



Efficacy and safety of AP-Bio®(KalmCold®) in participants with uncomplicated upper respiratory tract viral infection (common cold) – A phase III, double-blind, parallel group, randomized placebo-controlled trial[☆]

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ABSTRACT

Introduction: Kalmegh (*Andrographis paniculata*) is commonly used for treating uncomplicated Upper Respiratory Tract Infection (URTI) in complementary and alternative system of medicine. AP-Bio®(KalmCold®) is a standardized extract derived from the leaves of *A. paniculata*. This study was proposed to evaluate its efficacy using validated scales and objective measures.

Methods: Participants were randomized in a ratio of 1:1:1 to receive either AP-Bio® 200 mg/day, AP-Bio® 400 mg/day or placebo for 7 days. The primary outcome measure was Wisconsin Upper Respiratory Symptom Survey (WURSS-21) score. The secondary outcome measures were nasal mucous weight, nasal muco-ciliary clearance function and Interleukin-8 in nasal wash, as well as safety and tolerability.

Results: A total of n = 331 participants were screened and N = 300 participants were enrolled. The absolute WURSS-21 global score [mean (Standard Deviation - SD)] in the AP-Bio® 400 mg group [5.70 (5.31)] was less than the AP-Bio® 200 mg group [5.81 (4.83)] on Day-3. However, it was much higher in the placebo group [9.55 (14.27)]. AP-Bio® 400 mg group (Mean Difference - MD [Standard Error - SE] = -3.85 [1.52]; 95% CI = -6.85, -0.85; adjusted p = 0.034) and 200 mg group (MD [SE] = -3.74 [1.51]; 95% CI = -6.73, -0.76; adjusted p = 0.038) had significantly lower score than placebo. Similarly, on Day-3, the change in global score from baseline was significantly better in the AP-Bio® 400 mg group (MD [SE] = -3.91; [1.82] 95% CI = -7.50, -0.32; adjusted p = 0.038) and AP-Bio® 200 mg group (MD [SE] = -3.84 [1.97]; 95% CI = -7.72, -0.04; adjusted p = 0.044) in comparison to the placebo group. Nasal mucous weight, tissue paper counts used, and interleukin-8 showed a trend towards AP-Bio® groups having a favourable outcome when compared with placebo but did not reach statistical significance due to a small sample size. None of the study participants complained of any adverse physical symptoms. However, incident eosinophilia was noted in n = 20 participants on day 3. (n = 6 in AP-Bio® 200 mg group, n = 7 in AP-Bio® 400 mg group and n = 13 in placebo group; p = 0.181).

Conclusions: Participants in both the AP-Bio® dose groups showed positive tendency towards resolution of URTI symptoms when compared with placebo on Day-3 but not on Day-5 and Day-7.

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1. Introduction

Common cold can be defined as a “self-limited irritation and swelling of the upper airways with/without associated cough with no proof of pneumonia, lacking a separate condition to account for the patient symptoms, or with no history of COPD/ emphysema/ chronic bronchitis.”¹. Currently, there is no recommended treatment to reduce the duration of the course of the disease. However, *Andrographis paniculata* (Burm.f.) Nees (colloquially known as Kalmegh) has been widely used as a natural remedy for common cold/ uncomplicated upper respiratory tract infection (URTI). It is a native plant of India and Sri Lanka and has been proven to possess properties such as anti-microbial, antimalarial, anti-inflammatory, cytotoxic, antioxidant, anti-diabetic, immune modulatory, anti-angiogenic and hepato-renal protective activity².

The usefulness of *A. paniculata* in common cold has been shown by many studies³ and a meta-analysis of four randomized controlled trials (RCTs) showed that the difference in effect between *A. paniculata* and placebo was 10.85 points (95% Confidence Intervals [CI]: 10.36, 11.34 points, $P < 0.0001$) of the symptom severity score in favour of *A. paniculata*³. The interventional product in this study was AP-Bio® (KalmCold®) capsules which contains a proprietary quantified extract of the leaves of *A. paniculata* and it has also been previously tested for its efficacy and safety in the management of URTI with favourable outcomes⁴. However, in this study, efficacy was evaluated using the Visual Analogue Scale (VAS) score which is not validated⁴. Since there are no studies till date that evaluates its efficacy by using objective measures and validated scales, our aim was to address this lacuna in its development for clinical use. The primary objective of this study was to assess the efficacy of daily dose of AP-Bio® 200 mg and 400 mg in reducing the symptoms of common cold using Patient Related Outcome Measures (PROM) as assessed by the validated Wisconsin Upper Respiratory Symptom Survey (WURSS-21) in comparison to those receiving placebo over a 7-day period⁵. The secondary objectives were to assess certain objective efficacy measures (nasal mucous weight, nasal mucociliary clearance function and Interleukin (IL) -8 in nasal wash), as well as safety and tolerability when compared with placebo.

2. Materials and methods

2.1. Study design and study setting

This study was a double blind, triple arm, parallel group, randomized, placebo-controlled trial conducted between 11-11-2019 and 13-02-2020 in a single quaternary care teaching hospital of Mumbai, Maharashtra, India. It was completed before the first patient of novel Corona Virus Disease – 2019 (COVID-19) got reported in the state of Maharashtra, India (i.e., 09 March 2020).⁶

2.2. Participant eligibility criteria and recruitment strategy

All consenting adults of any gender, 18–60 years of age with a diagnosis of otorhinolaryngologist confirmed uncomplicated URTI and onset of symptoms within 48 h preceding the randomization were included in the study. Those with a history of allergic rhinitis, asthma, tuberculosis or any chronic diseases of the lungs and women of reproductive age who are pregnant, lactating or not willing for adequate contraception were excluded from the study. Further, any participant on medications or requiring medications such as antibiotics, anti-viral agents, anti-histaminics, steroids, decongestants, herbal remedies, Vitamin C or Zinc for the treatment of common cold and regular users of tobacco in any form (i.e., smoking or smokeless forms) were also excluded.

Participants were recruited from the community with the help of health workers affiliated with our institution, who were well versed with the local community in Mumbai city, India. The health worker sensitized the community about the ongoing study so that potential participants

can self-report to the health worker in case of symptom onset and the health workers also actively looked out for participants who show symptoms of common cold in the last 48 h.

2.3. Interventions

The Interventional product (IP) in this study was AP-Bio®, a quantified extract from leaves of *Andrographis paniculata* (Burm.f.) Nees developed by Natural Remedies Private Limited, Bangalore, India. It contains diterpene lactones (> 33%) with andrographolide (> 30.0% w/w). The content of 14-deoxy-11,12-didehydroandrographolide is not more than 15% of the total diterpene lactones as per the United States Pharmacopeia (USP). A unique and patented process is implemented to enhance the andrographolide content that is known to be responsible for the therapeutic benefit and limit the content of 14-deoxy 11, 12, didehydroandrographolide that is known to cause adverse effects^{3,4}. The composition and physico-chemical analysis adhere to the international quality requirements (US pharmacopeia and British pharmacopeia) which include analysis of solvent residue, heavy metals residue, pesticide residue, mycotoxin residue and microbial contamination. The entire manufacturing process and quality control procedures adheres to the procedures and protocols as approved and mandated by the Indian complementary and alternative drug regulatory authorities. The IP was available in the form of capsules supplied in sealed bottles and it was dispensed by the study investigators only once to the study participant on the day of randomization. Each capsule of AP-Bio® contained 100 mg or 200 mg of *A. paniculata* extract, microcrystalline cellulose and syloid as excipients. The placebo capsule contained microcrystalline cellulose and syloid. It was matched with investigational product in size, colour and weight. Based on the group of participant allocation, they were instructed to take the IP as mentioned below immediately after food intake as per standard Ayurveda practice⁷:

- Treatment arm 1: AP-Bio® 100 mg twice daily for 7 days
- Treatment arm 2: AP-Bio® 200 mg twice daily for 7 days
- Control Arm: Identical placebo twice daily for 7 days

The dose was selected based on the previous clinical trial and 400 mg was chosen to explore the differences in safety and efficacy of the IP at a higher dose^{3,4}. Normal Saline nasal drops and paracetamol 650 mg (not exceeding 4 g / day) as and when required were given as rescue medications. The used IP bottles were collected back on the last visit to ensure adherence by pill counting.

2.4. Preparation of AP-Bio®

The preparation of the extract is as outlined in the study by Saxena et al.,⁴. To summarize in brief, coarsely ground leaves of *A. paniculata* (Burm.f.) Nees were refluxed with ethanol, concentrated in distillator and dried under vacuum. The extract was then milled and sieved to get a uniform powdered extract of *A. paniculata*. The marc was extracted with water and distilled under vacuum ($\leq 75^\circ\text{C}$) until the total solid content reached approximately 15–20% (w/v) in the liquid. It was then spray dried to get the water extract of *A. paniculata*. The ethanolic and water extracts were then analysed for its composition of the active constituents, and it was blended to get AP-Bio with the required levels of active constituents by deriving an herb to extract ratio of 14:1. The phytochemical analysis of AP-Bio® using High Performance Liquid Chromatography (HPLC) is also already narrated in detail in the previous publication⁴. The analytical method was developed and validated in the National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited laboratory at the Natural Remedies Private Limited, Bengaluru, Karnataka, India to ensure selectivity, accuracy and precision.

2.5. Outcome measures

The primary outcome measure was symptom severity score assessed by WURSS-21 at baseline, 6 h, D2, D3, D5 and D7. WURSS-21 is an illness-specific health-related quality-of-life questionnaire outcomes instrument. It is a 21-item questionnaire with questions 2–11 reporting symptom severity (for symptoms such as runny nose, plugged nose, sneezing, sore throat, scratchy throat, cough, hoarseness, head congestion, chest congestion and feeling tired) and 12–20 reporting functional impairment (such as ability to think clearly, sleep well, breathe easily, walk or climb stairs or exercise, accomplish daily activities, work outside the home, work inside the home, interact with others, and live your personal life). Each question is marked using a 7-point severity scale with zero denoting no symptoms and seven denoting severe symptoms⁵. The WURSS-21 scale was translated into Hindi and Marathi by professional translators and cognitive debriefing interviews were conducted within the target population for harmonization and reconciliations based on feedbacks from these interviews as per the requirements of the licensor, the Wisconsin Alumni Research Foundation.

The following were the secondary outcome measures:

- Area under the curve (AUC) for WURSS-21 symptom score (computed from questions 2–11) with scores ranging from 0 to 70; AUC for WURSS-21 functional impairment score (computed from questions 12–20) with scores ranging from 0 to 63 and AUC for WURSS-21 global score (computed from questions 2–20) with scores ranging from 0 to 133, where 0 indicates best clinical outcome for all three computations. Using the WURSS-21 data at each time point assessed, the AUC was calculated over the 7 days from the start of intervention to treat common cold. It is a measure of the total symptom burden of the common cold suffered.⁵
- Visual Analogue Score (VAS) (0–100) of each individual symptom (cough, expectoration, nasal discharge, headache, fever, sore throat, earache, malaise/ fatigue, and sleep disturbance) at baseline, 6 h, and afterwards once daily at night till day 7 and VAS AUC: scores 0–900; 0 being the least score and 900 being the maximum score after summing the individual VAS scores of all 9 symptoms at a given time point.
- Adverse events (E.g., Nausea, vomiting, diarrhoea, abdominal pain, arthralgia, myalgia, rash, pruritis, allergy, abnormal liver or renal function tests, dyselektrolytemia, or any other new onset complaints adjudicated to be casually related to the study medication) and their respective WHO Uppsala Monitoring Centre Causality assessment.
- Time to achieve complete resolution of illness
- Change in C-Reactive Protein (CRP) on D3 from baseline
- Nasal mucous weight by daily tissue counts, nasal muco-ciliary clearance function and IL-8 in nasal wash (in $n = 20$ participants / group) at baseline and D3

2.6. Sample size

Based on the study by Saxena et al.⁴ the estimated Cohen's d was 1.63, effect size r was 0.63, and cohens f was 0.315. Thus, the total sample size calculated using ANOVA (F tests) with an alpha error of 5% and power of the study 80% for a three-arm study using the estimated Cohen's f was $n = 102$ (i.e., 34/ group). However, since the effect size used was based on a study utilizing subjective measure (VAS) and was not based on objective end points or validated scales to estimate the symptom severity scores, as there were no previous studies, we decided to include at least 100 participants in each arm. Thus, a total of $N = 300$ participants were recruited.

2.7. Randomization sequence allocation, allocation concealment and blinding

Computer generated randomization with fixed block size of 6 was

utilized and participants were allocated to three groups (AP-Bio® 200 mg, AP-Bio® 400 mg or Placebo) in a ratio of 1: 1: 1. The randomization sequence was generated at the clinical trial site by the study pharmacists who were unblinded under the guidance of a qualified doctor, a Clinical Pharmacologist by training, who was independent of the study team. Allocation concealment was ensured using sequentially numbered opaque sealed envelopes (SNOSE). The envelopes were opened by the researchers only at the time of intervention assignment after verifying the participant's eligibility to participate in the study. The participant and the researchers were blinded of the allocation, and it was strictly ensured that there was no contamination between the blinded and the unblinded teams at the site, as was the practice at the site for many previous and ongoing regulatory clinical trials.

2.8. Study procedures

Once eligibility was confirmed by the study physicians, socio-demographic characteristics, medical history, baseline WURSS-21 and visual analogue scale (VAS) score on symptom severity were collected from the participants by the study physicians. Subsequently, blood was collected for routine baseline laboratory investigations, and they were enrolled and randomized by the blinded researchers/ study doctors. The participants were sent home with a diary card to mark VAS once daily and also to capture adverse events – both solicited and unsolicited adverse events. The common AEs were listed in the diary for the participants to look out for and self-report. On D1, participants were asked to score VAS at 6 h (± 1 h) followed by once daily till D7. WURSS-21 was evaluated by the study team telephonically on D1 at 6 h and on D2 at 10.00 am (± 1 h) in the morning. In-person follow-up visits were conducted on D3, D5 and D7 (± 1 day) to document WURSS-21 and any adverse events. Routine haematology and biochemistry blood tests were repeated on D3 to assess the safety profile of the IP. In 20% of the participants in each group, special tests such as nasal mucociliary function tests, interleukin – 8 (IL-8) in nasal wash fluid and nasal mucous weight assessments were performed at baseline and D3 (± 1 day). All samples were discarded immediately after the laboratory analysis as per the standard processes followed for destruction of human biological samples.

2.9. Nasal muco-ciliary clearance function test

The test was conducted with the participant in sitting position. A 1 mm saccharin particle was placed under direct vision on the inferior nasal turbinate 1 cm from the anterior end. The participant was instructed not to sniff or blow out nose during the procedure and to avoid coughing or sneezing as much as possible. A stop clock was started immediately after placement of the saccharin particle, and it was stopped when the participant experienced the sweet taste. The time was recorded in completed minutes. A participant with good mucociliary function is expected to experience the sweet taste much earlier than those with abnormal mucociliary function.⁸

2.9.1. Procedure for nasal wash

Normal saline was used for the nasal wash. The participant was asked to sit leaning forward. A maximum of 10 ml saline was flushed in each of the nostril using a syringe. The fluid was collected in a clean container provided by the lab. The participant was further asked to blow his/her nose gently at the end through each of the nostrils.

2.10. Data management and quality assurance

All relevant data of each participant were recorded in a specially designed case record form (CRF) by the research study doctors at the site. Data entry was done in Microsoft Excel (Publisher: Microsoft Corporation, Redmond, Washington, USA, 2016) and 100% source data verification was done by the monitors who were not involved in data

collection or data entry and that the study was conducted as per the approved protocol without violating the rights and safety of the participants and in accordance with the ethics and regulatory guidelines. The monitors ensured authenticity, accuracy and completeness of the data. Analysis was performed with IBM SPSS Statistics for Windows, Version 20.0 (Publisher: IBM Corp., USA, 2011).

2.11. Statistical analysis plan

Modified Intention to treat analysis was performed to analyse both efficacy and safety parameters. For analysing efficacy, data of all randomized participants including those who were lost to follow-up until the time they were in the study were used and for analysing safety, data of all participants who at least took one dose of AP-Bio® was used. No imputations were done to treat missing values. The statistical significance was set at $p < 0.05$. The baseline demographic characteristics were summarized using descriptive statistics. Normal distribution of outcome measures was assessed using Shapiro-Wilk test. Between group differences at baseline were assessed using Chi-squared test for categorical variables and one-way ANOVA for continuous variables which were also normally distributed.

Outcome measures such as WURSS-21, VAS scores, AUCs and CRP were subjected to Brown Forsythe ANOVA⁹ with *post-hoc* Games Howell tests to evaluate for between-group differences, as the distribution was non-normal and heterogenous and these tests are free of these assumptions. Within group analysis was performed using Wilcoxon signed rank test for CRP and repeated measures ANOVA with *post-hoc* Games Howell test for WURSS-21, VAS scores and AUCs. Greenhouse-Geisser correction was used in the repeated measures ANOVA if the Mauchly's test of sphericity was violated.¹⁰ Time to complete resolution of illness was evaluated using Kaplan Meier's curve with log-rank test.

The other outcome measures such as nasal mucous weight, number of tissues used, nasal muco-ciliary clearance function tests and IL-8 were

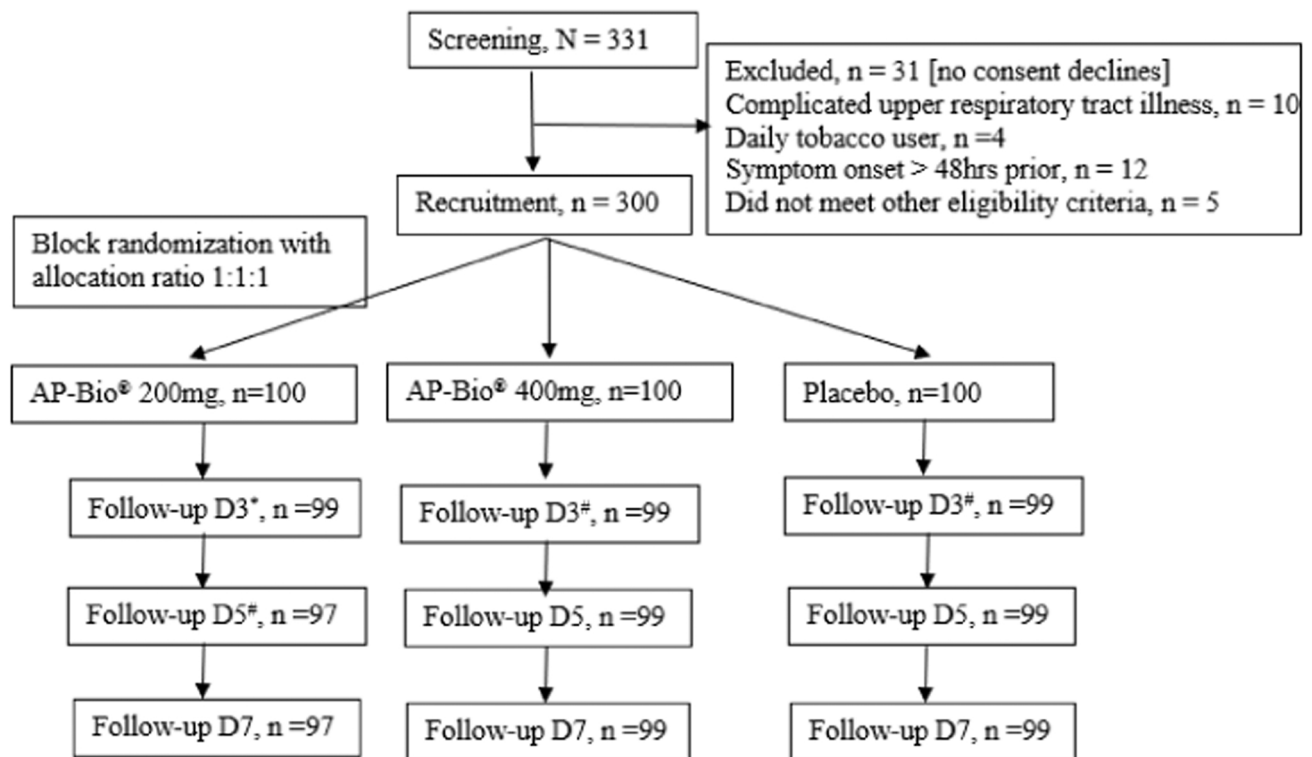
analysed between groups using Kruskal Wallis test and post hoc Mann-Whitney U test with Bonferroni's correction for multiple comparisons. The within group analysis was performed using Wilcoxon signed-rank test. Comparisons of important / significant outcome measures between groups were described using Mean (standard error [SE]) and for within group analysis using mean (SD) along with 95% CI in each analysis. The difference in the incidence of adverse events between the groups was assessed using Chi-Squared test. Wherever feasible, we preferred to use parametric alternatives with necessary corrections for violations of assumptions instead of rank based non-parametric tests as they are slightly less robust than the parametric and often result in loss of data due to conversion of actual data into ranks during the computational process.¹¹

3. Results

A total of $n = 331$ participants were screened and $N = 300$ were finally recruited between 11 Nov 2019 and 13 Feb 2020. Of this, $n = 295/300$ participants completed the entire trial as per the protocol. The reasons for screen failures and the participant flow during the study period is depicted in Fig. 1. Three participants from the AP-Bio® 200 mg group and one participant each from the placebo and AP-Bio® 400 mg groups did not complete the study. Except for one participant in the AP-Bio® 200 mg group who withdrew consent on Day 3 due to worsening symptoms, all other participants who did not complete the study were lost to follow-up i.e., the study team was unable to establish communication with the participant despite their best efforts. There were no other protocol deviations reported that would impact the data integrity or participant safety.

3.1. Baseline characteristics of study participants

The overall mean (SD) age was 29.33 (11.56) years, and the



* Participant withdrew consent # lost to follow-up.

Fig. 1. Participant flow diagram.

minimum and maximum ages were 18 and 59 years, respectively. Approximately 85% of participants in each group were men. The baseline demographic and clinical parameters, group wise, have been summarized in Table 1. The mean (SD) of baseline laboratory parameters is summarised in S1 Table and all ranges were within normal limits in all the three groups.

3.2. Changes in WURSS-21 score (primary outcome measure)

There was a statistically significant difference on D3 with regards to the symptom severity score and the global score. With regards to the symptom severity score on D3, AP-Bio® 400 mg group had significantly lower score than placebo (MD [SE] = -3.34 [1.28]; 95%CI = -5.86, -0.82; adjusted $p = 0.027$). Similarly, AP-Bio® 200 mg group also had significantly lower scores than placebo at D3 (MD [SE] = -3.22 [1.27]; 95% CI = -5.72, -0.71; adjusted $p = 0.033$). Similar findings were seen with the global score on D3 wherein, AP-Bio® 400 mg group (MD [SE] = -3.85 [1.52]; 95% CI = -6.85, -0.85; adjusted $p = 0.034$) and 200 mg group (MD [SE] = -3.74 [1.51]; 95% CI = -6.73, -0.76; adjusted $p = 0.038$) had significantly lower score than placebo. The absolute scores in the AP-Bio® 400 mg group was less than the AP-Bio® 200 mg group. There was no significant difference across the other time points between groups (Table 2).

The analysis with baseline adjusted WURSS-21 scores at D3 further corroborated these findings.

On D3, the change in symptom severity score from baseline was significantly better in the AP-Bio® 400 mg group (MD [SE] = -3.03 [1.36]; 95% CI = -5.72, -0.34; adjusted $p = 0.040$) and AP-Bio® 200 mg group (MD [SE] = -2.86 [1.45]; 95% CI = -5.72, -0.01; adjusted $p = 0.049$) when compared to placebo group. Similarly, on D3, the change in global score from baseline was significantly better in the AP-Bio® 400 mg group (MD [SE] = -3.91; [1.82] 95% CI = -7.50, -0.32; adjusted $p = 0.038$) and AP-Bio® 200 mg group (MD [SE] = -3.84 [1.97]; 95% CI = -7.72, -0.04; adjusted $p = 0.044$) in comparison to the placebo group.

In the within group analysis (S2 Table), the symptom severity scores reduced in all the three groups at 6 h when compared to baseline. However, in comparisons of D3 versus 6 h (MD [SD] = -1.94 [6.26], 95% CI = -3.18, -0.69; adjusted $p = 0.040$ for AP-Bio® 200 mg & MD [SD] = -2.01 [6.20], 95% CI = -3.24, -0.78; adjusted $p = 0.024$ for AP-Bio® 400 mg) and D3 versus D2 (MD [SD] = -2.06 [4.65], 95% CI =

-2.99, -1.13; adjusted $p < 0.001$ for AP-Bio® 200 mg & MD [SD] = -1.91 [5.29], 95% CI = -2.96, -0.86; adjusted $p = 0.007$ for AP-Bio® 400 mg), the symptom severity score had significantly fallen in both the AP-Bio® groups but had modestly increased in the placebo group. With regards to the functional impairment score, none of the comparisons yielded a significant difference. However, with regards to the global score, comparison of D3 versus D2 (MD [SD] = -1.97 [4.99], 95%CI = -2.97, -0.97; adjusted $p = 0.002$ for AP-Bio® 200 mg & MD [SD] = -1.98 [5.40], 95% CI = -3.05, -0.91; adjusted $p = 0.006$ for AP-Bio® 400 mg) revealed a significant fall in score in both the AP-Bio® groups but at the same time, it increased in the placebo group.

3.3. Change in individual WURSS-21 symptom score (secondary outcome measure)

The between groups analysis of observed values at a given timepoint for Individual WURSS symptom score of the individual symptom scores (S3 Table) have shown statistically significant difference between placebo and the AP-Bio® groups on day 3 for certain symptoms. AP-Bio® 200 mg group demonstrated a significantly lower score when compared with placebo for runny nose and sneezing. AP-Bio® 400 mg group demonstrated a significantly lower score when compared with placebo with regards to plugged nose, sneezing, cough and headache/head congestion. In addition, on day 5 AP-Bio® 200 mg group demonstrated a significantly lower score when compared with placebo for hoarseness of voice. The between groups analysis of change in score from baseline (baseline adjusted values) at a given time point for Individual WURSS symptom score (S4 Table) confirmed the above findings except for plugged nose that lost its statistical significance.

3.4. Visual analogue scale – score (secondary outcome measure)

AP-Bio® 400 mg group demonstrated a significant improvement on D2 when compared with placebo for expectoration and sore throat. It also demonstrated a significant improvement on D3 when compared with placebo for cough, expectoration, nasal discharge and sore throat. AP-Bio® 200 mg group showed a significant improvement against the placebo group with regards to nasal discharge on D3 and sore throat on D2. The VAS global score in the AP-Bio® 400 mg group was significantly low (MD [SE] = -30.26 [9.90], 95% CI = -53.77, -6.75, adjusted $p = 0.008$) on D3 when compared with placebo. However, in the AP-Bio® 200 mg group, although the scores were much less when compared to the placebo on D3, it did not reach statistical significance (MD [SE] = -23.70 [10.67], 95% CI = -48.98, 1.57 $p = 0.071$). The between groups analysis of observed values at a given timepoint for individual VAS score and the global VAS score is summarized in S5 Table.

With regards to the baseline adjusted values, the AP-Bio® 200 mg group significantly differed from the placebo group for Nasal Discharge on D3 and expectoration at 6 h, and on D2. Whereas the AP-Bio® 400 mg group, differed significantly from the placebo group for cough on D3; expectoration at 6 h, D2, and D3; nasal discharge on D2, and D3; Sleep disturbance on D5, and D7; Global VAS score at 6 h, D2 and D3. The baseline adjusted VAS global score in the AP-Bio® 200 mg group was significantly low on D3 (MD [SE] = -25.19 [9.84], 95% CI = -48.43, -1.95, adjusted $p = 0.030$) when compared with placebo. But in case of AP-Bio® 400 mg when compared with placebo, the baseline adjusted VAS global score was significantly low at 6 h, (MD [SE] = -23.06 [8.61], 95% CI = -43.40, -2.72, adjusted $p = 0.022$) on D2 (MD [SE] = -27.44 [9.26], 95% CI = -49.33, -5.56, adjusted $p = 0.010$) and on D3 (MD [SE] = -43.98 [10.17], 95% CI = -68.02, -19.94, adjusted $p < 0.001$). The between groups analysis of change in score from baseline (baseline adjusted values) at a given time point for VAS for the individual VAS score and the global VAS score is summarized in S6 Table.

The within group analysis of VAS global score is summarized in Table S7. The scores were consistently low in both the AP-Bio® groups at

Table 1
Baseline Demographic & Clinical Characteristics.

Characteristics	AP-Bio® 200 mg n = 100	AP-Bio® 400 mg n = 100	Placebo n = 100	P value
Age (years)				0.288 *
18 – 30	61	71	57	
31 – 45	21	19	33	
46–59	18	10	10	
Sex				0.915
Male	88	87	86	
Female	12	13	14	
Symptoms at presentation				
Runny Nose	64	68	67	0.824
Blocked Nose	60	62	66	0.671
Sneezing	50	40	51	0.226
Sore Throat	62	52	55	0.343
Scratchy throat	67	62	62	0.698
Cough	70	70	77	0.442
Hoarseness of voice	32	41	36	0.415
Head Ache/Head Congestion	42	40	40	0.946
Chest Congestion	19	16	11	0.284
Feeling tired	31	28	31	0.867

* Based on One-Way ANOVA (Analysis of Variance).

Table 2
Between group analysis of WURSS-21.

Time point		Symptoms Severity Score				Functional Impairment Score				Global Score			
		AP-Bio® 200 mg Mean (SD)	AP-Bio® 400 mg Mean (SD)	Placebo Mean (SD)	P value	AP-Bio® 200 mg Mean (SD)	AP-Bio® 400 mg Mean (SD)	Placebo Mean (SD)	P value	AP-Bio® 200 mg Mean (SD)	AP-Bio® 400 mg Mean (SD)	Placebo Mean (SD)	P value
Baseline	Observed value	13.80 (8.89)	13.75 (8.29)	14.06 (9.87)	0.967	2.34 (5.54)	2.20 (5.30)	1.83 (4.39)	0.766	16.14 (15.95)	15.95 (11.90)	15.89 (12.89)	0.989
6 h	Observed value	7.46 (5.41)	7.26 (4.21)	8.44 (6.40)	0.258	0.40 (1.58)	0.50 (1.24)	0.70 (2.31)	0.477	7.86 (6.24)	7.76 (4.81)	9.14 (7.89)	0.242
	Change from baseline	-6.34 (7.79)	-6.49 (7.92)	-5.62 (6.98)	0.686	-1.94 (5.14)	-1.70 (5.06)	-1.13 (3.60)	0.451	-8.28 (11.43)	-8.19 (11.25)	-6.75 (9.50)	0.530
Day 2	Observed value	7.57 (5.31)	7.16 (4.76)	8.47 (8.12)	0.317	0.34 (0.88)	0.52 (1.70)	0.55 (2.55)	0.683	7.91 (5.69)	7.68 (5.38)	9.02 (8.94)	0.338
	Change from baseline	-6.23 (9.42)	-6.59 (8.94)	-5.59 (8.88)	0.733	-2.00 (5.61)	-1.68 (5.21)	-1.28 (4.49)	0.609	-8.23 (13.52)	-8.27 (12.08)	-6.87 (11.47)	0.661
Day 3	Observed value	5.37 (4.63)	5.25 (4.91)	8.59 (11.78)	0.004*	0.43 (1.08)	0.45 (1.23)	0.96 (4.14)	0.262	5.81 (4.83)	5.70 (5.31)	9.55 (14.27)	0.004 [#]
	Change from baseline	-8.33 (9.52)	-8.50 (8.22)	-5.47 (8.87)	0.028 [@]	-1.85 (5.44)	-1.75 (5.01)	-0.87 (5.40)	0.357	-10.18 (13.43)	-10.25 (11.21)	-6.34 (10.33)	0.026 ^{&}
Day 5	Observed value	3.25 (3.72)	3.11 (3.36)	3.25 (3.88)	0.887	0.11 (0.47)	0.18 (0.63)	0.16 (0.51)	0.651	3.36 (3.76)	3.29 (3.60)	3.54 (4.75)	0.905
	Change from baseline	-10.45 (9.72)	-10.64 (9.35)	-10.68 (9.49)	0.984	-2.17 (5.58)	-2.02 (5.30)	-1.67 (4.34)	0.776	-12.63 (13.82)	-12.66 (12.81)	-12.35 (12.37)	0.983
Day 7	Observed value	1.52 (2.80)	1.04 (1.97)	2.13 (4.83)	0.080	0.11 (0.43)	0.15 (0.58)	0.19 (1.10)	0.763	1.63 (3.09)	1.19 (2.19)	2.32 (5.26)	0.101
	Change from baseline	-12.19 (9.12)	-12.71 (8.54)	-11.93 (9.30)	0.823	-2.17 (5.56)	-2.05 (5.32)	-1.64 (4.47)	0.746	-14.36 (13.36)	-14.76 (12.08)	-13.57 (11.82)	0.789

WURSS – Wisconsin Upper Respiratory Symptom Score; SD – Standard Deviation

Sample size: 100 in each group at baseline; 99 in each group at 6 hrs, day 2 and day 3; 97 in AP-Bio® 200 mg group & 99 each in AP-Bio® 400 mg and placebo group at day 5 and day 7.

* Post Hoc Games-Howell's Test: AP-Bio® 200 mg versus placebo, adjusted p = 0.033; AP-Bio® 400 mg versus placebo, adjusted p = 0.027; and AP-Bio® 400 mg versus AP-Bio® 200 mg, adjusted p = 0.982

[#]Post Hoc Games-Howell's Test: AP-Bio® 200 mg versus placebo, adjusted p = 0.038; AP-Bio® 400 mg versus placebo, adjusted p = 0.034; and AP-Bio® 400 mg versus AP-Bio® 200 mg, adjusted p = 0.988

[@] Post Hoc Games-Howell's Test: AP-Bio® 200 mg versus placebo, adjusted p = 0.049; AP-Bio® 400 mg versus placebo, adjusted p = 0.040; and AP-Bio® 400 mg versus AP-Bio® 200 mg, adjusted p = 0.990

[&] Post Hoc Games-Howell's Test: AP-Bio® 200 mg versus placebo, adjusted p = 0.044; AP-Bio® 400 mg versus placebo, adjusted p = 0.038; and AP-Bio® 400 mg versus AP-Bio® 200 mg, adjusted p = 0.999

all time points when compared with placebo.

3.5. Area under the curve (AUC) analysis for WURSS-21 and VAS (secondary outcome measure)

AP-Bio® 400 mg group demonstrated a significantly smaller symptom severity score AUC when compared to the placebo group (MD [SE] = -1211.76 [494.16], 95% CI = -2382.25, -41.27, adjusted p = 0.041). AP-Bio® 200 mg group, on the other hand, although showed a smaller area when compared with placebo, it did not reach statistical significance (MD [SE] = -1037.94 [514.63], 95% CI = -2255.59, 179.71, adjusted p = 0.111). Also, both AP-Bio® groups did not achieve statistical significance with regards to the Global WURSS-21 AUC, functional impairment score AUC and the VAS global score AUC when compared with placebo, although both the AP-Bio® groups

demonstrated a much smaller area when compared to that of the placebo. The results of the analysis of AUC for WURSS-21 and VAS is given in Table 3.

3.6. Exploratory outcome measure

The between group differences of nasal mucosal weight (g), number of tissue papers used (total number expressed as n), muco-ciliary clearance (min), nasal wash IL-8 (pg/ml) and serum hsCRP (mg/l) are summarized in Table 4. There was a trend towards a difference in nasal mucosal weight and tissue count on D3 between both the AP-Bio® groups and placebo but did not achieve statistical significance. The analysis of baseline adjusted values however showed that AP-Bio® 400 mg, significantly decreased the nasal mucosal weight on D3. (MD [SE] = -2.40 [0.95]; 95% CI = -4.32, -0.49; p = 0.015). The nasal

Table 3
Between group analysis for WURSS-21 Area under the Curve on Day 7.

	AP-Bio® 200 mg (n = 99)		AP-Bio® 400 mg (n = 100)		Placebo (n = 100)		P value
	Mean	SD	Mean	SD	Mean	SD	
Symptom severity score AUC	4025.64	2547.17	3851.82	2118.59	5063.58	4464.40	0.017*
Functional Impairment score AUC	255.27	412.65	311.76	653.07	433.44	1357.97	0.364
Global WURSS-21 Score AUC	4280.91	2677.23	4163.58	2393.86	5497.02	5158.90	0.017 [#]
VAS score AUC ^{\$}	21,616.67	27,904.15	20,286.36	20,168.52	31,456.00	50,819.56	0.056

WURSS – Wisconsin Upper Respiratory Symptom Score; SD – Standard Deviation; AUC – Area Under the Curve.

* Post Hoc Games-Howell's Test: AP-Bio® 200 mg versus placebo, adjusted p = 0.111; AP-Bio® 400 mg versus placebo, adjusted p = 0.041; and AP-Bio® 400 mg versus AP-Bio® 200 mg, adjusted p = 0.860

[#]Post Hoc Games-Howell's Test: AP-Bio® 200 mg versus placebo, adjusted p = 0.095; AP-Bio® 400 mg versus placebo, adjusted p = 0.053; and AP-Bio® 400 mg versus AP-Bio® 200 mg, adjusted p = 0.943

^{\$} Sample size: AP-Bio® 200 mg, n = 97; AP-Bio® 400 mg, n = 99; Placebo, n = 99

Table 4
Between group analysis of exploratory objective measures.

Time Point		AP-Bio® 200 mg (n = 20)		AP-Bio® 400 mg (n = 20)		Placebo (n = 20)		P value
		Mean	SD	Mean	SD	Mean	SD	
Nasal Mucosal weight (g)	Baseline	2.58	3.24	2.88	3.35	3.00	2.75	0.657
	Day 3	0.32	0.78	0.33	0.63	2.85	3.97	0.040 [^]
	Change from baseline	-2.26	3.07	-2.55	3.11	-0.15	2.87	0.046 ⁺
Tissue Count	Baseline	1.70	2.34	3.20	3.04	3.20	2.59	0.098
	Day 3	0.45	1.05	0.40	0.68	2.15	2.54	0.032 [ⓧ]
	Change from baseline	-1.25	1.92	-2.80	2.80	-1.05	3.19	0.083
Nasal Muco-ciliary time (min)	Baseline	27.10	6.36	26.00	7.55	26.60	7.30	0.571
	Day 3	25.75	5.85	25.45	6.79	25.05	3.71	0.969
	Change from baseline	-1.35	9.65	-0.55	6.86	-1.55	6.07	0.824
Nasal wash IL8 (pg/ml)	Baseline	336.54	665.41	97.21	115.00	170.52	334.75	0.536
	Day 3	258.35	523.31	68.38	87.36	269.92	512.75	0.283
	Change from baseline	-78.19	583.81	-28.83	103.87	99.40	456.56	0.577
Serum hsCRP (mg/L)	Baseline*	3.04	4.65	2.29	2.59	3.13	6.79	0.856 [@]
	Day 3 [#]	2.31	2.17	2.38	3.32	2.48	3.05	0.081 [@]
	Change from baseline	-0.62	3.45	0.13	2.79	-0.70	6.24	0.353 [@]

SD – Standard Deviation; IL – Interleukin; hsCRP – High sensitivity C-Reactive Protein

* n = 100 each in AP-Bio® 200 mg and 400 mg groups and n=99 in placebo group

n=98 each AP-Bio® 200 mg and 400 mg groups and n=100 in placebo group

@ Based on Brown Forsythe ANOVA. The p values of other parameters are based on Kruskal Wallis test

[^] p = 0.522 for AP-Bio® 200 mg versus AP-Bio® 400 mg; p = 0.024 for AP-Bio® 200 mg versus Placebo; p = 0.058 for AP-Bio® 400 mg versus Placebo.

Corrected level of significance p = 0.017

⁺ p = 0.671 for AP-Bio® 200 mg versus AP-Bio® 400 mg; p = 0.064 for AP-Bio® 200 mg versus Placebo; p = 0.015 for AP-Bio® 400 mg versus Placebo.

Corrected level of significance p = 0.017

& p = 0.618 for AP-Bio® 200 mg versus AP-Bio® 400 mg; p = 0.020 AP-Bio® 200 mg versus Placebo; p = 0.043 for AP-Bio® 400 mg versus Placebo. Corrected level of significance p = 0.017

muco-ciliary clearance time, nasal wash IL-8 and serum hsCRP similarly were not significantly different between the three groups on D3.

Within group analysis (S8 Table) demonstrated that there was a significant decrease from baseline in the nasal mucosal weight in both AP-Bio® 200 mg group (MD [SD] = -2.26 (3.07), 95% CI = -3.69, -0.82; p = 0.007) and AP-Bio® 400 mg group (MD [SD] = -2.55 (3.11), 95% CI = -4.00, -1.49; p = 0.001). Similarly, with regards to the tissue count both AP-Bio® 200 mg group (MD [SD] = -1.25 (1.92), 95% CI = -2.15, -0.35; p = 0.009) and AP-Bio® 400 mg group (MD [SD] = -2.80 (2.80), 95% CI = -4.11, -1.49; p = 0.001) showed a significant decrease on Day 3 when compared with baseline. Whereas there was no significant difference from baseline in placebo group for both nasal mucosal weight and tissue count. IL-8 values decreased in both the AP-Bio® groups over time, while on the other hand it increased in the placebo group although it did not achieve statistical difference.

3.7. Adverse events

None of the study participants complained of any major adverse physical symptoms attributable with certainty to the IP. A total of n = 8 participants reported to have dyspeptic symptoms (2 each in each of the IP groups and 4 in the placebo group) that resolved on its own without any intervention [WHO Uppsala Monitoring Centre Causality assessment: Possible]. Also, as stated earlier, one participant in the AP-Bio® 200 mg group withdrew consent due to lack of efficacy (Type-F Adverse drug reaction). On analysis of laboratory data, incident eosinophilia was noted in n = 20 participants on day 3. (n = 6 in AP-Bio® 200 mg group, n = 7 in AP-Bio® 400 mg group and n = 13 in placebo group; p = 0.181). The causality assessment for all these events in the interventional group were adjudicated as possible and for those events in the placebo group were adjudicated as unlikely. (S9 Table) There were no incident dyselektrolytemia and renal or liver injury. The mean (SD) of laboratory parameters in all the three groups post-intervention is summarized in S10 Table and all were within the normal range.

4. Discussion

The present study was conducted to evaluate the efficacy of AP-

Bio®/ KalmCold® a quantified extract from the leaves of *A. paniculata*, in reducing the duration and severity of symptoms in patients with uncomplicated URTI. There was a significant fall in the WURSS-21 score on D3 in both AP-Bio® groups when compared with placebo signifying that AP-Bio® helps decrease the symptom severity at a short duration, as early as D3. Our findings corroborate the results reported by Saxena et al. where there was a significant decrease in the VAS score between placebo and AP-Bio® 200 mg/day group on D5⁴. However, Saxena et al. reported that there was no difference between groups on D3, unlike our findings. This could be attributed to the potential of the virus that is related to the severity and duration of the symptoms. There are more than 200 viruses that are known to cause common cold and the predominant strain causing disease constantly keeps changing over time¹². Our findings are also in line with those studies that report the efficacy of other preparations of *A. paniculata*, who have reported significant decrease in the severity of symptoms especially sore throat and nasal secretions by D4^{13,14}.

The mean scores of WURSS-21 symptom severity at D5 and D7 of all the three groups were similar due to the self-limiting natural course of the illness. Although, the within-group analysis has showed a consistently lower mean scores in both the AP-Bio® groups in comparison to the placebo group during the entire seven day study duration, given the small differences in the effect size favouring AP-Bio® groups over the placebo group, the chosen sample size of n = 100 per group was not likely to have been adequate to prove statistical significance. Further, there was no significant difference between groups with regards to the functional impairment score as common cold is not a severe illness and patients often carry on with their regular routine¹². However, the minimal clinically important difference (MCID) for WURSS-21 is approximately 10⁵ and both AP-Bio® groups at D3 have demonstrated a fall in global score of approximately 10 units when compared with the baseline. We noted a fall in the score in all three groups in the within group analysis at 6 h and it is probably due to the placebo effect.

A similar effect was seen with the VAS scores as well, but the consistently lower score in the AP-Bio® groups when compared to the placebo group suggests that the reduction in the symptom severity score and global score in the AP-Bio® groups, is likely to be more than merely a placebo effect. We also report that all AUC findings, except for the

symptom severity score in AP-Bio® 400 mg group, did not achieve a statistical significance although they have much smaller areas when compared to the placebo. These findings are probably because the AUC curves from all the three groups merged by D5 due to the self-limiting nature of the illness.

The objective exploratory measures were assessed by us as a proof of concept. The stark decrease in tissue counts and nasal mucous weight in the AP-Bio® groups when compared with the placebo group is suggestive of decrease in nasal secretions. On a similar note, IL-8 has decreased in the AP-Bio® groups but on the contrary increased in placebo group. IL-8 has a strong correlation with the nasal domain of acute respiratory infection illness, especially with three specific symptoms namely runny nose, plugged nose, and sneezing¹⁵. It is an important cytokine of human nasal epithelium, readily produced upon virus infection and are high in individuals with acute respiratory infection. IL-8 concentration correlates with symptom severity/global severity as assessed by WURSS-21¹⁵. Thus, the decrease of IL-8 in both treatment groups correlates well with the decrease in symptoms although it did not achieve statistical significance due to a small sample size. The probable effects of AP-Bio on the common cold symptoms could be due to the immunomodulatory potential of *Andrographis* that regulates the innate and adaptive immune responses¹⁶.

AP-Bio® was also generally found to be safe and tolerable. There were no reports of any major physical ailments. However, laboratory tests revealed an incident eosinophilia in some patients even in the placebo group with no significant difference between the groups. This suggests that the eosinophilia is most likely independent of the IP and possibly a consequence of the URTI itself. There are reports that state that eosinophilic airway inflammation is common in acute URTI. This may explain the eosinophilia reported in the placebo group as well.¹⁷

The strengths of our study are that validated scale has been used to determine the efficacy and few objective measures were evaluated in a small subset. Further, the symptom duration prior to randomization was fixed thereby avoiding bias, especially given that the illness is self-limiting. The limitations are that an *a priori* sample size estimation using validated measures was not feasible as there were no studies evaluating the efficacy of AP-Bio® using such measures. However, a post-hoc power calculation using one way ANOVA fixed-effects omnibus model for the calculated effect size of 0.18 based on the D3 baseline adjusted WURSS-21 global score with an alpha error of 5% was determined to be 80%. Yet another limitation was that the objective measures were evaluated only in a small subset thereby posing challenge to prove statistical significance although favourable trends were noted in a few of these measures. Also, since most patients in all the three groups showed marked improvement by D5, more frequent evaluation timepoints in the first 4 days would have given a clear picture in the AUC analysis.

5. Conclusions

AP-Bio® (KalmCold®) is a quantified extract of *A. paniculata* that is intended for decreasing symptom severity of URTI. The intervention groups i.e., AP-Bio® 200 mg and 400 mg groups showed positive tendency towards resolution of symptoms of uncomplicated upper respiratory tract infections when compared with placebo on D3 with no safety concerns. However, this difference was not maintained on D5 and D7 due to the self-limiting nature of the disease and a small sample size not likely to have the adequate power to detect small differences to be picked up in favour of the AP-Bio® groups over the placebo group on those days. With regards to the exploratory objective measures such as the nasal mucous weight, number of tissue papers used, and the nasal wash IL-8 showed a trend towards significant difference in both the AP-Bio® groups when compared to the placebo group but did not achieve statistical significance probably due to a small sample size.

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CRediT authorship contribution statement

Jeffrey Pradeep Raj: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Resources, Software, Validation, Funding acquisition, Writing – original draft, Project administration. **Miteshkumar Maurya:** Data curation, Investigation, Writing – review & editing. **Nayana Nair:** Data curation, Investigation, Writing – review & editing. **Hetal Marfatia:** Data curation, Investigation, Writing – review & editing. **Rujuta Hadaye:** Data curation, Investigation, Writing – review & editing. **Nithya Gogtay:** Methodology, Investigation, Formal analysis, Writing – review & editing, Supervision.

Conflicts of Interest

The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

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Institutional Review Board Statement

The study was conducted in accordance with the principles of Good Clinical Practice that is based on the Declaration of Helsinki. The study also abided by the National Guidelines for Ethical Research in Human Participants (Indian Council of Medical Research Guidelines, 2017) and the New Drugs and Clinical Trials rules (Central Drugs Standard Control Organisation, 2019) of India. The Institutional Ethics Committee (IEC) approval was obtained before the commencement of the study (Reference Number: EC/PHARMA-11/2019) from the IEC-1 of Seth GS Medical College and King Edward Memorial Hospital, Mumbai, India. The study was also registered in the Clinical Trials Registry of India (CTRI/2019/10/021754) before the recruitment of the first participant.

Informed Consent Statement

A written informed consent was obtained from all the participants.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ctim.2023.102934](https://doi.org/10.1016/j.ctim.2023.102934).

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