVA test and the average mean in the groups Panchatulasi, placebo and control group was assessed. The average mean of the score was less in Pancatulasi group, compared to placebo and control. (1.96 for Panchatulasi, 3.13 for placebo and 2.75 for control, respectively).

VAS scores correlated with symptom scores for WURSS-21 and SF-8. There was a 25% (p=XX) reduction in Panchatulsi arm by day 4, and 50% reduction by day 6 (p=XX) in the VAS score. Placebo and control arms on the other hand, showed similar results with only 26% reduction in VAS by day 6.

Statistical analysis of IL-8 count showed that no significant change was detected in any of the patient groups, and variability was large. Most patients had high IL-8 values (< 29 µg/ml) at baseline, whereas some had low values at baseline (>29 µg/ml). On separate analysis of these two groups, it appeared that in the PT arm, patients with a low baseline IL-8 count had a minimum increase in IL-8 count, followed by control and placebo which had the maximum increase. Patients with a high IL-8 count at baseline showed minimal decrease in PT arm, followed by placebo and control which showed maximum mean reduction in IL-8.This could suggest regulation of IL-8 by Panchatulasi whereby IL-8 levels are not excessively stimulated or inhibited during the course of infection, due to the importance of such proinflammatory cytokines in resolution of the infection. Cytokines like IL-8 and IL-6 are generally elevated during

respiratory infections as compared to healthy subjects. IL-8 is a major neutrophil attractant, and promotes inflammation by recruitment of neutrophils to the site of infection. However, there is still uncertainty regarding the implications of cytokines in symptoms of common cold. For example, while some studies demonstrate an increase in IL-8 during early infection, other studies have shown IL-8 to peak around day 4-6 (Hayden *et al.*, 1998; Skoner *et al.*, 1999).

Although within the normal range for all patients, neutrophil count decreased slightly in PT arm (-2.48±0.06, p<0.05), whereas it increased in other treatment arms (p>0.05). Neutrophils are important first innate immune cells that migrate to the site of infection and help in containing infection *via* phagocytic activity, releasing antibacterial molecules and forming neutrophil extracellular traps (NETs) that degrade bacterial components. However, neutrophil products are also involved in stimulating mucus secretion and sneezing/coughing as they release inflammatory mediators such as neutrophil elastase and oxygen radicals which may contribute to the increase in mucus and airway fluid characteristic of respiratory infections like common cold. The small decrease in neutrophil count by day 8 in the treatment arm could, therefore, suggest faster resolution of the common cold symptoms with Panchatulasi drops.

4. Conclusion

Panchatulasi drops were effective in reducing the number of days of illness, and reducing the severity of symptoms significantly by day 4 compared to placebo and control. Overall, the study drug has no major side effects and could be an effective therapy for the treatment of common cold. Due to immunomodulatory effects, it may also be used on a regular basis for prevention of respiratory diseases and infections.

References

Astani, A.; Reichling. J. and Schnitzler, P. (2011). Screening for antiviral activities of isolated compounds from essential oils. Evidence-Based Complementary and Alternative Medicine, Article ID 253643, pp:8; doi:10.1093/ecam/nep187.

Aziba, P.; Bass. D. and Elegbe. Y. (1999). Pharmacological investigation of *Ocimum gratissimum* in rodents. Phytotherapy Research, **13**(5): 427-429.

Batt, D.; Ellis, G.P. and Luscombe, D.K. (1992). 1 5-Lipoxygenase inhibitors and their anti-inflammatory activities. **In**:Progress in Medicinal Chemistry, **29**:1-63.

Chiang, L.C.; Ng, L. and Cheng, T. (2005). Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. Clinical and Experimental Pharmacology and Physiology, **32**(10):811-816.

De Almeida, E.R.; Rafael, K.R.; Couto, G.B. and Ishigami, A.B. (2009). Anxiolytic and anticonvulsant effects on mice of flavonoids, linalool, and α-tocopherol presents in the extract of leaves of

Cissus sicyoides L.(Vitaceae). Journal of Biomedicine and Biotechnology, Article ID 274740, pp:6; doi:10.1155/2009/274740.

De Sutter, A.I.M.; Saraswat, A. and Van Driel, M.L. (2015). Antihistamines for the common cold. cochrane database of systematic reviews, issue 11. Art. No.: CD009345. DOI: 10.1002/14651858.CD009345.pub2

Doyle, W.J.; McBride, T.P.; Skoner, D.P.; Maddern, B.R.; Gwaltney, J.M. and Jr. Uhrin, M. (1988). A double-blind, placebo-controlled clinical trial of the effect of chlorpheniramine on the response of the nasal airway, middle ear and eustachian tube to provocative rhinovirus challenge. The Pediatric Infectious Disease Journal, 7(3):229-238.

Eccles, R. (2005). Understanding the symptoms of the common cold and influenza. The Lancet Infectious Diseases, 5(11):718-725.

Fendrick, A.M.; Monto, A.S.; Nightengale, B. and Sarnes, M. (2003). The economic burden of non-influenza-related viral respiratory tract infection in the United States. Archives of Internal Medicine, 163(4):487-494.

Gern, J.E.; Martin, M.S.; Anklam, K. A.; Shen, K.; Roberg, K. A.; Carlson-Dakes, K.T. and Adler, K. (2002). Relationships among specific viral pathogens, virus induced interleukin 8, and respiratory symptoms in infancy. Pediatric Allergy and Immunology, 13(6):386-393.

Harada, A.; Sekido, N.; Akahoshi, T.; Wada, T.; Mukaida, N. and Matsushima, K. (1994). Essential involvement of interleukin-8 (IL-8) in acute inflammation. Journal of Leukocyte Biology, 56(5): 559-564.

Hayden, F.G.; Fritz, R.; Lobo, M.C.; Alvord, W.; Strober, W. and Straus, S.E. (1998). Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. Journal of Clinical Investigation, 101(3):643.

Harish, B.; Singh, Aasin Maurya; Sathish Sadagopan; Kesavan, P.K.; Subasranjan Acharya and Shri Natarajan Arumugam (2016). Immunomodulatory and antimicrobial activity of a polyherbal composition, Panchatulasi drops, derived from essential oils of five species of basil. Ann. Phytomed., 5(2): Unpublished.

Heikkinen, T. and Järvinen (2003). The common cold. The Lancet, 361(9351):51-59.

Jackson, G.G.; Dowling, H.F.; Spiesman, I.G. and Boand, A.V. (1958). Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. AMA Arch. Intern. Med., 101:267-278.

Jeba Jeba, C.R.; Vaidyanathan, R. and Rameshkumar, G. (2011). Immunomodulatory activity of aqueous extract of *Ocimum sanctum* in rat. International Journal on Pharmaceutical and Biomedical Research, 2(1):33-38.

Knapp, H. R. (1990). Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor. New England Journal of Medicine, 323(25):1745-1748.

Kurian, R.; Arulmozhi, D.K.; Veeranjaneyula, A. and Bodhankar, A.L. (2006). Effect of eugenol on animal models of nociception. Indian Journal of Pharmacology, 38(5):341.

Leon, L.R. (2002). Invited review: Cytokine regulation of fever: studies using gene knockout mice. Journal of Applied Physiology, 92(6): 2648-2655.

National Health Interview Survey (1996). Current estimates from the National Health Interview Survey. Vital Health Stat., 10:7.

Mahajan, N.; Rawal, S.; Verma, M. and Alok, S. (2013). A phytopharmacological overview on Ocimum species with special emphasis on *Ocimum sanctum*. Biomedicine and Preventive Nutrition, 3(2):185-192.

Mondal, S.; Varma, S.; Bamola, V.D.; Naik, S.N.; Mirdha, B. N.; Padhi, M. M.; Mehta, N. and Mahapatra, S.C. (2011). Double-blinded randomized controlled trial for immunomodulatory effects of Tulsi (*Ocimum sanctum* Linn.) leaf extract on healthy volunteers. Journal of Ethnopharmacology, 136(3):452-456.

Myint, S. and Taylor-Robinson, D. (1996). Viral and other infections of the human respiratory tract. Chapman and Hall Ltd.

Manoharachary, C. and Nagaraju, D. (2016). Medicinal plants for human health and welfare. Ann. Phytomed., 5(1):24-34.

Netea, M. G.; Kullberg, B. J. and Van Der Meer, J. (2000). Circulating cytokines as mediators of fever. Clinical Infectious Diseases, 31(5): S178-S184.

Nicholson, K.G.; Kent, J.; Hammersley, V. and Cancio, E. (1997). Acute viral infections of upper respiratory tract in elderly people living in the community: Comparative, prospective, population based study of disease burden. Bmj, 315(7115):1060-1064.

Polito, A.J. and Proud, D. (1998). Epithelial cells as regulators of airway inflammation. Journal of Allergy and Clinical Immunology, 102(5): 714-718.

Röseler, S.; Holtappels, G.; Wagenmann, M. and Bachert, C. (1995). Elevated levels of interleukins IL-1β, IL-6 and IL-8 in naturally acquired viral rhinitis. European Archives of Oto-Rhino-Laryngology, 252(1): S61-S63.

Rosenstein, N.; Phillips, W.R.; Gerber, M.A.; Marcy, M.S.; Schwartz, B. and Dowell, S.F. (1998). The common cold-principles of judicious use of antimicrobial agents. Pediatrics, 101(1):181-184.

Skoner, D.P.; Gentile, D.A.; Patel, A. and Doyle, W.J. (1999). Evidence for cytokine mediation of disease expression in adults experimentally infected with influenza A virus. Journal of Infectious Diseases, 180(1):10-14.

Turner, R.B. (1997). Epidemiology, pathogenesis, and treatment of the common cold. Annals of Allergy, Asthma and Immunology, 78(6): 531-540.

Turner, R.B.; Weingand, K.W.; Yeh, C.H. and Leedy, D.W. (1998). Association between interleukin-8 concentration in nasal secretions and severity of symptoms of experimental rhinovirus colds. Clinical Infectious Diseases, 26(4):840-846.

World Medical Association (2013). WMA declaration of Helsinki: Ethical principles for medical research involving human subjects. Retrieved from http://www.wma.net/en/30publications/10policies/b3/.