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A randomized double blind placebo controlled clinical evaluation of extract of *Andrographis paniculata* (KalmCold™) in patients with uncomplicated upper respiratory tract infection

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ABSTRACT

A randomized, double blind placebo controlled clinical study was conducted to evaluate the efficacy of KalmCold™, an extract of *Andrographis paniculata*, in patients with uncomplicated upper respiratory tract infection (URTI). The assessment involved quantification of symptom scores by Visual Analogue Scale. Nine self evaluated symptoms of cough, expectoration, nasal discharge, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance were scored. A total of 223 patients of both sexes were randomized in two groups which received either KalmCold™ (200 mg/day) or placebo in a double blind manner. In both the treatments, mean scores of all symptoms showed a decreasing trend from day 1 to day 3 but from day 3 to day 5 most of the symptoms in placebo treated group either remained unchanged (cough, headache and earache) or got aggravated (sore throat and sleep disturbance) whereas in KalmCold™ treated group all symptoms showed a decreasing trend. Within groups, mean scores of symptoms in both the groups decreased significantly ($p \leq 0.05$) from day 1 to day 3 and day 5 while from day 3 to day 5 all symptoms except expectoration in placebo group did not improve significantly whereas in KalmCold™ treated group all symptoms improved significantly ($p \leq 0.05$) except earache. Comparing mean between both groups, all symptoms at day 1 and day 3 were found to be the same while at day 5 all symptoms except earache in KalmCold™ treated group improved significantly ($p \leq 0.05$) than placebo group. Similarly, within groups, overall scores of all symptoms in both the groups decreased significantly ($p \leq 0.05$) from day 1 to day 3 and day 5 while from day 3 to day 5 placebo group did not improve significantly whereas KalmCold™ treated group showed significant improvement ($p \leq 0.05$). On between groups analysis, KalmCold™ group showed significant reduction ($p \leq 0.05$) in overall symptom scores as compared to placebo group. In both placebo and KalmCold™ treated groups, there were only a few minor adverse effects with no significant difference in occurrence ($Z = 0.63$; $p > 0.05$). The comparison of overall efficacy of KalmCold™ over placebo was found to be significant ($p \leq 0.05$) and it was 2.1 times (52.7%) higher than placebo. The findings of this study revealed that KalmCold™ was effective in reducing symptoms of upper respiratory tract infection.

Introduction

Common cold (synonyms: acute coryza, acute viral nasopharyngitis) is a highly contagious, viral disease of the upper respiratory tract. More than 200 different viruses are reported as etiological agents for common cold and flu (NIAID, 2007) and among them rhino virus is reported to be the most common causative agent in humans (Hershenson and Johnston, 2006).

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Upper respiratory tract infection (URTI) is recognized as leading cause for absence from school and work (Melchior et al., 1997). As per the estimates of the Centers for Disease Control and Prevention, 22 million school days are lost annually in the United States due to the common cold (CDC, 2004). The general symptoms of URTI include cough, expectoration, nasal discharge, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance (Caceres et al., 1999; Eccles, 2005). No specific antiviral therapy or vaccination against the common cold is available at present. The existing symptomatic treatment with antibiotics, nasal decongestants, cough suppressants, anti histamines and analgesics is found to be of limited value as the agents mainly focus on relieving the symptoms rather than treat the condition (Caceres et al., 1999).

A vast number of medicinal plants of traditional use are reported to exhibit great potential of therapeutic applications and botanical products are being considered as important part of the health food market (Schilter et al., 2003). *Andrographis paniculata* Nees (*A. paniculata*) commonly known as Chiretta, King of Bitters or Kalmegh, has been widely used in traditional system of medicine in India. *A. paniculata* belonging to family Acanthaceae, is an annual herb native to peninsular India and Sri Lanka and is also distributed in different regions of South-east Asia, China, America, West Indies and Christmas Island (Lattoo et al., 2006). *A. paniculata* has been shown to possess wide spectrum of pharmacological properties (Mishra et al., 2007; Khare, 2007).

Herbal preparations that contain extract of *A. paniculata* as their principal ingredient are reported to improve the recovery from common cold and reduce its occurrence. The beneficial effects of *A. paniculata* in the management of common cold have been clearly validated in several clinical trials of recent past (Thamlikitkul et al., 1991; Melchior et al., 1997; Caceres et al., 1997; Melchior et al., 2000) and meta analyses of the clinical trials also pointed out to its favourable effectiveness (Coon and Ernst, 2004; Poolsup et al., 2004). Recent research has indicated that cold remedies which exhibit anti – inflammatory and antiviral milieu in the respiratory tract are imperative in the treatment of common cold (Mossad, 1998). The primary mechanisms of action of *A. paniculata* are attributed to the anti–inflammatory (Shen et al., 2002) and immunostimulant (Puri et al., 1993; Kumar et al., 2004) properties of its main active ingredient – Andrographolide. *A. paniculata* is listed in Indian Herbal Pharmacopoeia, Herbs of Commerce of American Herbal Products Association, and in Indian Pharmacopoeia (IDMA, 2002; AHPA, 2004; IPC, 2007). It has been used for long without any known toxicity and has a strong traditional usage from safety point of view (Puri, 2003). The available human studies on *A. paniculata* did not indicate any serious adverse effects (Coon and Ernst, 2004).

With the above perspectives, the study was conducted to evaluate the efficacy of extract of *A. paniculata* (KalmCold™) in patients with uncomplicated upper respiratory tract infection.

Materials and methods

Study design

The study was conducted at four different centers in India between May 2007 and November 2007. Due to the self-limiting nature of the illness, it was decided to introduce a placebo group and carry out the study in a double blind manner.

Investigational substance

The investigational substance used was capsule of KalmCold™, an extract from the leaves of *A. paniculata* Nees, developed by M/s Natural Remedies Pvt. Ltd., Bangalore, India. Each capsule of KalmCold™ (Sample code: 26 (APE-30); Batch No.: AP07/Lot 03) contained *A. paniculata* extract 100 mg and 200 mg of micro crystalline cellulose. The extract contained andrographolide (31.30% w/w), isoandrographolide (0.40% w/w), neoandrographolide (3.20% w/w), andrograpanin (0.60% w/w), skullcapflavone I (0.05% w/w), 7-O-methylwogonin (0.05% w/w) and total andrographolides (38.40% w/w). The content of 14-deoxy 11, 12, didehydroandrographolide was 2.80% w/w. The composition adheres to the international quality requirements which include analysis of solvent residue, heavy metals residue, mycotoxin residue, pesticide residue evaluation and microbial contamination. The placebo capsules (Sample code: 27 (APE-30)) contained 300 mg of micro crystalline cellulose. The test medication and the placebo were filled in 0 size blue hard gelatin capsules that could not be distinguished from each other. Both investigational substance and the placebo capsules were packed in blisters of sample size 10 capsules/blister pack and labeled with code numbers.

Preparation of KalmCold™

Coarse ground leaves of *A. paniculata* (300 kg) were charged into a stainless steel jacketed extractor fitted with a reflux condenser. Methanol (1200 L) was added to the extractor and the contents were refluxed for 3 h by providing steam in the jacket. The liquid extract was drained from the extractor into a separate vessel and fresh methanol (1000 L) was added to the extractor containing the marc. The extraction procedure as above was repeated two more times and the liquid extracts from each extraction step was separately subjected to distillation under vacuum (at $\leq 55\ ^{\circ}C$) until a thick paste with a total solid content of 40-50% (w/w) was obtained. Thick paste obtained from the three extraction steps were mixed and dried under vacuum ($\leq 65\ ^{\circ}C$) to get lumps of the extract. The extract lumps were then milled and sieved (# 40) to get a uniform powdered extract of *A. paniculata* (18 kg). To the marc contained in the extractor, water (1200 L) was added and the contents were refluxed for 3 h by providing steam in the jacket. The liquid extract was drained from the extractor into a concentrator and was subjected to distillation under vacuum (at $\leq 75\ ^{\circ}C$) until the total solid content in the liquid reached about 15 - 20% (w/v). The concentrated liquid was then spray dried to get the water extract of *A. paniculata* (10 kg). The alcohol and water extracts were then analyzed for the content of active constituents and blended to get KalmCold™ with the required levels of active constituents.

Analysis

KalmCold™ was subjected to physico-chemical and phytochemical analysis. The chemical analysis revealed that the moisture content was 4 - 5% (USP, 2007a), ash was 10% (USP, 2007b) and total bitters about 60% (IP, 1966) accounting to almost 75% of the composition. KalmCold™ on analysis by HPLC was found to contain the following constituents, *viz.*, andrographolide ($>$ 30.0% w/w), isoandrographolide ($>$ 0.3%, w/w), neoandrographolide ($>$ 1.0%, w/w), andrograpanin ($>$ 0.3%, w/w), 14-deoxy-11,12-didehydroandrographolide ($\leq$ 5.0%, w/w), skullcapflavone I ($>$ 0.05%, w/w) and 7-O-methylwogonin ($>$ 0.05%, w/w). One of the above constituents viz., 14-deoxy-11,

12-didehydroandrographolide has been reported to cause dose dependent hypotension (Zhang et al., 1998; Yoopan et al., 2007). Hence, content of 14-deoxy-11, 12-didehydroandrographolide was constrained to an upper limit of less than 5% w/w.

Isolation of phytochemical reference standards

The extract was subjected to liquid-liquid partitioning between ethyl acetate and water. The ethyl acetate layer was repeatedly chromatographed over silica gel using combinations of hexane: ethyl acetate and chloroform: methanol. Crystallization of different chromatographic fractions yielded andrographolide **(1)**, isoandrographolide **(2)**, neoandrographolide **(3)**, andrograpanin **(4)**, 14-deoxy-11, 12 didehydroandrographolide **(5)**, skullcapflavone I **(6)** and 7-O-methylwogonin **(7)**. Identification of these compounds was confirmed by comparing their ^{1}H and ^{13}C NMR data with literature (Jalal et al., 1979; Fujita et al., 1984; Kuroyanagi et al., 1987; Matsuda et al., 1994; Jayakrishna et al., 2001). Purity of the isolated compounds was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) for their use as reference standards. The HPLC purity of the isolated constituents was found to be $>98.0\%$ by area normalization.

Standard and sample solution preparation

Standards **(1-7)** were prepared by weighing 10 mg of the individual standards and dissolving them separately in 10 ml with methanol (HPLC grade, Qualigens make). The stock solution was suitably diluted to obtain 0.5 mg/ml of 1 and 0.1 mg/ml of **2-7** as a mix and individual solution. Sample solution was prepared by dissolving 200 mg of KalmCold™ in 100 ml of methanol.

Methodology

HPLC method was adopted for analyzing **1-7** compounds. The analytical method was validated for specificity, linearity, precision, accuracy, and range of quantification (ICH, 2005). A known volume (20 µL) of the individual, mixed standard and sample solutions were injected to the HPLC system (Shimadzu, Model LC 2010 A, Japan) consisting of quaternary pump with UV detector, auto injector and column oven with class LC solution software. The stationary phase was an octadecylsilane column (C18, 5 µm, 250 x 4.6 mm, Hibar RT, Lichrosphere 100, Merck). The mobile phase consisted a mix of phosphate buffer (Solvent A) [prepared by dissolving 0.136 g of potassium dihydrogen orthophosphate (KH_2PO_4) in 900 ml of HPLC grade water (obtained from ''Arium'' Sartarious water purification system) and 0.5 ml of orthophosphoric acid (H_3PO_4) (AR grade, Rankem) then the final volume was made upto 1000 ml] and acetonitrile (Solvent B) (HPLC grade, Qualigens make). Both acetonitrile and the phosphate buffer was filtered separately through 0.45 µ membrane filter and degased by sonicating for 3 min. The solvent A and B were mixed in such a manner that the concentration of solvent B was increased from 5 - 45% as linear gradient in the first 18 min. From 18 to 25 min. the concentration of solvent B was increased from 45 - 80% as a linear gradient. The flow rate of mobile phase was maintained at 1.5 ml per minute throughout the analysis and the detector wave length was kept at 223 nm, and chromatogram was recorded. Quantification of **1** to **7** was achieved by external standard method. The peaks in the mixed standards and in KalmCold™ were identified by injecting the individual standard solutions (Fig. 1).

Participants

The required sample size for difference between two means i.e. for a two sample t-test was estimated according to Snedecor and Cochran (1989). The required sample size for each arm of KalmCold™ or placebo was a minimum of 92 subjects or a total 184 for the whole study. As drop outs are common in clinical trial of a self-limiting condition, around 20% more subjects were added in each group. Two hundred and twenty three participants of both sexes (143 males and 80 females) aged between 18 and 60 years were enrolled for the study. The clinical symptoms of upper respiratory tract infection are characteristically heterogeneous in measurement (Poolsup et al., 2004) and are categorized as 'early' or 'later' symptoms based on the occurrence/onset, which varies from immediate to several days (Eccles, 2005). Such symptoms of common cold varies the sample size between individual symptom scores. Based on this, the sample size of this study was calculated considering any difference in the overall symptoms scores between the two treatments.

Informed consent

The objectives along with detailed procedures of the study were explained to all the participants in their own simple, communicable language. All the participants were requested to understand and sign the informed consent form before randomization procedure. All these patients were provided the clinical symptom assessment/Visual Analogue Scale (VAS**;** scale from 0-100 wherein the value '0' indicates no symptoms and '100' meaning the highest severity of symptoms) presented in local language or English for ease of understanding of the evaluation procedure. In addition, any queries/doubts, raised by trial subjects prior to signing the consent form were clarified by the investigator. Copy of the informed consent was given to the subjects participating in the study. Consent was taken by the investigators of the clinical trial.

The patients were recruited according to the following inclusion and exclusion criteria:

Inclusion criteria

- Subjects in the age group of 18-60 years, of either sex.
- Subjects who have given written informed consent.
- Subjects predominantly suffering from two or more symptoms of common cold (cough, expectoration, running nose, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance).
- Subjects agreed to come for follow-up on day 3 and day 5 irrespective of any relief or side effects.

Exclusion criteria

- Patients who were unable to give voluntary consent.
- Patients who were ill more than three days with common cold
- Patients with diabetes or with serious ailments of heart, liver, kidney or brain
- Subjects suffering from immunologically associated diseases such as multiple sclerosis, polyarthritis or rheumatic or other autoimmune diseases.
- Subjects suffering from accompanying illnesses such as bronchiolitis, pneumonia, pleuritis, septic infections, special bacterial infections as pneumoconiosis, angina tonsilaris, sublingual fever higher than 40.5 °C, sinusitis or any other infections.
- Subjects who had any allergy or were allergic to any medication.

- Patients who were pregnant or lactating.
- Subjects who were unable to complete follow-up on day 3/5.
- Subjects on any medication that would affect evaluation like anti-histamines, steroids, Over-the-counter (OTC) or herbal medicines for control of the symptoms of URTI.
- Patients addicted to smoking, tobacco/pan masala chewing or alcohol.

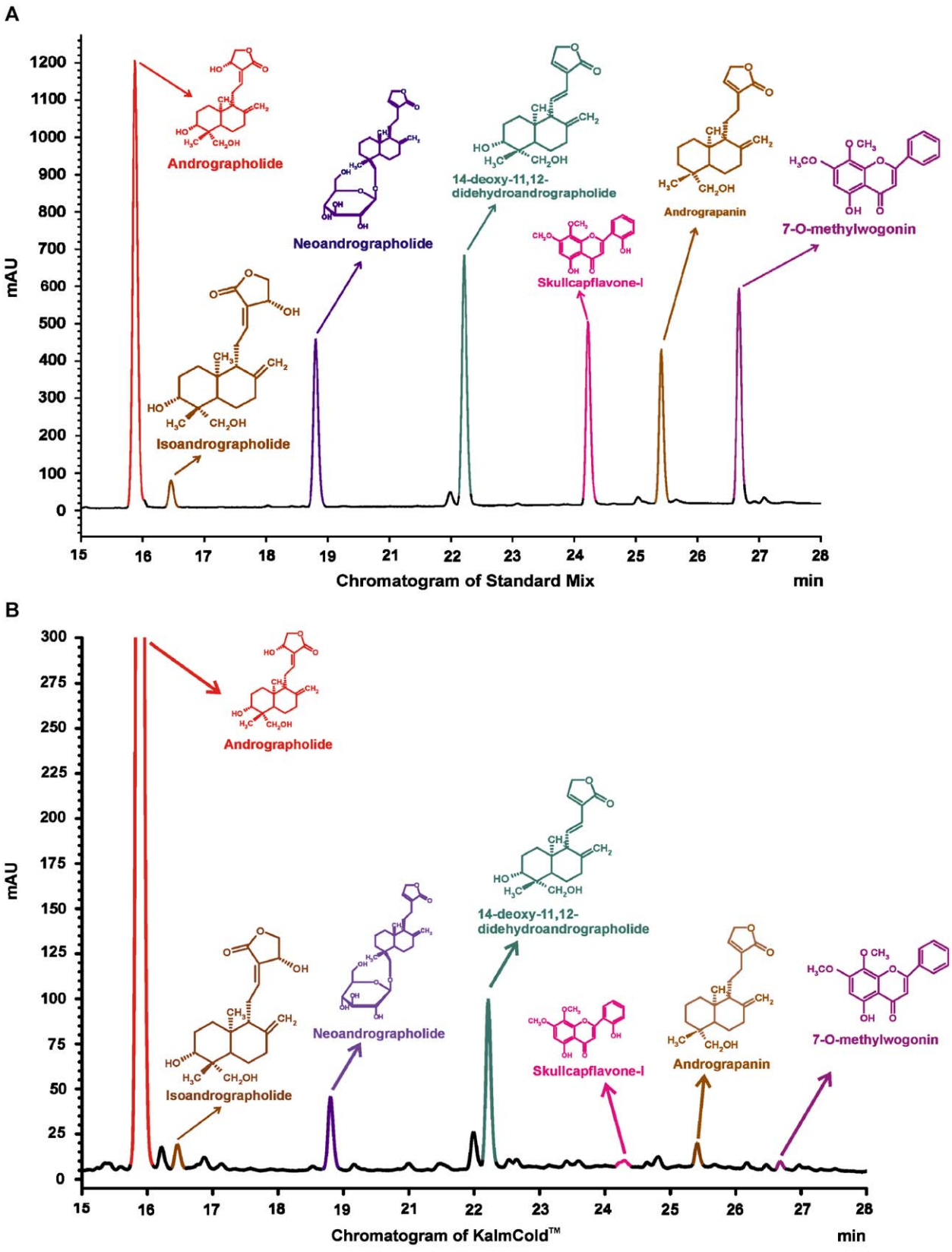

Fig. 1. HPLC chromatogram of standard mix (A) and KalmCold™ (B).

Randomization

The subjects enrolled for clinical trial were allotted to placebo and KalmCold™ groups using a simple randomization procedure. A computer aided random series programme was used to generate the random allocation sequence, which is a list of unique integer random numbers identified as patient code. The unique integer random numbers were then mentioned in respective places (blister pack containing either placebo or KalmCold™) as per the random allocation sequence. The random allocation sequence was generated at Natural Remedies, Bangalore from where the labeled and packed materials for clinical trial were dispatched to study centre. The entire process was carried out in completely concealed manner and all concerned in study centre viz., investigator, participants and staff were unaware of the sequence. The participants fulfilling the inclusion/exclusion criteria of the study and after obtaining the written informed consent were enrolled by the study investigator at the study centre and subsequently the pharmacist dispensed the study medication to the participants taking into consideration the order of enrolment and as per the random allocation sequence. The investigator, participants and pharmacist dispensing the interventions were all blinded to group assignment. The blinding process was maintained till all the data were compiled and confirmed for accuracy and then forwarded to the statistician for analysis.

Dosage regimen and dose rationale

Each patient was handed over one pack containing 10 capsules. Each patient was advised to take one capsule containing 100 mg of actives with a glass of water twice a day after breakfast and dinner (total of 200 mg of actives per day) for a period of five days. The dosage was based on the published clinical trials carried out on *A. paniculata* for the mentioned indication.

Symptomatic assessment of efficacy

Symptoms were assessed on day 1, 3 and 5 of treatment period. Each individual was requested for his/her availability to the investigator for routine follow up and for final clinical examination. The following outcome measures were assessed on each occasion: Cough, expectoration, nasal discharge (running nose), headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance. The patient was asked to grade each individual symptom on VAS. On subsequent follow up, the participants were shown a fresh sheet on which their symptoms were re-graded without referring to the previous rating. The scores of each symptom present on day 1, 3 and 5 of assessment were recorded. The improvement or otherwise was assessed by the changes in the scores over subsequent days.

Safety evaluation

A record of all adverse events reported by the patients was maintained. Patients in the study were instructed and or specifically asked to report to the investigators in the event of any adverse events which the patient or the investigator may attribute to the test substance treatment during start of treatment and follow up on day 3 and day 5.

Restrictions

The study imposed restriction of co-administration of any other drugs viz. antibiotics, cough remedies, anti-histamines or any other herbal/OTC product for control of the symptoms of URTI. No special diet/change in dietary pattern and activity was advised during the study period. Subjects were requested not to participate in any other clinical study during the trial period.

Statistics

Two hundred and twenty participants were considered for the final statistical analysis. Patients' characteristics of two groups were compared by independent samples t-test. Individual symptom scores recorded at different time intervals for each group (Placebo and KalmCold™) were analyzed using paired samples t-test (within groups). Similarly, independent samples t-test was applied to compare the individual symptom scores between the groups. For each periods and treatments, the overall symptoms scores were calculated and for this, each symptom score in an individual was pooled on a particular day and was considered as total symptom score of that individual. The average of total symptom of all the subjects was then calculated and considered as mean scores of overall symptoms. The overall symptom score were chosen to overcome the clinical heterogeneity in the measurement and scoring of symptoms of upper respiratory tract infection (Poolsup et al., 2004). Similarly, the effect on each symptom and overall symptoms is the mean difference of respective symptoms scores at day 1 and day 5. The effect size is the mean difference of effect between placebo and KalmCold™. To interpret the results, a percent mean change of effect of KalmCold™ over placebo was evaluated as

$$\text{Effect size}(\%) = [(\text{KalmCold}^{TM} - \text{Placebo})/\text{KalmCold}^{TM}]\text{X}100$$

In patients, wherein the overall symptoms scores was increased from day 1 to day 5 were considered as 'Aggravated' while in subjects the overall symptoms scores remain same at day 1 and day 5 as 'Unchanged'. The symptoms severity of patients and adverse effects observed in two groups were analysed by proportion Z test using correction for continuity (Zar, 1974). The above statistical applications were performed using SPSS software. A two-tailed (α=2) probability value $p \leq 0.05$ was considered to be statistically significant.

Results

Patients' characteristics

Out of 234 subjects assessed for eligibility, a total of 223 patients who fulfilled the selection criteria and willing to give informed consent were enrolled in the study and randomized to either placebo (n=111; 68 males and 43 females) or KalmCold™ (n=112; 75 males and 37 females) groups. The demographic characteristics of all available patients are summarized in Table 1. On comparison, mean characteristics of both the groups at baseline (day 1) were found to be similar.

Drop-outs

Out of 223 enrolled patients at baseline, three patients in placebo group did not turn up for follow up and hence excluded from analysis of symptom scores. Since the number of subjects in both the groups were almost similar it was considered appropriate to exclude them from overall assessment as justified in practice. Thus symptom scores of 108 patients in placebo and 112 in test group were analysed statistically.

Symptom scores

The symptom scores of all available patients in both placebo and KalmCold™ groups were summarized in Table 2. There was no significant difference between the individual symptom scores of participants of KalmCold™ group as compared to placebo group on day 1. In both the groups, baseline (day 1) mean score of cough was the maximum while the score for sleep disturbance the minimum. In both the treatments, mean scores of all symptoms showed a decreasing trend from day 1 to day 3 but from day 3 to day 5 most of the symptoms in placebo treated group either remained unchanged (cough, headache and earache) or got aggravated (sore throat and sleep disturbance) whereas in KalmCold™ treated group all symptoms showed a decreasing trend.

Comparing mean within groups, mean scores of symptoms in both the groups decreased significantly ($p \leq 0.05$) from day 1 to day 3 and day 5 while from day 3 to day 5 all symptoms except expectoration in placebo group did not improve significantly whereas in KalmCold™ treated group all symptoms improved significantly ($p \leq 0.05$) except earache. Comparing mean between both groups, the effect of both the treatments on all symptoms at day 3 was found to be the same i.e. treatments did not differ significantly while at day 5, all symptoms except earache in KalmCold™ treated group improved significantly ($p \leq 0.05$) than placebo group (Table 2).

Similarly, within groups, overall scores of all symptoms in both the groups decreased significantly ($p \leq 0.05$) from day 1 to day 3 and day 5 while from day 3 to day 5 placebo group did not improve significantly whereas KalmCold™ treated group showed significant improvement ($p \leq 0.05$). On between groups analysis, KalmCold™ group showed significant reduction ($p \leq 0.05$) in overall symptom scores as compared to placebo group (Table 2).

The effect (i.e. mean difference from baseline to final assessment) of placebo and KalmCold™ treatment on individual symptoms of common cold was presented in Table 2. The comparison showed that the effect of KalmCold™ over placebo was significant ($p \leq 0.05$) for all parameters except earache. The overall effect size (i.e. mean difference of effect between two groups) of KalmCold™ (d=81.97, SE=10.09, t=8.13, $p \leq 0.05$; 95% CI: 62.09 – 101.85) on symptom scores was found to be significantly different ($p \leq 0.05$) and 2.1 (52.7%) times higher than placebo.

Table 1
Summary of demographic information

Variables	Placebo	KalmCold™	t-value
Patients (M/F)	68 / 42	75 / 37	-
Age (yr)	32.42 ± 1.10	34.36 ± 0.97	1.33[ns]
Weight (kg)	56.52 ± 1.00	56.23 ± 0.81	0.22[ns]
Height (cm)	162.96 ± 0.66	162.46 ± 0.65	0.55[ns]
Heart rate (min)	81.21 ± 0.72	80.94 ± 0.76	0.26[ns]
BP diastolic (mmHg)	124.55 ± 1.07	125.09 ± 0.94	0.38[ns]
BP systolic (mmHg)	79.23 ± 0.58	79.16 ± 0.58	0.08[ns]

Values are expressed as mean ± SEM; n=110 in placebo and n=112 in KalmCold™

[ns] – Non significant.

Symptom severity

The overall symptoms aggravated or unchanged in patients of two treatment groups were summarized in Table 3. In placebo group (out of 108 patients), symptoms of fourteen patients got aggravated (i.e. increased at the end of final evaluation as compared to baseline) while one in KalmCold™ group (out of 112). The proportions of patients whose symptoms got aggravated was found to be significantly (Z = 3.28; $p \leq 0.05$)

Table 2
Effect of KalmCold™ on symptoms of common cold evaluated using VAS.

Symptoms	Treatments	Day 1		Day 3		Day 5		Effect[X]		Effect size[Y] (KalmCold™- Placebo)
		n	Mean scores	n	Mean scores	n	Mean scores	n	Difference in mean scores (Day 1 – Day 5)	
Cough	Placebo	89	55.06 ± 1.55	91	36.65 ± 1.66*	99	35.91 ± 1.60*	89	19.16 ± 1.70	15.45
	KalmCold™	89	57.87 ± 1.50	89	39.16 ± 1.64*	89	23.26 ± 1.81*,#,@	89	34.61 ± 1.64@	
Expectoration	Placebo	59	44.15 ± 1.60	59	32.03 ± 1.68*	65	29.85 ± 1.44*,#	57	16.58 ± 1.77	12.61
	KalmCold™	65	47.08 ± 1.95	67	31.42 ± 1.94*	63	17.14 ± 2.32*,#,@	62	29.19 ± 2.19@	
Nasal discharge	Placebo	79	44.87 ± 1.84	78	29.49 ± 1.79*	82	26.65 ± 1.53*	76	18.95 ± 2.33	13.52
	KalmCold™	70	42.79 ± 2.14	71	25.70 ± 1.84*	70	11.50 ± 1.93*,#,@	69	32.46 ± 2.16@	
Headache	Placebo	62	40.08 ± 2.28	67	26.49 ± 2.03*	68	26.99 ± 1.80*	57	16.14 ± 2.22	12.11
	KalmCold™	64	40.86 ± 2.50	64	23.75 ± 2.49*	61	11.56 ± 1.97*,#,@	60	28.25 ± 2.10@	
Fever	Placebo	87	43.22 ± 1.97	86	27.03 ± 1.98*	80	23.88 ± 1.66*	76	21.32 ± 2.30	14.32
	KalmCold™	92	46.36 ± 1.93	91	25.22 ± 2.00*	87	11.03 ± 1.73*,#,@	86	35.64 ± 1.72@	
Sore throat	Placebo	70	46.57 ± 2.28	72	30.35 ± 2.31*	74	33.99 ± 2.04*	66	14.17 ± 1.84	17.73
	KalmCold™	82	46.46 ± 1.81	83	27.77 ± 2.07*	82	15.00 ± 1.88*,#,@	79	31.90 ± 1.42@	
Earache	Placebo	9	32.22 ± 5.21	7	18.57 ± 4.59*	7	17.14 ± 7.47*	6	18.33 ± 7 .03	21.67
	KalmCold™	4	42.50 ± 16.52	4	22.50 ± 10.31[ns]	5	6.00 ± 4.00[ns]	4	40.00 ± 14.14	
Malaise/ Fatigue	Placebo	52	39.52 ± 2.67	51	28.04 ± 2.75*	47	24.26 ± 2.30*	40	18.88 ± 3.48	15.53
	KalmCold™	53	44.81 ± 2.88	53	23.87 ± 2.58*	52	10.29 ± 1.64*,#,@	50	34.40 ± 2.72@	
Sleep disturbance	Placebo	16	28.13 ± 2.28	15	14.67 ± 1.33*	13	16.15 ± 3.31*	13	13.85 ± 4.46	12.31
	KalmCold™	16	27.50 ± 2.14	17	12.35 ± 1.61*	15	3.33 ± 2.11*,#,@	13	26.15 ± 2.90@	
Overall	Placebo	108	216.20 ± 7.21	108	144.35 ± 5.91*	108	142.69 ± 6.34*	108	73.52 ± 6.98	81.97
	KalmCold™	112	222.14 ± 6.99	112	134.82 ± 5.76*	112	66.65 ± 5.60*,#,@	112	155.49 ± 7.26@	

Symptom scores are expressed as mean ± SEM.

* $p \leq 0.05$ Symptom scores of day 1 Vs Symptom scores of day 3 and day 5.
# $p \leq 0.05$ Symptom scores of day 3 Vs Symptom scores of day 5.
@ $p \leq 0.05$ Placebo Vs KalmCold™.
[ns] – Non significant.
[X] Effect – Difference in mean scores between day 1 and day 5.
[Y] Effect size – Difference in mean effect between placebo and KalmCold™ .

Table 3
Summary of symptom severity observed in patients.

Symptom severity	Placebo	KalmCold™	Z-value
Aggravated	14 (13.0%)	1 (0.9%)	3.28*
Unchanged	3 (2.8%)	1 (0.9%)	0.54ns
Total	17 (15.7%)	2 (1.8%)	3.44*

Percentage of patients experienced the symptom severity is presented in parenthesis.

* $p \leq 0.05$ Placebo Vs KalmCold™; n=108 in placebo and n=112 in KalmCold™.

ns – Non significant.

higher in placebo group than KalmCold™ group. The symptoms of three patients in placebo group and one in KalmCold™ group remained unchanged (i.e. same at baseline and at the end of the treatment) and the difference in proportions of symptoms unchanged between two groups were found to be the same ($Z=0.54$, $p > 0.05$). Overall, a total of seventeen patients in placebo group and two patients in KalmCold™ group did not show response to the treatments and the difference in the proportions was found to be significantly higher in placebo group than KalmCold™ group ($Z=3.44$, $p \leq 0.05$).

Adverse effects

The KalmCold™ group had total of six patients suffering from minor adverse effects, one patient each with vomiting, epistaxis, urticaria and three with diarrhoea. Of the three with diarrhoea, in addition one each had nausea or lethargy. The placebo group had three patients with adverse effects, one each with diarrhoea, vomiting (both mild in severity) and moderate rigor. The adverse effects between two groups were found to be same ($Z=0.63$, $p > 0.05$). In eight patients the effects were mild and isolated, and in one patient the effect was moderate and isolated. Except for vomiting (patient in KalmCold™ group) and urticaria, all other effects stopped spontaneously without any medical aid.

Discussion

Upper respiratory tract infections, caused by viruses, are most common diseases of human beings and on an average, adult are reported to have two to five common colds each year and school children from seven to ten colds per year (Eccles, 2005). In the light of gaining popularity of herbal preparations as remedies for various ailments, safe and effective herbal products would be of immense help. *A. paniculata* has been used in traditional system of medicine for promoting healthy immune system (Puri et al., 1993). It is incorporated as a principal ingredient in a number of proprietary preparations (Mishra et al., 2007). It is also recommended for treatment of fever associated with infectious diseases in Chinese and Thai traditional medicine (Coon and Ernst, 2004).

In the present study, the effect of extract of *A. paniculata* (KalmCold™; 100 mg, twice daily for 5 days) in patients with uncomplicated URTI was evaluated using VAS as the changes in pre and post-treatment scores of symptoms viz., cough, expectoration, nasal discharge, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance. The efficacy assessment of KalmCold™ essentially involved improvements in self evaluated scores of symptoms as most treatments for URTI are symptomatic. Clinical studies on the effectiveness of new treatments generally focus on changes in individual symptom scores as the main parameter of efficacy than any alterations in viral load. Additionally, it is also imperative for clinical evaluations on novel treatments for URTI to reveal outcomes in terms of decrease in intensity/duration of symptoms since these end points are the key benefits for patients in distress (Eccles, 2005). The results, as a whole, showed that reduction in symptom severity scores were indicative of effect of KalmCold™.

A cursory view at the intricate display of anti-inflammatory, immunostimulant and anti-pyretic properties exhibited by *A. paniculata* explains its effect on common cold. Cough, a common symptom associated with URTI is believed to be caused by inflammatory mediators on airway sensory nerve endings (Jacoby, 2004). Clinical studies on common cold included intensity/frequency of cough as one of the primary outcome measures and reported that *A. paniculata* extract alone or in combination with *Acanthopanax senticosus* (*A. senticosus*) was superior to placebo (Caceres et al., 1999; Melchior et al., 2000). The findings of the current study indicated that cough was graded with maximum VAS scores in both groups and KalmCold™ has shown progressive efficacy during the treatment period. Similar significant decrease in severity was noticed for expectoration also. Nasal secretion (rhinorrhoea), an early symptom of URTI that often accompanies sneezing, varies with the time course of infection and the severity of inflammatory response (Eccles, 2005). Significant reduction in severity scores of nasal discharge was observed after 5 days of treatment with KalmCold™ and was in accordance with the findings of Caceres et al. (1999) and Gabrielian et al. (2002).

A double blind study has shown that significant improvements from shivering (feverishness) was achieved in patients with common cold at day 4 of treatment with standardized extract of *A. paniculata* (Kan Jang; 1200 mg/day). In the current trial, similar reduction in fever scores was observed with KalmCold™ on day 5 (Hancke et al., 1995). Sore throat, in particular relevance to rhinoviral infections, is related with the formation of bradykinin in the upper airway in response to viral infection (Proud et al., 1988). As is evident from Table 2, sore throat was found to be significantly reduced by KalmCold™ treatment on day 5. Beneficial effects of *A. paniculata* in alleviating sore throat were demonstrated in previous studies (Thamlikitkul et al. 1991; Hancke et al., 1995; Gabrielian et al., 2002).

Cytokines released from immune cells can be considered as the common mediators of headache associated with infections (Smith, 1992). Treatment with KalmCold™ decreased the symptom scores of headache from 40.86 ± 2.50 (day 1) to 11.56 ± 1.97 (day 5). KalmCold™ also resulted in significant reduction ($p \leq 0.05$) in symptoms scores of malaise/fatigue than placebo group indicating the effectiveness of the treatment over the physical and psychological entities of common cold reportedly influenced by the cytokine cascade (Mahoney and Ball, 2002). Gabrielian et al. in 2002 found that general malaise was significantly improved in test group wherein participants took composition containing 85 mg standardized extract of *A. paniculata* and 10 mg of *A. senticosus* extract for 5 days. Likewise, sleep disturbance, a manifestation of sickness behaviour syndrome induced by the cytokines (Capuron and Miller, 2004) was also significantly decreased in the KalmCold™ group.

From Table 2, it is evident that no significant differences in mean base line scores of symptoms (day 1) between KalmCold™ and placebo group during commencement of the trial. However, given the fact that the overall symptoms scores reduced significantly in synchronization with the effect size on day 5 confirmed the benefits of KalmCold™. Contrarily, placebo group showed no further relief in outcome measures except for expectoration on day 5. The minimal incidence of worsening or persistence of the clinical condition during final evaluation observed in KalmCold™ group (1.8%) in contrast to the substantial proportion of the placebo group (15.7%) also authenticated the efficacy of KalmCold™. Additionally, our findings are

supported by the earlier reports of clinical studies that revealed significant improvements in patients with URTI (the overall effect) upon short term treatment with *A. paniculata* extract alone or with *A. senticosus* (Hancke et al., 1995; Caceres et al., 1999; Melchior et al., 2000; Gabrielian et al., 2002). Meta analyses of clinical trials also showed that *A. paniculata* extract alone or in combination with *A. senticosus* was more effective than placebo and can be an appropriate alternative for treatment of uncomplicated cases of URTI (Coon and Ernst, 2004; Poolsup et al., 2004).

As regards the safety aspects, treatment with KalmCold™ was found to be well tolerated in human subjects. The side effects observed in the present study were mild, spontaneously recovered and did not require any medical aid except for vomiting and urticaria. Similar events were also recorded in published literature (Thamlikitkul et al., 1991; Melchior et al., 1997; Calabrese et al., 2000; Coon and Ernst, 2004).

In conclusion, the findings showed that KalmCold™ treatment significantly decreased all the symptom scores except for earache whereas in the placebo group the symptoms were either unchanged or got aggravated after day 3 during the study period. In the present study, treatment with KalmCold™ for uncomplicated URTI revealed the potentials of extract of *A. paniculata* and was found to be 2.1 times or 52.7% more effective than the placebo.

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References

American Herbal Products Association (AHPA), 2004. *Andrographis paniculata*. In: McGuffin, M. (Ed.), Herbs of Commerce. American Herbal Products Association, USA, pp. 13.

Caceres, D.D., Hancke, J.L., Burgos, R.A., Sandberg, F., Wikman, G.K., 1999. Use of visual analogue scale measurements (VAS) to assess the effectiveness of standardized *Andrographis paniculata* extract SHA-10 in reducing the symptoms of common cold. A randomized double blind-placebo study. Phytomedicine 6, 217–223.

Caceres, D.D., Hancke, J.L., Burgos, R.A., Wikman, G.K., 1997. Prevention of common colds with *Andrographis paniculata* dried extract. A pilot double blind trial. Phytomedicine 4, 101–104.

Calabrese, C., Berman, S.H., Babish, J.G., Ma, X., Shinto, L., Dorr, M., Wells, K., Wenner, C.A., Standish, L.J., 2000. A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytother. Res. 14, 333–338.

Capuron, L., Miller, A.H., 2004. Cytokines and psychopathology: lessons from interferon-alpha. Biol. Psychiatry 56, 819–824.

Centers for Disease Control and Prevention (CDC), 2004. Fact sheet: stopping germs at home, work and school. Available from: <http://www.cdc.gov/germstopper/materials/home_work_school.pdf>.

Coon, J.T., Ernst, E., 2004. *Andrographis paniculata* in the treatment of upper respiratory tract infections: a systematic review of safety and efficacy. Planta Med. 70, 293–298.

Eccles, R., 2005. Understanding the symptoms of the common cold and influenza. Lancet Infect. Dis. 5, 718–725.

Fujita, T., Fujitani, R., Takeda, Y., Takaishi, Y., Yamada, T., Kido, M., Miura, I., 1984. On the diterpenoids of *Andrographis paniculata*: X-ray crystallographic analysis of andrographolide and structure determination of new minor diterpenoids. Chem. Pharm. Bull. 32, 2117–2125.

Gabrielian, E.S., Shukarian, A.K., Goukasova, G.I., Chandanian, G.L., Panossian, A.G., Wikman, G., Wagner, H., 2002. A double blind, placebo-controlled study of *Andrographis paniculata a* fixed combination Kan Jang in the treatment of acute upper respiratory tract infections including sinusitis. Phytomedicine 9, 589–597.

Hancke, J., Burgos, R., Caceres, D., Wikman, G., 1995. A double-blind study with a new monodrug Kan Jang: decrease of symptoms and improvement in the recovery from common colds. Phytother. Res. 9, 559–562.

Hershenson, M.B., Johnston, S.L., 2006. Rhinovirus infections: more than a common cold. Am. J. Respir. Crit. Care Med. 174, 1284–1285.

Indian Drug Manufacturer's Association (IDMA), 2002. *Andrographis paniculata*, Indian Herbal Pharmacopoeia. Indian Drug Manufacturer's Association, Mumbai, pp. 57-69.

Indian Pharmacopoeia (IP), 1966. Kalmegh, Monographs. The Pharmacopoeia of India, pp. 385-387.

International Conference on Harmonisation (ICH, 2005). International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: Text and methodology Q2 (R1). Current step 4 version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005).

Jacoby, D.B., 2004. Pathophysiology of airway viral infections. Pulm. Pharmacol. Ther. 17, 333–336.

Jalal, M.A.F., Overton, K.H., Rycroft, D.S., 1979. Formation of three new flavones by differentiating callus cultures of *Andrographis paniculata*. Phytochemistry 18, 149–151.

Jayakrishna, G., Harikishore, P., Rao, C.V., Gunasekar, D., Blond, A., Bodo, B., 2001. Two new 2' – oxygenated flavonoids from *Andrographis elongata*. Chem. Pharm. Bull. 49, 1555–1557.

Khare, C.P., 2007. *Andrographis paniculata*. In: Khare, C.P. (Ed.), Indian Medicinal Plants, An Illustrated Dictionary. Springer, (India), New Delhi, pp. 49–50.

Kumar, R.A., Sridevi., K., Kumar, N.V., Nanduri, S., Rajagopal, S., 2004. Anticancer and immunostimulatory compounds from *Andrographis paniculata*. J. Ethnopharmacol. 92, 291–295.

Kuroyanagi, M., Sato, M., Ueno, A., Nishi, K., 1987. Flavonoids from *Andrographis paniculata*. Chem. Pharm. Bull. 35, 4429–4435.

Lattoo, S.K., Khan, S., Dhar, A.K., Choudhary, D.K., Gupta, K.K., Sharma, P.R., 2006. Genetics and mechanism of induced male sterility in *Andrographis paniculata* (Burm. f.) Nees and its significance. Curr. Sci. 91, 515–519.

Mahoney, T., Ball, P., 2002. Common respiratory tract infections as psychological entities: a review of the mood and performance effects of being ill. Aust. Psychol. 37, 86–94.

Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A., Nishi, K., 1994. Cell differentiation-inducing diterpenes from *Andrographis paniculata* Nees. Chem. Pharm. Bull. 42, 1216–1225.

Melchior, J., Palm, S., Wikman, G., 1997. Controlled clinical study of standardized *Andrographis paniculata* extract in common cold – a pilot trial. Phytomedicine 3, 315–318.

Melchior, J., Spasov, A.A., Ostrovskij, O.V., Bulanov, A.E., Wikman, G., 2000. Double-blind, placebo-controlled pilot and phase III study of activity of standardized *Andrographis paniculata* Herba Nees extract fixed combination (Kan jang) in the treatment of uncomplicated upper-respiratory tract infection. Phytomedicine 7, 341–350.

Mishra, S.K., Sangwan, N.S., Sangwan, R.S., 2007. *Andrographis paniculata* (Kalmegh): a review. Phcog Rev. 1, 283–298.

Mossad, S.B., 1998. Treatment of the common cold. BMJ 317, 33–36.

National Institute of Allergy and Infectious Diseases (NIAID), 2007. Common cold. <http://www3.niaid.nih.gov/topics/commonCold/cause.htm>.

Poolsup, N., Suthisisang, C., Prathanturarug, S., Asawamekin, A., Chanchareon, U., 2004. *Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials. J. Clin. Pharm. Ther. 29, 37–45.

Proud, D., Reynolds, C.J., Lacapra, S., Kagey-Sobotka, A., Lichtenstein, L.M., Naclerio, R.M., 1988. Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. Am. Rev. Respir. Dis. 137, 613–616.

Puri, A., Saxena, R., Saxena, R.P., Saxena, K.C., Srivastava, V., Tandon, J.S., 1993. Immunostimulant agents from *Andrographis paniculata*. J. Nat. Prod. 56, 995–999.

Puri, H.S., 2003. Kalmegh. In: Hardman, R. (Ed.), Rasayana: Ayurvedic Herbs for Longevity and rejuvenation. Taylor & Francis Group, London and New York, pp. 151–156.

Schilter, B., Andersson, C., Anton, R., Constable, A., Kleiner, J., O'Brien, J., Renwick, A.G., Korver, O., Smit, F., Walker, R., 2003. Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. Food Chem. Toxicol. 41, 1625–1649.

Shen, Y.C., Chen, C.F., Chiou, W.F., 2002. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. Br. J. Pharmacol. 135, 399–406.

Smith, R.S., 1992. The cytokine theory of headache. Med. Hypotheses 39, 168–174.

Snedecor, G.W., Cochran, W.G., 1989. In: Statistical Methods 8th Ed. Iowa State Press, Ames.

Thamlikitkul, V., Dechatiwongse, T., Theerapong, S., Chantrakul, C., Boonroj, P., Punkrut, W., Ekpalakorn, W., Boontaeng, N., Taechaiya, S., Petcharoen, S., Riewpaiboon, W., Reiwpaiboon, A., Tenambergen, E.D., 1991. Efficacy of *Andrographis paniculata*, Nees for pharyngotonsillitis in adults. J. Med. Assoc. Thai. 74, 437–442.

The Indian Pharmacopoeia Commission (IPC), 2007. Kalmegh. Indian Pharmacopoeia, Vol. III. The Indian Pharmacopoeia Commission, Ghaziabad, pp. 2044.

United States Pharmacopeia (USP), 2007a. Water determination, Physical tests and determinations, USP 30 – NF 25. The United States Pharmacopeial Convention, Rockville, MD, pp. 385-386.

United States Pharmacopeia (USP), 2007b. Articles of botanical origin, Chemical Tests and Assays, USP 30 – NF 25. The United States Pharmacopeial Convention, Rockville, MD, pp. 198-199.

Zar, J.H., 1974. Biostatistical Analysis, Prentice-Hall. Inc. Englewood Cliffs, NJ pp.296-298..