Study Protocol for Lemon Essential Oil Inhalation in Attention-Deficit/Hyperactivity Disorder (ADHD)

STUDY PROTOCOL

Official Title: Lemon Essential Oil Inhalation Improves Sustained Attention in Children With Attention-Deficit/Hyperactivity Disorder: A Placebo-Controlled Trial

Protocol Version: 1.0

Document Date: June 10, 2021

This document represents the full study protocol, including study design, methodology, and statistical analysis plan.

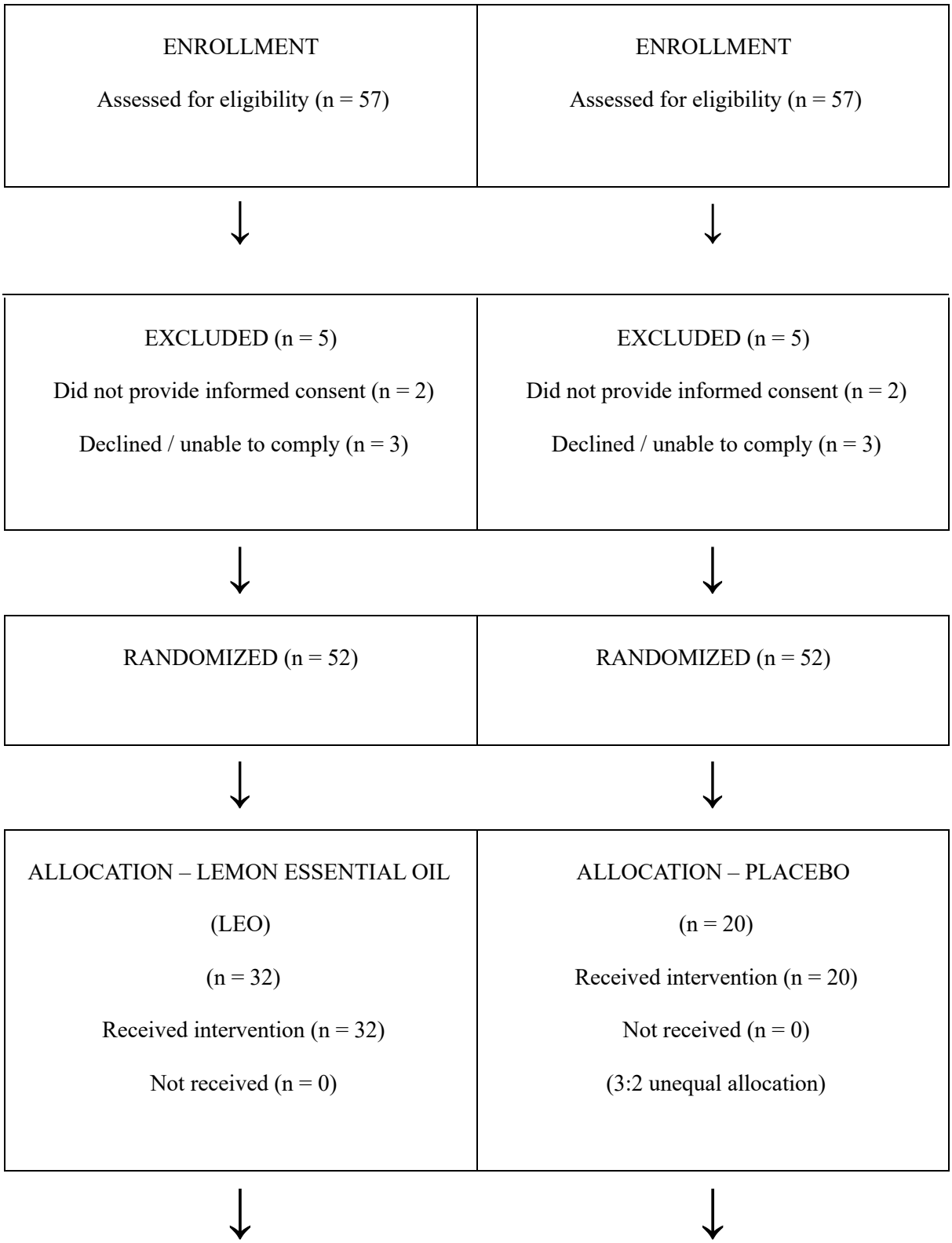
METHODOLOGY

Study Design

This study is a prospective, parallel-group, randomized, placebo-controlled, single-blind clinical trial designed to evaluate the acute effects of Lemon Essential Oil (LEO) inhalation on sustained attention and visual memory in children diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD).

The primary outcome was defined as the change in percentile scores on the D2 Attention Test concentration performance (KZ) from baseline to day 30. The secondary outcome was the change in the number of correct responses on the Benton Visual Memory Test (BVRT). The trial was conducted between February 15, 2022, and September 30, 2023, at the Child Psychiatry and Pediatric Outpatient Clinics of Istanbul Çam ve Sakura City Hospital. All assessments took place in a standardized, low-stimulus environment and adhered to the principles of the Declaration of Helsinki (2013 revision) and CONSORT 2010 reporting standards. The intervention protocol was documented according to the TIDieR checklist.

No protocol amendments were made after initial ethics approval.


ENROLLMENT
Assessed for eligibility (n = 57)
ENROLLMENT
Assessed for eligibility (n = 57)
EXCLUDED (n = 5)
Did not provide informed consent (n = 2)
Declined / unable to comply (n = 3)
EXCLUDED (n = 5)
Did not provide informed consent (n = 2)
Declined / unable to comply (n = 3)
RANDOMIZED (n = 52)
RANDOMIZED (n = 52)
ALLOCATION – LEMON ESSENTIAL OIL
(LEO)
(n = 32)
Received intervention (n = 32)
Not received (n = 0)
ALLOCATION – PLACEBO
(n = 20)
Received intervention (n = 20)
Not received (n = 0)
(3:2 unequal allocation)

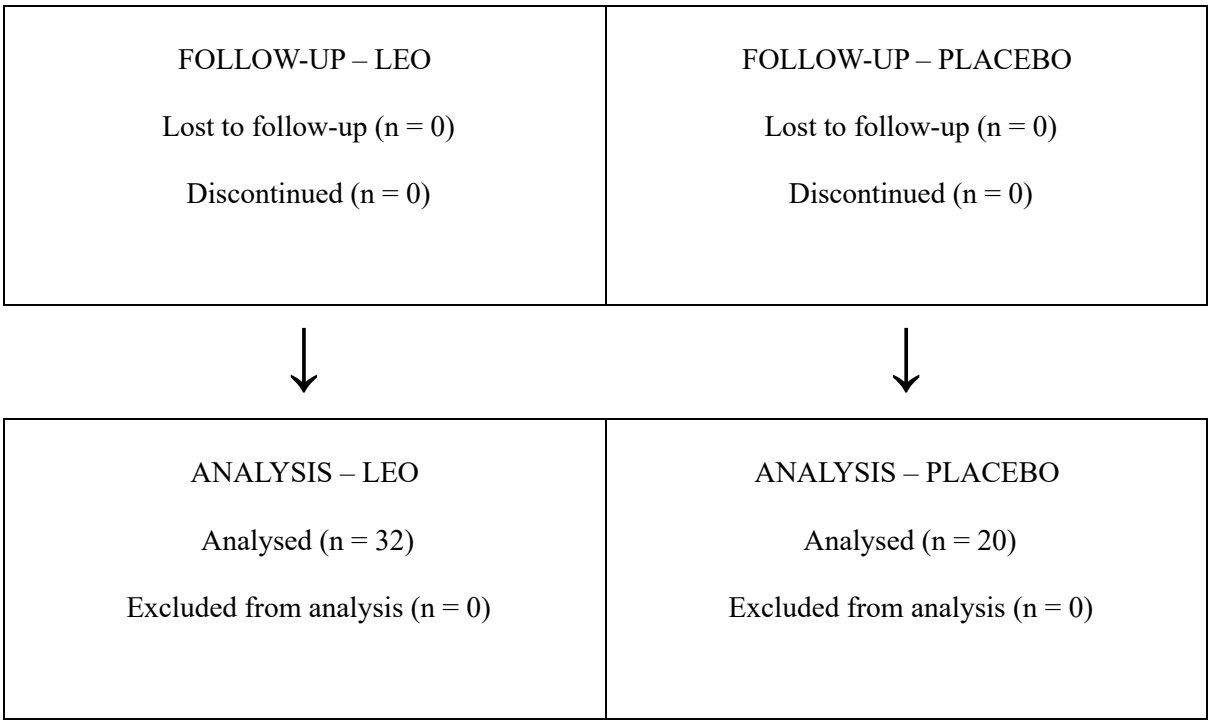


Figure 1. CONSORT 2010 flow diagram of participant enrollment, randomization, follow-up, and analysis.

Participants and Sample Size

A total of 57 children aged 9 to 16 years were screened. Inclusion criteria were: (1) newly diagnosed ADHD according to DSM-5 criteria, (2) medication-naive status, (3) absence of chronic medical or psychiatric comorbidities, and (4) capacity to complete standardized cognitive assessments. Written parental consent and child assent were required.

Five children were excluded due to lack of consent (n=2) or inability to comply with the protocol (n=3), resulting in 52 participants who were randomized into two groups: LEO (n = 32) and placebo (n = 20). All participants were assessed by a board-certified child and adolescent psychiatrist. ADHD severity was classified as mild to moderate, with no indication for pharmacological treatment based on AACAP (2020) and NICE (2019) guidelines.

A priori power analysis using G*Power determined a minimum of 19 participants per group to detect a between-group difference (Cohen's d = 0.88, $\alpha = 0.05$, power = 0.95). The final sample exceeded this requirement, and no dropouts occurred; thus, per-protocol and intention-to-treat analyses were equivalent.

Randomization was stratified by age and sex using a 3:2 allocation ratio (LEO:placebo).

A computer-generated randomization list was prepared by an independent statistician. Allocation concealment was ensured through serially numbered opaque sealed envelopes (SNOSE).

This was a single-blind study: outcome assessors and data analysts were blinded to group allocation. Participant blinding was partially effective due to the distinctive lemon scent.

Application Procedure

All assessments were conducted in a temperature-controlled (approx. 22°C), odor-neutral clinical room. Participants and caregivers avoided scented products 24h before testing. Rooms were ventilated 10 minutes before each session.

Cognitive testing was conducted by the same clinical psychologist trained in standardized protocols. Citrus allergies were screened in all participants beforehand.

Session 1 (Baseline):

• D2 Attention Test

• Benton Visual Memory Test

Session 2 (Day 30):

• LEO group: 5 drops of 100% pure Citrus limon essential oil were placed on a sterile cotton pad, held ~5 cm from the nose for 5 minutes. • Placebo group: Same procedure using distilled water.

All interventions were administered between 09:00–12:00. Dosage, duration, and distance were standardized. Protocol adherence was monitored with a checklist. No deviations occurred.

Participants were monitored for 20 minutes post-inhalation. No adverse effects were observed.

Assessment Tools

D2 Attention Test: Turkish version. Measures sustained attention. KZ score (correct responses minus errors) used.

Benton Visual Memory Test (BVRT): Turkish-validated version. Children view shapes for 10 seconds, then select the match from four options. 15 items in total.

Both tests have established reliability in Turkish populations.

Statistical Analysis Plan

All statistical analyses were performed using SPSS software (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation or median (interquartile range), as appropriate. Categorical variables were presented as frequencies and percentages. Normality assumptions were evaluated prior to selecting parametric or non-parametric tests. Between-group comparisons were performed using independent samples t-test or Mann–Whitney U test, depending on data distribution. Within-group comparisons were conducted using paired t-test or Wilcoxon signed-rank test. To evaluate intervention effects while adjusting for baseline values, analysis of covariance (ANCOVA) was applied. Effect sizes were calculated using Cohen's d. All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant. No missing data were observed; therefore, complete-case analysis was performed.

Ethical Considerations: Approval from Medipol University GETAT Ethics Committee (Decision No: 21, Date: June 10, 2021). Parental consent and child assent were obtained. The study was classified as "minimal risk."