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Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ('CUPID' study): Protocol for a randomised, double-blind, placebo-controlled, crossover, three-arm, proof-of-concept trial.

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Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ('CUPID' study): Protocol for a randomised, double-blind, placebo-controlled, crossover, three-arm, proof-of-concept trial.

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Ethics and dissemination: Human Research Ethics Committee approval has been granted by Bellberry Limited (2021-08-907). Study findings will be disseminated in a peer-reviewed open-access journal and at academic conferences.

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Abstract (258/300 words)

Objective: Insomnia is the most prevalent sleep disorder with few effective pharmacotherapies. Recent preclinical studies, as well as anecdotal evidence in humans, suggests that cannabinol (CBN), a cannabinoid formed through oxidation of delta-9-tetrahydrocannbinol (THC), has potential as an intervention for insomnia disorder. Despite this, the effects of CBN, in isolation, on sleep have yet to be systematically studied in humans. **Methods:** The present randomised, double-blind, placebo-controlled, single-dose, three-arm, crossover, proof-of-concept study investigates CBN effects on sleep and next-day function in twenty participants with clinician diagnosed insomnia disorder and Insomnia Severity Index [ISI] Score ≥15. Participants will receive a single fixed oral liquid dose of 30 mg CBN ('ECS310' 1.5%), 300 mg CBN ('ECS310' 15%), and matched placebo, in random order on three treatment nights each separated by a two-week washout period. Participants will undergo overnight sleep assessment using in-laboratory polysomnography (PSG) and next-day neurobehavioral function tests. The primary outcome of the study is wake after sleep onset (WASO) in minutes measured using PSG, compared to placebo. Secondary outcomes include changes to traditional sleep staging, sleep onset latency, and absolute spectral power during non-rapid eye movement (NREM) sleep. Tertiary outcomes include changes to sleep spindles during NREM sleep, arousal indices, absolute spectral power during rapid eye movement (REM) sleep, and subjective sleep quality. Safety-related and exploratory outcomes include changes to next day driving performance, alertness and reaction time, overnight memory consolidation, subjective drug effects, mood, postural balance, subjective and objective sleepiness, and plasma, urinary, and salivary cannabinoid concentrations. The study will provide novel preliminary data on CBN safety and efficacy in insomnia disorder, which may inform larger clinical trials in the future. ClinicalTrials.gov Identifier: NCT05344170.

Key words: Power Spectral Analysis; Resting Wake Electroencephalography; Psychomotor Vigilance Test; Karolinska Drowsiness Test; Karolinska Sleepiness Scale; Leeds Sleep Evaluation Questionnaire; Standard Deviation of Lateral Position; Liquid chromatography–Mass Spectrometry.

Strengths and limitations of the study

- This is the first clinical study to investigate the effects of CBN isolate on sleep using gold-standard objective measures, validated subjective measures, and rigorous design methodology.
- We will test two active doses (30 and 300 mg CBN) that have been guided by preclinical data and CBN product marketing/consumer testimony.
- Study participants will have clinician-diagnosed insomnia disorder and will undergo extensive screening, including an overnight diagnostic sleep study (PSG), to rule out other sleep disorders that commonly co-occur with insomnia.
- Carry-over effects may occur between study conditions. A recent crossover study demonstrated oral cannabinoids can persist in plasma for >4 weeks (at 1500 mg doses); [1] however, our washout period (2-weeks) was chosen to minimise participant burden (participants cannot undergo insomnia treatment before [3-months] or during [~2 months] the study).
- The study is a single-dose in-laboratory proof-of-concept design. Results may, therefore, lack ecological validity. Repeated CBN dosing may be necessary to effectively treat insomnia.

Background

Insomnia disorder is characterised by unwelcome subjective difficulty falling asleep, maintaining sleep, and/or achieving restorative sleep – where symptoms persist for 3-months and are accompanied by daytime sequalae of dysfunction and/or distress.[2] Insomnia disorder is the most prevalent sleep disorder which affects between 10–30% of adults[3] and persists in 37% of cases at 5-year follow-up.[4] Insomnia disorder increases risk of mental and physical illness, notably depression [5] and dementia [6]. The economic burden of insomnia disorder is estimated at >$13 billion per annum in Australia.[7]

Insomnia disorder treatments are typically behavioral or pharmacological in nature. Cognitive behavioural therapy for insomnia (CBTi) is the first-line treatment, the efficacy of which is confirmed in recent meta-analyses. [8] Nevertheless, significant barriers to CBTi access exist including limited providers, high cost,[9] and delayed perception of subjective benefits, with treatment adherence essential to attain desired outcomes.[10] CBTi may also be less effective in individuals with short sleep duration (i.e., <6 hours/night).[11] As such, sedative-hypnotics are commonly used in primary care.[8] While effective in the short-term, well-documented side effects include daytime sedation, psychomotor impairment, addiction/dependence, and premature mortality.[12] Therefore, there exists a clear need for safe pharmacological insomnia disorder treatments.

Cannabis sativa is a complex plant containing numerous potentially therapeutic chemical compounds, including over 140 constituent '*cannabinoids*'.[13] As historical legal prohibitions against cannabis are relaxed, cannabinoids are increasingly used to aid sleep.[14] The best-researched cannabinoids are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD); however, in recent times, *cannabinol* (CBN) has become recognized as a putative 'sleep-enhancing' cannabinoid.[15] CBN is produced via the nonenzymatic oxidation of THC and is therefore present at higher concentrations in aged (rather than 'young') cannabis plant material.[16] This is significant as aged cannabis plant material is anecdotally reported to induce sleep.[17] CBN products are also being sold as sleep aids in unregulated markets; [18] however, a recent narrative review of CBN highlighted insufficient evidence to support sleep-related claims, despite a plausible mechanism of action described below.

THC and CBN exert some of their physiological effects via interactions with the *endogenous cannabinoid system* (ECS):[13] comprising of signaling molecules termed '*endocannabinoids*' (e.g., anandamide, 2-Arachidonoylglycerol [2-AG]), their receptors, and enzymes.[19, 20] The ECS is involved in regulating myriad biological processes, including circadian rhythmicity[21] and systematic administration of anandamide has been shown to promote sleep in rodents and humans [19, 22, 23] via activation of the cannabinoid receptor 1 (CB_1R).[22, 24] As such, the CB_1R is considered a promising therapeutic target.[25] The pharmacological effects of THC occur via partial agonism at CB_1R[26] and can include intoxication, sedation and changes in sleep architecture.[19]. CBN also has agonist actions at CB_1R, albeit approximately 10-times less potent than THC[26], which may potentially account for the low levels of intoxication reported in historic CBN studies. This lack of intoxication potentially renders CBN a more practical and safer alternative to THC as a sleep aid.[27]

Recent preclinical (unpublished) research generated by the Lambert Initiative for Cannabinoid Therapeutics has investigated the effects of CBN isolate on sleep. Long-Evans rats with implanted electrodes were administered CBN (10, 30, and 300 mg/kg intraperitoneally) or a positive control (Zolpidem). CBN (10 mg/kg) initially decreased rapid eye movement (REM) sleep quantity; however, 4 hours after administration, a significant increase in the percentage of REM and non-REM (NREM) sleep occurred. In a follow-up chronic dosing study, the delayed effects on sleep followed a similar

pattern to plasma concentrations of CBN metabolites (11-OH-CBN and 11-COOH-CBN) which target CB_1R with greater affinity than CBN.[28] These delayed effects suggest that CBN and its metabolites could reduce wake after sleep onset (WASO).

Two recent clinical studies have administered small quantities of CBN as a component of multi-cannabinoid formulations.[29, 30] A crossover randomized controlled trial (RCT), with a one-week washout, investigated two-weeks of an oral liquid containing 20 mg THC, 2 mg CBN, and 1 mg CBD in 20 adults with insomnia disorder. The drug significantly improved insomnia disorder symptoms (Insomnia Severity Index [ISI] -5.1 points, p=0.0001, d=0.94) compared to placebo, with no significant changes to polysomnography (PSG).[29] In an interventional open-label study, current medicinal cannabis users with self-report sleep difficulty reported improved sleep (assessed via an unvalidated subjective survey and a validated non-contact at-home tracking device) after three weeks of nightly administration of an oral capsule containing 10 mg THC and 5 mg CBN.[30] The effects of CBN cannot be deconvoluted from those of THC in these studies. We are not aware of any studies to-date that have tested the effects of CBN isolate on objectively measured human sleep.

Study design and aim

Using a randomised, double-blind, placebo-controlled, three-arm, crossover, single-site, proof-of-concept study design, we will investigate the acute effects of oral dose CBN on sleep and next-day function in 20 participants with clinician-diagnosed insomnia disorder. The primary study aim is to investigate the effects of CBN (30 and 300 mg) versus placebo on sleep in insomnia disorder. The study will indicate the safety and feasibility of CBN as a pharmacological therapy and generate preliminary data to inform larger clinical trials. Many outcome measures used in insomnia disorder clinical trials are known to be susceptible to placebo effects. A placebo control is scientifically and ethically defensible choice as effects of CBN on human sleep are unknown and could not be properly elucidated if an active treatment such as Zolpidem was the comparator rather than placebo.

Study outcomes

The primary study outcome is WASO minutes measured using in-laboratory overnight PSG from lights out until the last epoch scored as any stage of sleep by an experienced polysomnographic technician in accordance with American Academy of Sleep Medicine (AASM) 2015 Sleep Scoring criteria (Version 2.2).[31] Additional study outcomes are as follows (see ClinicalTrials.gov Identifier: NCT05344170):

1. Secondary outcomes include changes to sleep microarchitecture, sleep onset latency (SOL) minutes, and absolute spectral power during NREM sleep, measured using in-laboratory PSG.
2. Tertiary outcomes include sleep spindle events during NREM sleep, arousal indices, absolute spectral power during REM sleep, and subjective sleep quality.
3. Safety outcomes include assessment of next day neurobehavioral function (subjective drug effects, mood, memory, alertness, resting electroencephalography (EEG) power after sleep, reaction time, simulated driving performance, and postural sway).
4. Exploratory outcomes will include plasma, urinary, and cannabinoid concentrations, as well resting EEG power before sleep.

Safety

The investigational product, CBN, is currently a Schedule 9 'Prohibited Substance' in Australia.[32] The safety profile of CBN can be inferred from a number of human studies conducted between the 1970-90's. These studies explored oral dose ranges up to 1200 mg CBN isolate and some involved repeated daily dosing with CBD for up to 4 weeks.[33-36] There were no notable safety concerns, aberrations or toxicity concerns from any of these studies, with no adverse changes in parameters such as heart rate, blood pressure, body temperature, respiratory rate, perception, intoxication and postural stability.[33-36] None of the studies reported effects on sleep or sedation, and residual next-day effects of CBN (such as adverse effects on driving performance and cognitive function) are also unknown and will be explored in the current study. CBN is currently being investigated as an analgesic agent (ClinicalTrials.gov Identifier NCT03675971) and topical treatment for epidermolysis bullosa (ClinicalTrials.gov Identifier: NCT04908215) in ongoing clinical trials. We will monitor and review safety reports from these trials as they become available.

Methods

Participants, interventions, and outcomes

Trial setting

The trial Sponsor and site is the Woolcock Institute of Medical Research, a specialist sleep and respiratory research institute and clinic (Sydney, Australia). The study funder is the Lambert Initiative for Cannabinoid Therapeutics, University of Sydney (Sydney, Australia), a philanthropically funded centre for cannabinoid science. Recruitment commenced in August 2022 and is planned to continue for 1.5 years. The first participant was randomised on the 13th of October 2022. The study received Bellberry Limited Human Research Ethics Committee (2021-08-907; Version 2.1, 01-Aug-2022) approval and is registered on ClinicalTrials.gov (Identifier: NCT05344170; 25-April-2022). The clinical trial protocol is available from the first author on request. Significant changes to the study protocol will be documented on ClinicalTrials.gov. The study protocol was developed according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement.[37] The Therapeutics Goods Administration (TGA) has acknowledged the use of CBN in this study through the Clinical Trial Notification (CTN) scheme (CTN Number: C T-2022-CTN-00543-1). The study investigators have led the design and conduct of this trial, will conduct the analyses, and make all publication decisions.

Participant eligibility

Eligible participants will be aged between 25-65 years with physician diagnosed insomnia disorder as per the International Classification of Sleep Disorders-3 (ICSD-3)[38] and Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[39] criteria as determined by the study physician (see Box 1). The age range was selected to minimise age-related changes to sleep architecture.[40] As cannabinoids have been shown to improve symptoms (e.g., anxiety, pain) that commonly impair sleep (i.e., causing 'secondary' insomnia disorder),[41] a 'primary' insomnia disorder population is being recruited for this study.

Box 1

Inclusion and exclusion criteria for study participants

Eligible individuals must fulfill the following criteria:

1. Between 25 – 65 years of age
2. Insomnia Severity Index (ISI) score ≥ 15 at the point of eligibility screening
3. Insomnia disorder (symptoms occurring at least 3 times per week and present for longer than 3 months) as determined by the study physician
4. Ability to take oral medication
5. Provision of signed and dated informed consent form
6. Stated willingness to comply with all study procedures and availability for the duration of the study

Individuals who meet any of the following criteria will be ineligible to participate in the study:

1. Medical condition or medication that is the cause of the insomnia disorder as determined by the study physician
2. Known hypersensitivity to cannabis or cannabinoid products (including if this becomes evident during the trial)
3. Reported use of cannabis or cannabinoid products within the past 3 months as confirmed by at least one negative urine drug screen (UDS) (or at the study physician's discretion)
4. Sleep apnoea (defined as Apnoea Hypopnea Index [AHI] >15 and Oxygen Desaturation Index [ODI]>10) as confirmed by polysomnography (PSG) at screening
5. Sleep-related movement disorder as determined by the study physician
6. Delayed or advanced sleep phase syndrome (based on actigraphy and sleep diary) as confirmed screening
7. Any medical condition that produces an abnormal EEG (i.e., epilepsy, brain injury)
8. Clinically relevant cardiovascular abnormalities as determined by the study physician and a 12-lead electrocardiogram (ECG) at screening
9. Shift work or trans meridian travel (two time zones) within the last month
10. History of major psychiatric disorder in the past 12 months at the study physician's discretion, except clinically managed mild depression and/or anxiety
11. History of suicide attempt or current suicide ideation (score greater than 1 on Q9 of the Patient Health Questionnaire [PHQ-9])[42]
12. Pregnancy or lactating. All female volunteers will be required to complete a urine pregnancy test at screening and instructed to use a reliable form of contraception while participating in the project
13. History of drug or alcohol dependency or abuse within approximately the past 2 years
14. Use of CNS-active drugs (cannabis, amphetamines, cocaine, antidepressants, opioids, benzodiazepines) in the past 3 months as confirmed by a positive urine drug test at screening or at the study physician's discretion
15. Use of medications that may have a clinically significant impact upon the metabolism and excretion of cannabinoids as determined by the study physician (e.g., CYP450 enzyme inducers/inhibitors
16. Excessive caffeine use that in the opinion of the study physician contributes to the participant's insomnia disorder, or the inability to abstain from caffeine use 24 hours prior to each overnight sleep study
17. Inability to refrain from alcohol consumption 24 hours prior to each overnight sleep study
18. Individuals with nicotine dependence (i.e., daily smokers)
19. Medical conditions that result in frequent need to get out of bed (e.g., sleep walking, nocturia)

20. Psychological or behavioural treatment for insomnia disorder, including cognitive behavioural therapy for insomnia, within 3 months before screening (excluding sleep hygiene advice)
21. Occupational or judicially ordered drug screening
22. Has held an unrestricted driving license <1 year
23. Cannot speak English fluently

Patient and Public Involvement

Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Intervention

Study doses

Participants will receive a single fixed 2 mL oral dose of: (1) 30 mg CBN ('ECS310' 1.5% containing 15 mg/mL CBN); (2) 300 mg CBN ('ECS310' 15% containing 150 mg/mL CBN); and (3) matched placebo across three separate overnight Treatment Sessions, in randomised order, two-hours prior to habitual sleep time. The lower dose was selected to match the CBN products available in the United States and supported by customer testimonies.[18] The higher dose was informed by unpublished preclinical research generated by the Lambert Initiative for Cannabinoid Therapeutics using interspecies scaling calculations.

Investigational product

The investigational drug ('ECS310') is a liquid formulation of CBN suspended in medium chain triglycerides (MCT) oil. The placebo contains excipient MCT oil only. The maximum detectable THC content of the active investigational products will be ≤ 150 µg. Neither of the study drugs nor matched placebo will contain any other cannabinoids, minor cannabinoids or terpenes/terpenoids. The study drug and matched placebo are expected to be identical in their visual appearance, taste, and smell. The MCT oil contains paprika oleoresin colorant to ensure the double-blind is maintained. The Sponsor purchased the investigational product from BOD Australia, who sourced investigational products from Medropharm, Switzerland.

Recruitment and retention

We aim to recruit 20 participants over an 18-month period. The sample size has been determined arbitrarily as the trial is of pilot nature. Participants will be recruited from the Woolcock Institute Volunteer Database, social media, and locally displayed physical study advertisements. Specialised participant recruitment services may be used where necessary. Participants will be reimbursed a fixed amount equivalent to that used in previous clinical trials with similar participant involvement and loss of working hours.[43] To encourage retention, participants will receive reimbursement upon completion of the final Treatment Session. Study advertisements will include an embedded link and QR code to the study website (described below).

Eligibility screening

Eligibility assessment will be performed in three steps undertaken by the study team at the Woolcock Institute of Medical Research (see Figure 1 *Study Flow Diagram*):

1. *On-line and telephone pre-screening*. Interested individuals will be directed to the study webpage. They will provide electronic consent before completing an online pre-screening questionnaire to assess initial eligibility (e.g., age, location, ISI score, trans meridian travel, shift work). Potentially suitable individuals will be invited to provide contact details and

provided with the Participant Information Statement so that the trial coordinator may conduct a brief telephone interview before scheduling screening appointments. Individuals deemed unsuitable will be provided with support resources.

2. *Screening visit 1*. Willing and potentially suitable participants will attend an on-site medical screening. The study physician will obtain written informed consent before conducting a screening interview to compile medical history, assess suitability against study eligibility criteria (Box 1), and diagnose insomnia disorder (ICSD-3[38] and DSM-V[39]). The following validated self-report questionnaires will be used to evaluate eligibility: ISI,[44] a measure of insomnia disorder symptom severity over the past two-weeks; Epworth Sleepiness Scale (ESS),[45] a measure of sleep propensity in daily life; Hospital Anxiety and Depression Scale (HADS),[46] a measure of anxiety and depression; and, the Patient Health Questionnaire-9 (PHQ-9),[42] an instrument for screening and monitoring the severity of depression and suicidal ideation. An electrocardiogram (ECG) and urinary screen (pregnancy and recent alcohol, cannabis, cocaine, benzodiazepines, opiates, or amphetamines, use) will also be conducted.
3. *Screening visit 2*. Participants will be required to complete a clinical overnight PSG sleep study to rule out the presence of other sleep disorders (obstructive sleep apnoea or sleep-related movement disorder, see Box 1). Diagnostic PSG results within the previous 12-months may be used where weight has not changed substantially in that time (at discretion of the Study). Diagnostic sleep studies will be scored by an experienced sleep technician[31] and reviewed by the study physician.

Insert Figure 1 here

Figure 1. *Study flow diagram*

Randomisation, allocation concealment, and blinding

Participants are being allocated to one of six possible treatment orders in a 1:1:1:1:1:1 ratio using a pre-populated randomisation schedule generated by the study epidemiologist (NSM). The six-orders will constitute a balanced Latin square. Sequences will be computer-generated and stored in a password-protected system inaccessible to blinded study personnel (centralised computerised randomisation). Allocation concealment will be managed by the unblinded study epidemiologist and an independent staff member who will not have any contact with participants, nor involvement in day-to-day trial activities. A study physician may request the study epidemiologist be unblinded in the event of a medical emergency. Participants who are enrolled in the study, but not yet randomised, will be assigned a unique screening number. Randomisation will occur at the outset of the first Treatment Session assuming eligibility persists. Working under the study physician, the trial coordinator will record the decision to randomise the patient. The study database will then issue an irrevocable randomisation number for that participant. Investigational product is stored in opaque, sequentially numbered containers labelled with the patient randomisation number and dose to be administered at each Treatment Session to maintain blinding of all patients, staff, and outcome assessors. The drug manufacturer packaged and labelled the investigational product according to the randomisation list generated by the study epidemiologist.

Pre-Treatment Session procedures

Participants are asked to maintain a regular sleep-wake schedule for one week prior to each Treatment Session. To confirm this, participants wear a wrist-worn actigraphy monitor (GENEActive, Activinsights, United Kingdom) and complete a daily sleep diary (Karolinska Sleep Diary [KSS])[47]

throughout this period. Participants are required to abstain from consuming cannabis, cannabinoids, and other drugs throughout the entire study; and avoid alcohol and caffeine 24 hours prior to Treatment Sessions. Urinary drug, alcohol (DrugCheck NxStep OnSite, USA) and pregnancy (SureStep, Germany) screening occurs at the outset of each Treatment Session.

Treatment Session procedures

Participants arrive at the Woolcock Sleep Clinic in the early evening (~17:00-18:00, depending on habitual lights out), where they remain until late morning (~11:00) the following day, as per the Treatment Session schedule in Figure 2. Standardised meals are provided. All Treatment Sessions are separated by a washout period of approximately 2-weeks, to mitigate carry-over effects between doses.[1]

Study drug administration

After a brief medical examination, the study physician prescribes and dispenses the study drug, drawing a fixed 2 mL volume of solution into a pre-labelled amber plastic syringe. Participants are administered the randomly allocated study drug approximately 2-hours prior to habitual bedtime. The trial coordinator observes participants self-administer the study drug to ensure accuracy of dosing. The dose is documented in participant records. Timing of administration has been informed by delayed onset of effects observed with oral dosing of other cannabinoids[48] and the abovementioned preclinical study of CBN.

Data collection

Study measures are described below, in order of assessment (see Table 1).

Table 1. *A summary of assessments completed throughout the investigation*

Measure (ordered first to last)	Screening	Treatment Session 1-3		
		At-home sleep monitoring	Night	Day
Informed consent	●			
Medical examination	●		●	
Electrocardiogram	●			
Baseline questionnaires (ISI, ESS, HADS & PHQ-9)	●			
Urinary drug and alcohol screen	●		●	
Pregnancy test	●		●	
Overnight PSG (clinical EEG)	●		●	
Actigraphy		●		
Sleep diary (KSD)		●		
Oral fluid drug screen (Securetech DrugWipe)			●	●
Quantisal saliva collection			●	●
Blood plasma collection			●	●
Urine collection			●	●
Mood (POMS)			●	●
Drug effects (DEQ)			●	●
Balance (posturography)			●	●
Memory (FTT & WPT)			●	●
KDT with EEG			●	●
Sleep questionnaires (LSEQ, RCSQ, & KSS)				●
Simulated driving performance				●
PVT				●

Note. DEQ=Drug Effects Questionnaire; EEG=electroencephalography; ESS=Epworth Sleepiness Scale; ISI=Insomnia Severity Index; HADS=Hospital Anxiety Depression Scale; KSD=Karolinska Sleep Diary; FTT=Finger Tapping Task; LSEQ= Leeds Sleep Evaluation Questionnaire; KSS, Karolinska Sleepiness Scale; PHQ-9=Patient Health Questionnaire-9; PVT=Psychomotor Vigilance Task; RCSQ=Richard Campbell Sleep Questionnaire; WPT=Word Pairs Task.

Insert Figure 2 here

Figure 2. *Schedule of events during each Treatment Session.* CBN = Cannabinol; DEQ=Drug Effects Questionnaire; EEG=electroencephalography; ESS=Epworth Sleepiness Scale; ISI=Insomnia Severity Index; HADS=Hospital Anxiety Depression Scale; KSD=Karolinska Sleep Diary; FTT=Finger Tapping Task; LSEQ=Leeds Sleep Evaluation Questionnaire; KSS=Karolinska Sleepiness Scale; PHQ-9=Patient Health Questionnaire-9; POMS= Profile of Mood States; POST=Posturography; PSG=Polysomnography; PVT=Psychomotor Vigilance Task; UDS=Urinary Drug Screen; WPT=Word Pairs Task.

Blood collection and plasma cannabinoid levels

Venous blood samples will be taken at baseline (~17:00 hours) and upon wake (~08:45 hours immediately after the simulated driving task). Samples are drawn into EDTA vacutainers (Becton, Dickinson and Company, New Jersey, USA) and centrifuged (2500×g for 10 min at 4°C). Supernatant plasma is aliquoted into 1.5 mL Eppendorf Tubes and stored at -80ºC in the Woolcock Institute of Medical Research until study completion when samples will be transferred to the Lambert Initiative Laboratory. Samples will undergo liquid chromatography–mass spectrometry (LC–MS/MS) analysis, using previously published methodology.[49, 50] Plasma will be analysed for cannabinoids (e.g., CBN, THC, CBD), their metabolites (e.g., 11-OH-CBN, 11-COOH-CBN, 11-OH-THC, THC-COOH), endocannabinoids and related molecules (e.g., 2-AG and anandamide). We are testing for other cannabinoids and metabolites to verify abstinence from other cannabinoids and because the investigational product may contain minuscule quantities of THC (≤ 150 µg).

Urine collection and urinary cannabinoid levels

Urine collection occurs at baseline, 2-hours post administration (~22:00 hours), upon wake (~07:00 hours), and prior to participant discharge (11:00 hours). Urine (~6 mL) is aliquoted into 1.5 mL Eppendorf Tubes and stored at -80ºC before being transferred to the Lambert Initiative Laboratory for quantification of cannabinoid, cannabinoid metabolites, and endocannabinoids at study completion. LC–MS/MS analyses will be conducted using similar methodology to plasma.[49, 50] It is important to conduct urinary analyses as the pharmacokinetic profile of CBN is not well described.

Salivary drug testing

Oral fluid testing occurs at baseline, 30-minutes post administration, as well as immediately after the driving simulator task (~08:45 hours) using the DrugWipe® 5S (DW5s; Securetec, Neubiberg, Germany) and Quantisal™ collection device (Immunalysis, Pomona, CA). The DW5s collects oral fluid (approximately 10–20 µL) from the tongue. The DW5s is commonly used in routine roadside drug-tests; however, demonstrates variable sensitivity (22-89%), specificity (50-100%), and accuracy (53-94%).[51] While ECS310 contains negligible quantities of THC, similarities in chemical structure between CBN and THC may conceivably lead to false-positive test results thay would be a concern should CBN become widely used by patients.[52] As such, the Quantisal™ device is used to collect oral fluid from participants (~1 ml) which will be used in LC–MS/MS quantification of cannabinoid (CBN and THC) and metabolite concentrations. Samples will be placed in a stabilising buffer and stored at 4ºC before being transferred to the Lambert Initiative Laboratory for analysis. Analyses will occur within three-months of collection.

Cognitive and psychomotor performance

Overnight declarative and procedural memory consolidation is assessed at each Treatment Session using the Word Pairs Task [WPT] and Finger Tapping Task [FTT], respectively. For both tasks, memory encoding during a learning phase occurs prior to drug administration (19:30 hours) with next-day retest occurring at approximately 09:00 hours. As THC can acutely impair cognitive function and memory (with prolonged use), it is necessary to examine CBN effects in this regard. Contrary to oral dosing studies, in one study, CBN reportedly caused a mild intoxication when administered intravenously at high doses (1.2mg/min)[53]. To investigate intoxication induced bodily sway, postural balance is being measured using the stabilometric balance platform (Advanced Medical Technologies Inc., AccuSwayPLUS Balance platform, MA, USA), which measures centre of pressure in anterior-posterior (y-axis) and medial-lateral (x-axis) directions using four strain gauge load cells at the bottom of the device. Centre of pressure will be assessed in eyes open and eyes closed conditions. Postural balance assessment takes less than 5 minutes to complete and will be repeated 3 times (i.e., baseline, 60-minutes post drug administration, and next-day ~09:30 hours). The psychomotor vigilance test (PVT) is a commonly used reaction time based measure of attention and fatigue related changes in alertness[54] involving a hand-held box with a red-light emitting diode display of a three-digit millisecond counter (PVT-192, Ambulatory Monitoring Inc, Adsley, NY, USA). During the 10-minute task, participants are instructed to respond as quickly as possible to stimuli appearing at variable intervals (ranging 2-10 seconds). The PVT will be repeated twice during next-day testing (09:30 and 10:30 hours).

Self-report questionnaires

Mood is being evaluated via the Profile of Mood States (POMS; 37-items with 5-point Likert scales)[55] questionnaire, a commonly used rating scale of transient and distinct moods. The Drug Effects Questionnaire (DEQ; 7-items with unipolar 100 mm visual analogue scales [VAS])[56] is used to evaluate subjective drug effect; including strength of drug effect, feeling stoned ('high'), liking/disliking the drug effect, feeling sedated, and feeling anxious. The POMS and DEQ are completed at baseline, 60-minutes post drug administration (21:00 hours), and the next day at 09:30 and 10:30 hours. The Leeds Sleep Evaluation Questionnaire (LSEQ; 10-items with 100 mm VAS)[57] is a validated measure of the previous night's sleep quality (sleep onset, maintenance, and quality; including behaviour following waking) in comparison to usual. The Richard Campbell Sleep Questionnaire (RCSQ; 5-item with 100 mm VAS)[58] is used to measure perceived sleep depth, sleep latency (time to fall asleep), number of awakenings, and sleep efficiency and quality. The LSEQ and RCSQ are administered immediately after waking (~07:00 hours).

Subjective and objective sleepiness

Karolinska Sleepiness Scale (KSS) and Karolinska Drowsiness Test (KDT) are used to measure subjective and objective sleepiness, respectively.[59] The KSS is a single-item rating scale assessing current sleepiness, ranging from 1 (*Extremely alert*) to 9 (*Extremely sleepy – fighting sleep)*. KSS scores will be compared to the objective drowsiness ascertained using the KDT. During the KDT, resting awake EEG is measured while participants focus on a wall marker, with eyes open and closed across 2-minute intervals. The KSS and KDT are administered immediately prior to sleep (~23:00 hours) and upon wake (~07:00 hours).

Polysomnography

Sleep is assessed using PSG (Grael PSG, Compumedics, USA), a multiparametric tool used for monitoring and diagnosing sleep disorders. PSG consists of 18 channels recording biophysical changes that occur during sleep, including brain activity (via EEG); muscle activity or skeletal muscle activation (electromyography [EMG]); eye movement (electrooculogram [EOG]); cardiac function (ECG);

respiratory effort using chest and abdominal belts; and digital pulse oximetry. To improve participant comfort/retention, pulse oximetry and the nasal cannula and thermistor will only be used to detect sleep disordered breathing during screening (Visit 2). PSG equipment will be fitted by an experienced sleep technician prior to sleep and will be removed the next morning once the KDT has been completed. Sleep and associated events will be scored according to AASM 2015 criteria in 30 second epochs.[31] Each patient's three polysomnograms will be scored by the same sleep technician.

Simulated driving performance

Next-day driving performance is measured at approximately 08:00 hours using a fixed-based driving simulator (Hyperdrive, Adelaide, Australia) equipped with standard vehicle controls (described elsewhere.[1, 43]) and using custom-built scenario developed using the SCANeR Studio Simulation Engine (version 2022.2 r25, AVSimulation, Paris, France). The 30-minute driving scenario incorporates three independent epochs: (1) a 'car following' drive (~7 minutes duration) that has previously demonstrated sensitivity to THC-induced impairment;[60] (2) a 'highway' drive (~17 minutes duration); and (3) a novel divided-attention drive (~6 minutes duration). The 6-minute divided-attention task employed in epoch 3 has been previously validated,[61] and is designed to replicate a casual mobile-phone conversation that does not require mental rehearsal or recall intervals of greater than 3 seconds. During the divided-attention task, pre-recorded five-word sentences will be played every 10 seconds (through a hands-free speaker). Participants immediately indicate whether the sentence was sensical (e.g., "*the truck delivered the package*") or non-sensical (e.g., "*the octopus burned the onions*") in nature; and 7 seconds later recall the last word in the sentence. The main outcome measure for all epochs of the driving task is standard deviation of lateral position (SDLP). This is a widely used measure of vehicular control that is sensitive to drug effects, with previous reports showing a THC dose-dependent increase in SDLP (higher represents more erratic driving), particularly in occasional cannabis users.[60, 62] Secondary outcomes for each driving epoch include: (epoch 1) average and standard deviation of car-following headway (i.e., following distance) and speed coherence (i.e., correlation between speed of lead and following car); (epochs 2 and 3) average speed, minimum and maximum speed, and standard deviation of speed (SDSP). The driving scenarios were programmed by investigator C.I.

Adverse events

Safety outcome measures are described above. Blood pressure is measured four times during each Treatment Session (~17:00, 18:30, 21:00, and 09:30 hours). Adverse event collection occurs via standardised interview at three timepoints (prior to discharge, and 7-hours and 7-days post-discharge). Passive adverse event collection occurs throughout the study protocol via open-ended interview and is recorded on paper form. A study physician will assess the severity and causality of each adverse event.

Post-Treatment Session care

Participants will be discharged from the research facility at approximately 11:00 hours and then contacted by the study co-ordinator that evening (18:00 hours), and one-week after each Treatment Session, to assess wellbeing and record any adverse events (including changes to sleep). Upon study completion, if deemed necessary in the study physician's opinion, a clinical follow-up will be arranged for participants on an individual basis.

Data management

Any information obtained for the purpose of this research that could identify participants will be treated as confidential and securely stored adhering to guidelines of the Sponsor, HREC, NHMRC National Statement on Ethical Conduct in Human Research (2007) and Note for Guidance on Good Clinical

Practice (CPMP/ICH-135/95). All onboarding, consent and questionnaire data will be captured through SPARDAC™ (Single Page Application - Research Data Capture) developed by Wappsystem Pty Ltd and hosted on Amazon Web Services in Sydney, NSW. Participant data will be identified by unique codes. The code linking participant data to identity (e.g., name, date of birth, contact details) will be stored securely with password protection, and will not be accessible via the internet. Paper files will be stored in locked storage cabinets on-site. Digital participant data will be stored on a secure electronic data system, that is regularly backed up with disaster recovery features. All data will be stored securely for at least 15 years. Only study investigators will have access to participant data. Data monitoring occurs on a regular basis by the study investigators. Data integrity is being enforced through a variety of mechanisms including data rules, range checks, and consistency checks against data already stored in the database. Written documentation of changes will be available through electronic logs and audit trails. Study safety will also be internally monitored and evaluated regularly, with adverse events documented and reported according to Sponsor and HREC requirements. The decision to terminate the study lies with the principal investigators and will be based on the recruitment target and safety data. The PIs and trial coordinator will monitor data integrity throughout the trial.

Trial management structure

This is a pilot study and does not require an intricate management structure. The advisory committee provide advice or complete discrete tasks (Table 2). The management of day-to-day trial activities is overseen by the principal investigators and the trial coordinator (IL, CH, & BY). This trial does not have an independent data safety monitoring committee because it is a single-site single-night small sample study with a medication that is thought to have a low side-effect profile in a group of patients that are known not to be at high risk of life-threatening events.[63]

Roles and responsibilities

Table 2. Investigator roles and responsibilities

Investigator	**Role**
Dr Camilla Hoyos	Principal Non-Medical Investigator
Professor Brendon Yee	Principal Medical Investigator
Professor Iain McGregor	Advisory committee member (Pharmacologist and Cannabinoid Expert)
Professor Ronald Grunstein	Advisory committee member (Sleep Physician)
Professor Bandana Saini	Advisory committee (Study Pharmacist)
Associate Professor Nathaniel Marshall	Advisory committee (Study Statistician/Epidemiologist)
Associate Professor Christopher Gordon	Advisory committee member (Registered Nurse and Sleep Disorder Expert)
Dr Angela D'Rozario	Advisory committee member (Sleep Neurobiology Expert/Consultant)
Dr Danielle McCartney	Driving Simulator and Cannabinoid Expert
Dr Chris Irwin	Advisory committee member (Driving Simulation Expert)
Ms Anastasia Suraev	Advisory committee member (Cannabinoid and Sleep Expert)

Ms Isobel Lavender	Clinical Trial Coordinator

Statistical analyses

Statistical analyses will be completed using a commercial statistical package (e.g., SPSS, SAS, etc). Outcome data will be analysed using Linear Mixed Models due to expected inter-participant variability with repeated measures and handle missing data. Where baseline (i.e., 'pre-drug') data exist for the outcome being analysed, those scores will also be included in the model as a covariate. Participants will be random factors. Fixed factors will be treatment (placebo, 30 mg and 300 mg CBN) and the arm of the study. Analysis will be by intention to treat. Difference in least squared means inside the treatment main effect will only be investigated for the hypotheses that the two active treatments are superior to placebo. We will not present a p-value comparison for the two active doses. The critical p-values for each of these hypotheses is set at 0.05 (two-tailed). Effect sizes will be calculated as partial eta squared (η_p^2), Cohen's d, and Hedges' g, where appropriate. The statistical code used to test the primary and 3 secondary outcomes will be written and published on a publicly available website before the completion of data collection.[64] We will not undertake any interim or stratified analyses.

Dissemination policy and access to data

Study findings will be disseminated via scientific peer-reviewed publications, conferences, and media, as applicable. Information will be provided such that participants cannot be identified. Non-identifiable Individual Participant Data (IPD) will become available one year after study completion and will be available upon reasonable request to the principal investigators.

Author contributions

IL, AS, DM, CI, AD, CG, NSM, ISM, RRG, BY, and CMH were involved in methodological design and creation of the study protocol. BY and CMH are the principal investigators (medical and non-medical, respectively) who have overall responsibility for the design, conduct and decision to submit for publication. NSM is the study epidemiologist and statistician who will design and publish the analysis plan in collaboration with IL. IL is the trial coordinator responsible for collecting trial data. IL drafted the manuscript. Investigator roles and responsibilities are outlined in Table 1. All authors have read and approved the final manuscript.

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Competing interest statement

ISM is the Academic Director of the Lambert Initiative for Cannabinoid Therapeutics, University of Sydney. ISM is a consultant for Kinoxis Therapeutics, Psylo Ltd, and Emyria and is an inventor on several patents relating to novel cannabinoid and non-cannabinoid therapeutics unrelated to insomnia. He has received consulting fees from the Medicinal Cannabis Industry Australia (MCIA) and acts as an expert witness in legal cases involving cannabis-related issues. AS has received consulting fees from the Medicinal Cannabis Industry Australia (MCIA) and Haleon. RRG has received discounted investigational products for an unrelated clinical trial from Neurim Pharmaceuticals Inc and received investigational product and matched placebo from Teva Pharmaceutical in unrelated clinical trials. He has received funding for lectures for Pfizer, Teva, Jazz and Eisai in the past 3 years. The other authors have no competing interests to disclose. The Woolcock Institute Sleep and Chronobiology Research Group has received research support from Avadel, Nyxoah, Idorsa, ResMed, BOD Australia, and Philips.

Patient consent for publication

Not required.

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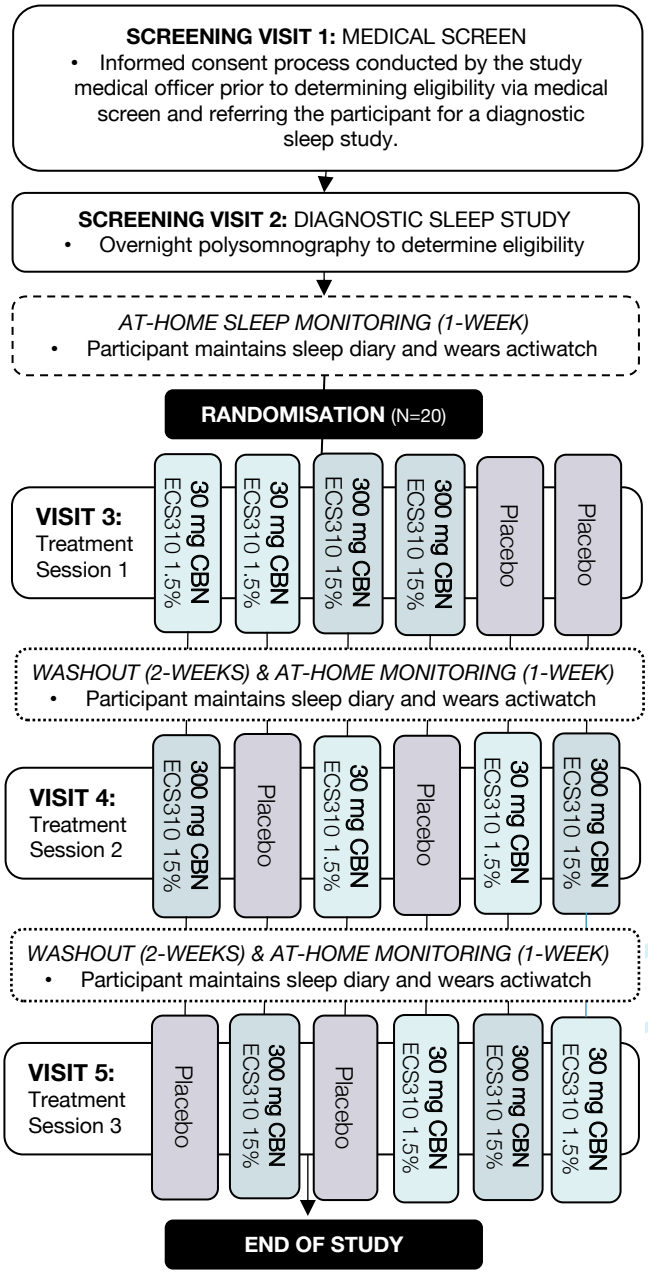
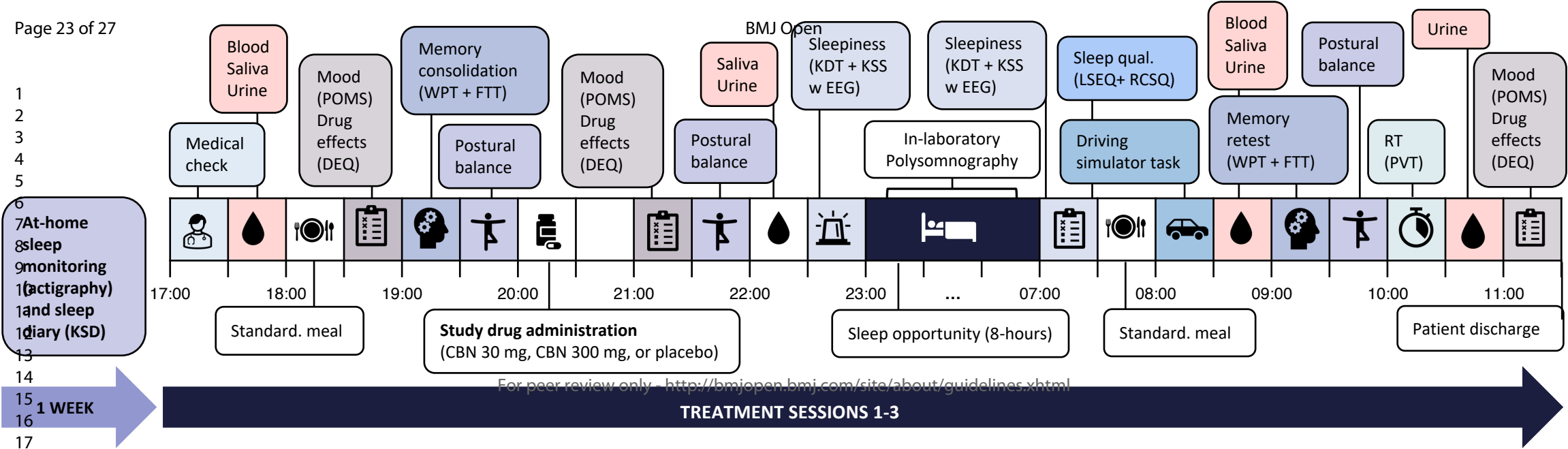

SCREENING VISIT 1: MEDICAL SCREEN
• Informed consent process conducted by the study medical officer prior to determining eligibility via medical screen and referring the participant for a diagnostic sleep study.
SCREENING VISIT 2: DIAGNOSTIC SLEEP STUDY
• Overnight polysomnography to determine eligibility
AT-HOME SLEEP MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
RANDOMISATION (N=20)
VISIT 3: Treatment Session 1
30 mg CBN ECS310 1.5%
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
300 mg CBN ECS310 15%
Placebo
Placebo
WASHOUT (2-WEEKS) & AT-HOME MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
VISIT 4: Treatment Session 2
300 mg CBN ECS310 15%
Placebo
30 mg CBN ECS310 1.5%
Placebo
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
WASHOUT (2-WEEKS) & AT-HOME MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
VISIT 5: Treatment Session 3
Placebo
300 mg CBN ECS310 15%
Placebo
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
30 mg CBN ECS310 1.5%
END OF STUDY



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1,5
	2b	All items from the World Health Organization Trial Registration Data Set	In trial registration
Protocol version	3	Date and version identifier	5
Funding	4	Sources and types of financial, material, and other support	1,2,5,16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,5,14-16
	5b	Name and contact information for the trial sponsor	1 and trial registration
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	5,16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14-16

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-7 (Box 1)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	6-7 (Box 1)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7, 13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6-7 (Box 1)
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	4, trial registry
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 1; Figures 1-2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13-14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13-14
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13-14
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13-14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	13-14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1,2,5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	5

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7-8
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13-14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	1,16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	16
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	16; trial registry
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ('CUPID' study): Protocol for a randomised, double-blind, placebo-controlled, crossover, three-arm, proof-of-concept trial.

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Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ('CUPID' study): Protocol for a randomised, double-blind, placebo-controlled, crossover, three-arm, proof-of-concept trial.

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Ethics and dissemination: Human Research Ethics Committee approval has been granted by Bellberry Limited (2021-08-907). Study findings will be published as open-access in a peer-reviewed journal and presented at academic conferences.

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Abstract (277/300 words)

Objective: Insomnia is the most prevalent sleep disorder, with few effective pharmacotherapies. Anecdotal reports and recent preclinical research suggest that cannabinol (CBN), a constituent of *Cannabis sativa* derived from delta-9-tetrahydrocannbinol (THC), could be an effective treatment. Despite this, the isolated effects of CBN on sleep have yet to be systematically studied in humans. **Methods:** The present protocol paper describes a randomised, double-blind, placebo-controlled, single-dose, three-arm, crossover, proof-of-concept study which investigates the effects of CBN on sleep and next-day function in 20 participants with clinician-diagnosed insomnia disorder and an Insomnia Severity Index [ISI] Score ≥15. Participants receive a single fixed oral liquid dose of 30 mg CBN, 300 mg CBN, and matched placebo, in random order on three treatment nights; each separated by a two-week washout period. Participants undergo overnight sleep assessment using in-laboratory polysomnography and next-day neurobehavioral function tests. The primary outcome is wake after sleep onset (WASO) minutes. Secondary outcomes include changes to traditional sleep staging, sleep onset latency, and absolute spectral power during non-rapid eye movement (NREM) sleep. Tertiary outcomes include changes to sleep spindles during NREM sleep, arousal indices, absolute spectral power during rapid eye movement (REM) sleep, and subjective sleep quality. Safety-related and exploratory outcomes include changes to next-day simulated driving performance, subjective mood and drug effects, postural sway, alertness and reaction time, overnight memory consolidation, pre and post subjective and objective sleepiness, and plasma, urinary, and salivary cannabinoid concentrations. The study will provide novel preliminary data on CBN efficacy and safety in insomnia disorder, which will inform larger clinical trials. Human Research Ethics Committee approval has been granted by Bellberry Limited (2021-08-907). Study findings will be disseminated in a peer-reviewed journal and at academic conferences. ClinicalTrials.gov Identifier: NCT05344170.

Key words: Power Spectral Analysis; Resting Wake Electroencephalography; Psychomotor Vigilance Test; Karolinska Drowsiness Test; Karolinska Sleepiness Scale; Leeds Sleep Evaluation Questionnaire; Standard Deviation of Lateral Position; Liquid Chromatography–Mass Spectrometry.

Strengths and limitations of the study

- This study uses a randomised, double-blind, placebo-controlled, crossover design to investigate two active doses of CBN isolate on sleep and neurobehavioral function over ~17-hours using gold-standard objective measures, validated subjective measures, and rigorous design methodology.
- Study participants undergo extensive screening with a sleep physician to confirm insomnia disorder diagnosis, including an overnight diagnostic polysomnogram to rule out other sleep disorders that commonly co-occur with insomnia.
- The study washout period (2-weeks) was chosen to minimise participant burden, as participants cannot undergo insomnia treatment before (3-months) or during (~2 months) the protocol; however, this may lead to carry-over effects between doses as a recent crossover study demonstrated oral cannabinoids can persist in plasma for >4 weeks (1500 mg; single dose).
- The study involves a single-dose, in-laboratory proof-of-concept design and results may, therefore, lack ecological validity (repeated CBN dosing may be necessary to effectively treat insomnia).

Background

Insomnia is a sleep disorder characterised by subjective difficulty falling asleep, maintaining sleep, and/or achieving restorative sleep, where symptoms persist for ≥3-months and are accompanied by daytime sequalae of dysfunction and/or distress.[1] Insomnia disorder is the most prevalent sleep disorder which affects between 10–30% of adults[2] and persists in 37% of cases at 5-year follow-up.[3] Insomnia disorder increases the risk of mental and physical illnesses such as depression [4] and dementia.[5] The economic burden of insomnia disorder is estimated at >$13 billion per annum in Australia.[6]

Insomnia disorder treatments are typically behavioral or pharmacological in nature. Cognitive behavioural therapy for insomnia (CBTi) is the first-line treatment and its efficacy has been confirmed in recent meta-analyses.[7] Nevertheless, significant access barriers to CBTi exist including limited providers, high cost,[8] and delayed perception of subjective benefits.[9] CBTi may also be less effective in individuals with shorter sleep duration (i.e., <6 hours/night).[10] As such, sedative-hypnotics are commonly used in primary care.[7] While effective in the short-term, well-documented side effects include daytime sedation, psychomotor impairment, addiction/dependence, and premature mortality.[11] There is a clear need for safe pharmacological insomnia disorder treatments.

Cannabis sativa is a complex plant containing numerous potentially therapeutic chemical compounds, including over 140 constituent '*cannabinoids*'.[12] As historical legal prohibitions against cannabis are relaxed, cannabinoids are being increasingly used to aid sleep.[13] The most well researched cannabinoids are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD); however, in recent times, *cannabinol* (CBN) has become recognized as a putative 'sleep-enhancing' cannabinoid.[14] CBN is produced via the nonenzymatic oxidation of THC and is therefore present at higher concentrations in aged (rather than 'young') cannabis plant material.[15] This is significant as aged cannabis plant material is anecdotally reported to induce sleep.[16] CBN products are also being sold as sleep aids in unregulated markets.[17] Importantly, a recent narrative review of CBN highlighted insufficient evidence to support sleep-related claims, despite a plausible mechanism of action.

THC and CBN exert some of their physiological effects via interactions with the *endogenous cannabinoid system* (ECS)[12], comprising of signaling molecules termed '*endocannabinoids*' (e.g., anandamide, 2-Arachidonoylglycerol [2-AG]), their receptors, and enzymes.[18, 19] The ECS is involved in regulating a myriad of biological processes including circadian rhythmicity[20]. Systematic administration of anandamide has been shown to promote sleep in rodents and humans [18, 21, 22] via activation of the cannabinoid receptor 1 (CB_1R).[21, 23] As such, the CB_1R is considered a promising therapeutic target.[24] The pharmacological effects of THC occur via partial agonism at CB_1R[25] and can include acute intoxication, psychomotor impairment, sedation, and changes in sleep architecture.[18] CBN also has agonist actions at CB_1R, albeit approximately 10-times less potent than THC[25] which may potentially account for the low levels of intoxication reported in historic CBN studies.[26, 27] Minimal intoxication could render CBN a more practical and safer alternative to THC as a sleep aid.

Two recent clinical studies administered small quantities of CBN in multi-cannabinoid formulations.[28, 29] The first, a crossover randomized controlled trial (RCT) with a one-week washout, investigated two-weeks of an oral liquid containing 20 mg THC, 2 mg CBN, and 1 mg CBD in 20 adults with insomnia disorder. The drug significantly improved insomnia disorder symptoms (Insomnia Severity Index [ISI] -5.1 points, p=0.0001, d=0.94) compared to placebo, with no significant changes to polysomnography (measured on night 14 of dosing).[28] In an interventional open-label

study, current medicinal cannabis users with subjective sleep difficulty reported improved sleep (assessed via an unvalidated subjective survey and a validated non-contact at-home tracking device) after three weeks of nightly administration of an oral capsule containing 10 mg THC and 5 mg CBN.[29] Importantly, the effects of CBN cannot be disentangled from those of THC in these studies. We are not aware of any clinical studies to-date that have tested the isolated effects of CBN on objectively measured human sleep.

Importantly, a recently published conference abstract reported on a preclinical study in which CBN isolate (10, 30, and 100 mg/kg intraperitoneally compared with zolpidem 10 mg/kg as a positive control) increased the proportion of non-rapid eye movement (NREM) sleep and sleep bout duration 4-hours post administration in Long-Evan rats.[30] With the lowest dose, biphasic effects were observed – CBN (10mg/kg) initially decreased rapid eye movement (REM) sleep proportion; however, 4 hours after administration, a significant increase in the percentage of REM sleep occurred. These results suggest CBN could reduce wake after sleep onset (WASO), given such delayed effects on sleep.

Study design and aim

Here we described the protocol for a randomised, double-blinded, placebo-controlled, three-arm, crossover, single-site, proof-of-concept study design to investigate the acute effects of oral CBN on sleep and next-day function in 20 participants with clinician-diagnosed insomnia disorder. The primary study aim is to investigate the effects of CBN (30 and 300 mg) versus placebo on sleep in insomnia disorder. The study evaluates the safety and efficacy of CBN as a pharmacological therapy and generate preliminary data to inform larger clinical trials. Many outcome measures used in insomnia disorder clinical trials are known to be susceptible to placebo effects. A placebo control is scientifically and ethically defensible choice as the effects of CBN on human sleep are unknown and could not be properly elucidated if an active treatment such as zolpidem was the comparator rather than placebo.

Study outcomes

The primary study outcome is WASO measured in minutes using in-laboratory overnight polysomnography, from the first epoch after lights out until the last epoch, scored as any stage of sleep by an experienced polysomnographic technician in accordance with American Academy of Sleep Medicine (AASM) 2020 Sleep Scoring criteria (Version 2.6).[31] Secondary study outcomes include:

1. Traditional sleep staging: Proportion of the sleep opportunity scored at the 5 stages (wake, and N1, N2, N3, and REM sleep) between lights out and lights on, measured using overnight in-laboratory polysomnography, scored by a polysomnography technician in accordance with AASM Sleep Scoring criteria.
2. Sleep Onset Latency (SOL): SOL measured in minutes using in-laboratory polysomnography, calculated from the time of lights out to the first sleep epoch as scored by a polysomnographic technician in accordance with AASM Sleep Scoring criteria.
3. Absolute Electroencephalographic (EEG) Power During NREM Sleep: Spectral power of delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequency ranges between treatment arms. Power spectral analysis will be applied to EEG signals from polysomnography after artefacts are detected and removed.

Tertiary, safety, and exploratory study outcomes are described in Box 1 (see ClinicalTrials.gov Identifier: NCT05344170).

Box 1. *Tertiary, safety, and exploratory study outcomes*

Tertiary outcomes

1. **Sleep Spindles During Non-Rapid Eye Movement (NREM) Sleep**: Sleep spindle and slow oscillation events in NREM sleep from in-laboratory overnight polysomnography. A sleep spindle and slow oscillation detection algorithm will be applied to electroencephalography (EEG) signals from polysomnography after artefacts are detected and removed. Comparisons between each CBN dose versus placebo.
2. **EEG Arousal Index:** Number of cortical arousals captured via the electroencephalogram per hour of sleep scored by the polysomnographic technician on the polysomnogram in accordance with AASM Sleep Scoring criteria. Comparisons between each CBN dose versus placebo.
3. **Absolute EEG Power During Rapid Eye Movement (REM) Sleep:** Spectral power of delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequency ranges between treatment arms. Power spectral analysis will be applied to EEG signals from polysomnography after artefacts are detected and removed. Comparisons between each CBN dose versus placebo.
4. **Next-Day Post Wake Subjective Sleep Evaluation (LSEQ):** RCSQ score. LSEQ score. LSEQ scores range from 0-100, with higher scores indicating better subjective experience. Assessed within 1 h after wake (comparison between each CBN dose versus placebo).
5. **Next-Day Post Wake Subjective Sleep Evaluation (RCSQ):** RCSQ score. RCSQ scores range from 0-100, with higher scores indicating better subjective experience. Assessed within 1 h after wake (comparison between each CBN dose versus placebo).

Safety outcomes

1. **Standard Deviation of Lateral Position (SDLP) During Next-day Post-Wake Simulated Drive:** SDLP ("weaving") is measured across the 'standard', 'car following', and 'divided attention' sub-sections of a ~30 minute simulated driving task. Assessed within 2 h after wake (comparison between each CBN dose versus placebo)
2. **Speed During Next-day Post-Wake Simulated Drive**: Average speed and standard deviation of speed is measured across the 'standard' and 'divided attention' sub-sections of a ~30 minute simulated driving task. Assessed within 2 h after wake (comparison between each CBN dose versus placebo).
3. **Distance Headway During Next-day Post-Wake Simulated Drive**: Average distance headway (i.e., distance between the driver's vehicle and vehicle immediately in front) and standard deviation of distance headway is measured across the 'car following' sub-section of a ~30-minute simulated driving task. Assessed within 2 h after wake at both treatment sessions (comparison between each CBN dose versus placebo).
4. **Subjective Mood Evaluation**: The Abbreviated Profile of Mood States (POMS) consists of 40 items measuring domains of 'tension', 'depressed', 'anger', 'vigour', 'fatigue', and 'concentration'. Participants respond to each item using 5-point Likert scales ranging from 0 (Not at all) to 4 (Extremely). A total mood disturbance score is calculated by summing negative domains and subtracting positive domains. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose versus placebo).
5. **Subjective Drug Effects:** The Drug Effects Questionnaire (DEQ) assesses the extent to which participants feel a drug effects, feel high, like the effects, dislike the effects, want more of the substance, and feel sedated, on self-rating 100mm visual analogue scales. A total mood disturbance score is calculated by summing negative domains and subtracting positive domains. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose versus placebo).
6. **Postural Sway:** Centre-of-pressure (COP) during computerised static posturography. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose versus placebo).
7. **Behavioural Alertness and Reaction Time:** Psychomotor Vigilance Test (PVT) is administered twice the next-day (comparison between each CBN dose versus placebo).

8. **Overnight Verbal Declarative Memory Consolidation**: Word pair recall scores measured using the computerised Word Pairs Task (WPT). Administered pre-drug administration and next-day (comparison between each CBN dose versus placebo).
9. **Overnight Procedural Memory Consolidation**: Motor sequence learning measured using the computerised Finger Tapping Task (FTT). Administered pre-drug administration and next-day (comparison between each CBN dose versus placebo).
10. **Resting Wake EEG Power After Sleep:** Resting wake EEG power during the Karolinska Drowsiness Test (KDT) upon wake: delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequency ranges. Power spectral analysis is applied to EEG signals from polysomnography, after artefacts are detected and removed. Comparison between each CBN dose versus placebo.
11. **Subjective Sleepiness After Sleep:** The Karolinska Sleepiness Scale (KSS) is a 10-item measure of subjective drowsiness. Participants respond to each item using a 9-point Likert scale ranging from 1 (Extremely alert) to 9 (Extremely sleepy). Higher scores are indicative of increased drowsiness. The KSS will be collected in accordance with the KDT protocol but will not be analysed due to insufficient statistical power.

Exploratory outcomes

1. **Resting Wake Electroencephalography (EEG) Power Before Sleep** (Acute Effects of CBN). Resting wake EEG power during the KDT prior to sleep: delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequency ranges. Power spectral analysis is applied to EEG signals from polysomnography, after artefacts are detected and removed. Comparison between each CBN dose versus placebo.
2. **Subjective Sleepiness Before Sleep** (Acute Effects): The KSS is a 10-item measure of subjective drowsiness. Participants respond to each item using 9-point Likert scales ranging from 1 (Extremely alert) to 9 (Extremely sleepy). Higher scores are indicative of higher drowsiness. The KSS will be collected in accordance with the KDT protocol but will not be analysed due to insufficient statistical power.
3. **Plasma Cannabinoid Concentrations**: Presence of cannabinoids (CBN, delta-9-tetrahydrocannabinol [THC], and cannabidiol [CBD]) (e.g., 11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD), and endocannabinoids and related molecules (e.g., 2-Arachidonoylglyceroland anandamide) and their metabolites in plasma samples.
4. **Urinary Cannabinoid Concentrations:** Presence of cannabinoids (CBN, THC, and CBD) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD) in urine samples.
5. **Salivary Cannabinoid Concentrations:** Presence of cannabinoids (THC, CBN) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC) in saliva samples.

Safety

The investigational product, CBN, is currently a Schedule 9 'Prohibited Substance' in Australia.[32] The safety profile of CBN can be inferred from human studies conducted between 1973 and 1987. These studies explored oral doses up to 1200 mg CBN with some administering repeated daily doses of CBN for up to 4 weeks.[26, 33-35] There were no notable safety concerns, aberrations or toxicity concerns from these studies, with no adverse changes in parameters such as heart rate, blood pressure, body temperature, respiratory rate, perception, intoxication and postural stability.[26, 33-35] None of these studies measured sleep, sedation, or residual next-day effects of CBN (such as adverse effects on driving performance and cognitive function). These measures are obtained in the current study. CBN is currently being investigated as an analgesic agent (ClinicalTrials.gov Identifier: NCT03675971) and topical treatment for epidermolysis bullosa (ClinicalTrials.gov Identifier: NCT04908215). An RCT of oral CBN isolate (compared with combination CBN/CBD and placebo doses) on the subjective sleep

of healthy adults was retrospectively registered in May 2022 (ClinicalTrials.gov Identifier: NCT05839964). We will monitor and review safety reports from these trials as they become available.

Methods

Participants, interventions, and outcomes

Trial setting

The trial Sponsor and site is the Woolcock Institute of Medical Research, a specialist sleep and respiratory research institute and clinic (Sydney, Australia). The study funder is the Lambert Initiative for Cannabinoid Therapeutics, University of Sydney (Sydney, Australia), a philanthropically funded centre for cannabinoid science. Recruitment commenced in August 2022 and is anticipated to conclude in late 2023. The first participant was enrolled the 24th of August 2022 and the first participant was randomised on the 13th of October 2022. The study has ethical approval from Bellberry Limited Human Research Ethics Committee (HREC; 2021-08-907; Version 2.1, 01-Aug-2022) and is registered on ClinicalTrials.gov (Identifier: NCT05344170; 25-April-2022). The clinical trial protocol is available on request. Significant changes to the study protocol will be documented on ClinicalTrials.gov. The study protocol was developed according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement.[36] The Therapeutics Goods Administration (TGA) has acknowledged the use of CBN in this study through the Clinical Trial Notification (CTN) scheme (CTN Number: CT-2022-CTN-00543-1).

Participant eligibility

Eligible participants are aged between 25-65 years with physician diagnosed insomnia disorder as per the International Classification of Sleep Disorders-3 (ICSD-3)[37] and Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[38] criteria as determined by the study physician (see Box 2). The age range was selected to minimise age-related changes to sleep architecture.[39] As cannabinoids have been shown to improve symptoms (e.g., anxiety, pain) that commonly impair sleep (i.e., causing 'secondary' insomnia disorder),[40] a 'primary' insomnia disorder population is being recruited for this study.

Box 2

Inclusion and exclusion criteria for study participants

Eligible individuals must fulfill the following criteria:

1. Between 25 – 65 years of age
2. Insomnia Severity Index (ISI) score ≥ 15 at eligibility screening
3. Insomnia disorder (symptoms occurring at least 3 times per week and present for longer than 3 months) as determined by the study physician
4. Ability to take oral medication
5. Provision of signed and dated informed consent form
6. Stated willingness to comply with all study procedures and availability for the duration of the study

Individuals who meet any of the following criteria are ineligible to participate in the study:

1. Medical condition or medication that is the cause of the insomnia disorder as determined by the study physician
2. Known hypersensitivity to cannabis or cannabinoid products (including if this becomes evident during the trial)

3. Reported use of cannabis or cannabinoid products within the past 3 months as confirmed by at least one negative urine drug screen (UDS) (or at the study physician's discretion)
4. Sleep apnoea (defined as Apnoea Hypopnea Index [AHI] >15 and Oxygen Desaturation Index [ODI]>10) as confirmed by polysomnography at screening
5. Sleep-related movement disorder as determined by the study physician
6. Delayed or advanced sleep phase syndrome (based on actigraphy and sleep diary) as confirmed during screening
7. Any medical condition that produces an abnormal EEG (i.e., epilepsy, brain injury)
8. Clinically relevant cardiovascular abnormalities as determined by the study physician and a 12-lead electrocardiogram (ECG) at screening
9. Shift work or trans meridian travel (two time zones) within the last month
10. History of major psychiatric disorder in the past 12 months at the study physician's discretion, except clinically managed mild depression and/or anxiety
11. History of suicide attempt or current suicide ideation (score greater than 1 on Q9 of the Patient Health Questionnaire [PHQ-9])[41]
12. Pregnancy or lactating. Female participants are required to complete a urine pregnancy test at screening and treatment sessions and all participants are instructed to use a reliable form of contraception throughout the study duration
13. History of drug or alcohol dependency or abuse within approximately the past 2 years
14. Use of CNS-active drugs (cannabis, amphetamines, cocaine, antidepressants, opioids, benzodiazepines) in the past 3 months as confirmed by a positive urine drug test at screening or at the study physician's discretion
15. Use of medications that may have a clinically significant impact upon the metabolism and excretion of cannabinoids as determined by the study physician (e.g., CYP450 enzyme inducers/inhibitors
16. Excessive caffeine use that in the opinion of the study physician contributes to the participant's insomnia disorder, or the inability to abstain from caffeine use 24 hours prior to each overnight sleep study
17. Inability to refrain from alcohol consumption 24 hours prior to each overnight sleep study
18. Individuals with nicotine dependence (i.e., daily smokers)
19. Medical conditions that result in frequent need to get out of bed (e.g., sleep walking, nocturia)
20. Psychological or behavioural treatment for insomnia disorder, including cognitive behavioural therapy for insomnia, within 3 months before screening (excluding sleep hygiene advice)
21. Occupational or judicially ordered drug screening
22. Has held an unrestricted driving license <1 year
23. Cannot speak English fluently

Patient and Public Involvement

Patients and/or the public were not involved in the design, conduct, reporting, nor dissemination plans of this research.

Intervention

Study doses

Participants receive a single fixed 2 mL oral liquid dose of: (1) 30 mg CBN ('ECS310' 1.5% containing 15 mg/mL CBN); (2) 300 mg CBN ('ECS310' 15% containing 150 mg/mL CBN); and (3) matched placebo. Treatments are administered across three separate overnight Treatment Sessions in randomised order two-hours prior to habitual lights out. The lower dose was selected to match the CBN products

available in the United States and supported by customer testimonies.[17] The higher dose was informed by a preclinical study of CBN isolate and sleep.[30]

Investigational product

The investigational drug ('ECS310') is a liquid formulation of CBN suspended in medium chain triglyceride (MCT) oil. The placebo contains MCT oil only. The maximum detectable THC content of the active investigational products is ≤ 150 µg. Neither the study drug nor matched placebo contain any other cannabinoids or terpenes/terpenoids. The study drug and matched placebo are expected to be identical in their visual appearance, taste, and smell. The MCT oil contains paprika oleoresin colorant to ensure the double-blind is maintained and prevent oxidation. The Sponsor purchased the investigational product from BOD Australia (Sydney, Australia), who sourced investigational products from Medropharm (Zürich, Switzerland). Stability studies supporting the shelf life applied to the drug product were conducted in accordance with the International Conference on Harmonization (ICH) Guideline Q1A Stability Testing of new Drug Substances and Products and are ongoing. The CBN solution stability is 24 months at 15-25°C, 60% humidity, and away from sunlight.

Recruitment and retention

We aimed to recruit 20 participants over an 18-month period. The sample size was selected with consideration for practical factors such as time, cost, and resource allocation (rather than using formal statistical techniques) as it is pilot in nature. Participants are recruited from social media, the Woolcock Institute Volunteer Database, and locally displayed physical study advertisements. Participants are reimbursed a fixed amount equivalent to that used in previous clinical trials with similar participant involvement and loss of working hours.[42] To encourage retention, participants receive reimbursement upon completion of the final Treatment Session. Study advertisements include an embedded link and QR code to the study website (described below).

Eligibility screening

Eligibility assessment is performed in three steps undertaken by the study team at the Woolcock Institute of Medical Research (see Figure 1 *Study Flow Diagram*):

1. *On-line and telephone pre-screening*. Interested individuals are directed to the study webpage. They provide electronic consent before completing an online pre-screening questionnaire to assess initial eligibility (e.g., age, location, ISI score, transmeridian travel, shift work). Potentially suitable individuals can enter their contact details and are provided the Participant Information Statement so that the trial coordinator may conduct a brief telephone interview before scheduling screening appointments. Individuals deemed unsuitable are provided with support resources.
2. *Screening visit 1*. Willing and potentially suitable participants attend an on-site medical screening. The study physician obtains written informed consent before conducting a screening interview to compile medical history, assess suitability against study eligibility criteria (Box 2), and diagnose insomnia disorder (ICSD-3[37] and DSM-V[38]). The following validated self-report questionnaires are used to evaluate eligibility: ISI,[43] a measure of insomnia disorder symptom severity over the past two-weeks; Epworth Sleepiness Scale (ESS),[44] a measure of sleep propensity in daily life; Hospital Anxiety and Depression Scale (HADS),[45] a measure of anxiety and depression; and, the Patient Health Questionnaire-9 (PHQ-9),[41] an instrument for screening and monitoring the severity of depression and suicidal ideation. An electrocardiogram (ECG) and urinary screen (pregnancy and recent alcohol, cannabis, cocaine, benzodiazepines, opiates, or amphetamines, use) are also conducted. Participants must agree to

the study protocol, including use of contraception and refraining from driving until the day after Treatment Session discharge.

3. *Screening visit 2*. Participants are required to complete a clinical overnight polysomnography to rule out the presence of other sleep disorders (obstructive sleep apnoea or sleep-related movement disorder, see Box 2). Diagnostic polysomnography results within the previous 12-months may be used where weight has not changed substantially in that time (at discretion of the study physician). Diagnostic sleep studies are scored by an experienced sleep technician[46] and reviewed by the study physician.

Insert Figure 1 here

Figure 1. *Study flow diagram*

Randomisation, allocation concealment, and blinding

Participants are allocated to one of six possible treatment orders in a 1:1:1:1:1:1 ratio using a pre-populated randomisation schedule generated by the study epidemiologist (NSM). The six-orders constitute a balanced Latin square. Sequences were computer-generated and are stored in a password-protected system inaccessible to blinded study personnel (centralised computerised randomisation). Allocation concealment is managed by the unblinded study epidemiologist and an independent staff member who do not have any contact with participants, nor involvement in day-to-day trial activities. A study physician may request to be unblinded in the event of a medical emergency. Participants who are enrolled in the study, but not yet randomised, are assigned a unique screening number. Randomisation occurs at the outset of the first Treatment Session assuming participant eligibility persists. Working under the study physician, the trial coordinator records the decision to randomise the patient. The study database then issues an irrevocable randomisation number for that participant. Investigational product is stored in opaque sequentially numbered containers labelled with the patient randomisation and Treatment Session number to maintain blinding of all patients, staff, and outcome assessors. The drug manufacturer packaged and labelled the investigational product according to the randomisation list generated by the study epidemiologist.

Pre-Treatment Session procedures

Participants are asked to maintain a regular sleep-wake schedule (i.e., lights out between 22-23:00 each evening) for one week prior to each Treatment Session. Participants wear a wrist-worn actigraphy monitor (GENEActive, Activinsights, United Kingdom) and complete a daily sleep diary (Karolinska Sleep Diary [KSS][47] modified to capture additional information, such as naps, alcohol/caffeine consumption, and GENEactiv removal times) throughout this period. If the sleep schedule is significantly irregular during the 7-night leadup (e.g., > 2 hours on > 2 nights), the principal medical and non-medical investigators may reschedule the visit. Participants are required to abstain from consuming cannabis, cannabinoids, and other CNS-active drugs throughout the entire study; and avoid alcohol and caffeine 24 hours prior to Treatment Sessions. Urinary drug, alcohol (DrugCheck NxStep OnSite, USA), and pregnancy (SureStep, Germany) screening occurs at the outset of each Treatment Session.

Treatment Session procedures

Participants arrive at the Woolcock Sleep Clinic in the early evening (~17:00-18:00 depending on habitual lights out), where they remain until late morning (~10-11:00) the following day, as per the Treatment Session schedule in Figure 2. Standardised meals are provided. All Treatment Sessions are separated by a washout period of ≥ 2-weeks to minimise carry-over effects between doses.[48]

Study drug administration

After a brief medical examination, the study physician prescribes and dispenses the study drug, drawing a fixed 2 mL volume of liquid into a pre-labelled amber plastic syringe. Participants self-administer the randomly allocated study drug 2-hours prior to lights out. The trial coordinator observes drug administration to ensure accuracy of dosing. The dose is documented in participant records and accountability logs. Timing of administration has been informed by delayed onset of effects observed with oral dosing of other cannabinoids[49] and delayed effects observed in a preclinical study of CBN.[30]

Data collection

Study measures are described below (see Table 1).

Table 1. *A summary of assessments completed throughout the investigation.*

Measure (ordered first to last)	Screening	Treatment Session 1-3		
		At-home sleep monitoring	Night	Day
Informed consent	●			
Medical examination	●		●	
Baseline questionnaires (ISI, ESS, HADS & PHQ-9)	●			
Electrocardiogram	●			
Urinary drug and alcohol screen	●		●	
Pregnancy test	●		●	
Overnight polysomnography (clinical EEG)	●		●	
Actigraphy		●		
Sleep diary (KSD)		●		
Oral fluid drug screen (Securetech DrugWipe)			●	●
Quantisal saliva collection			●	●
Blood plasma collection			●	●
Urine collection			●	●
Mood (POMS)			●	●
Drug effects (DEQ)			●	●
Balance (posturography)			●	●
Memory (FTT & WPT)			●	●
KDT with EEG			●	●
Sleep questionnaires (LSEQ, RCSQ, & KSS)				●
Simulated driving performance				●
PVT				●

Note. DEQ=Drug Effects Questionnaire; EEG=electroencephalography; ESS=Epworth Sleepiness Scale; FTT=Finger Tapping Task; HADS=Hospital Anxiety Depression Scale; ISI=Insomnia Severity Index; KSD=Karolinska Sleep Diary; KSS, Karolinska Sleepiness Scale; LSEQ= Leeds Sleep Evaluation Questionnaire; PHQ-9=Patient Health Questionnaire-9; POMS=Profile of Mood States; PSG=polysomnography; PVT=Psychomotor Vigilance Task; RCSQ=Richard Campbell Sleep Questionnaire; WPT=Word Pairs Task.

Insert Figure 2 here

Figure 2. *Schedule of events during each Treatment Session.*
Note. DEQ=Drug Effects Questionnaire; EEG=electroencephalography; FTT=Finger Tapping Task; ISI=Insomnia Severity Index; KSD=Karolinska Sleep Diary; KSS=Karolinska Sleepiness Scale; LSEQ= Leeds Sleep Evaluation Questionnaire; POMS=Profile of Mood States; POST=Posturography; PSG=polysomnography; PVT=Psychomotor Vigilance Task; RCSQ=Richard Campbell Sleep Questionnaire; UDS=Urinary Drug Screen; WPT=Word Pairs Task.

Blood collection and plasma cannabinoid levels

Venous blood samples are taken at baseline (~17:00) and approximately 13-hours post administration (~08:45). Samples are drawn into EDTA vacutainers (Becton, Dickinson and Company, New Jersey, USA) and centrifuged (2500×g for 15 min at 4°C). Supernatant plasma is aliquoted into 1.5 mL Eppendorf Tubes and stored at -80ºC in the Woolcock Institute of Medical Research until study completion when samples will be transferred to the Lambert Initiative Laboratory. Samples will undergo liquid chromatography–mass spectrometry (LC–MS/MS) analysis, using previously published methodology.[50-52] Plasma will be analysed for cannabinoids (e.g., CBN, THC, CBD), their metabolites (e.g., 11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD), and endocannabinoids and related molecules (e.g., 2-AG and anandamide). THC and CBD and their metabolites are being tested to verify abstinence from common cannabinoids, notwithstanding the trace quantities of THC that the investigational product may contain (≤ 150 µg/2ml).

Urine collection and urinary cannabinoid levels

Urine collection occurs at baseline (~17:00), and approximately 90-minutes (~21:30), 11-hours (~07:00), and 14-hours (~10:00) post drug administration. Urine (~6 mL) is aliquoted into 1.5 mL Eppendorf Tubes and stored at -80ºC before being transferred to the Lambert Initiative Laboratory for quantification of cannabinoids (primarily THC, CBD and CBN) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD), and endocannabinoids at study completion. LC–MS/MS analyses will be conducted using our previously described methods .[50, 51] It is important to conduct urinary analyses to further characterise the pharmacokinetic profile of CBN, which is currently not well described.

Salivary drug testing

Oral fluid testing occurs at baseline (~17:00), and at approximately 75-minutes (~21:15) and 13-hours (~08:55) post drug administration using the DrugWipe® 5S (DW5s; Securetec, Neubiberg, Germany) and Quantisal™ collection (Immunalysis, Pomona, CA) devices. The DW5s collects oral fluid (approximately 10–20 µL) from the tongue. The DW5s is commonly used in routine roadside drug tests; however, demonstrates variable sensitivity (22-89%), specificity (50-100%), and accuracy (53-94%).[53] While the investigational product contains negligible quantities of THC, similarities in chemical structure between CBN and THC may conceivably lead to false-positive test results that would be a concern should CBN become widely used by patients.[54] As such, the Quantisal™ device is used to collect oral fluid from participants (~1 mL) which will be used in LC–MS/MS quantification of cannabinoid (CBN and THC) and metabolite concentrations. Samples are placed in a stabilising buffer and stored at 4ºC before being transferred to the Lambert Initiative analytical chemistry laboratory for analysis. Analyses occur within three-months of collection.

Cognitive and psychomotor performance

Overnight declarative and procedural memory consolidation is assessed at each Treatment Session using the Word Pairs Task [WPT] and Finger Tapping Task [FTT], respectively. For both tasks, memory encoding during a learning phase occurs prior to drug administration (~19:15-20:00) with next-day retest (~09:00). As THC can acutely impair cognitive function and memory, it is necessary to examine CBN effects in this regard. Contrary to oral dosing studies, in one study, CBN reportedly caused a mild intoxication when administered intravenously at high doses (1.2mg/min)[27]. To investigate possible intoxication-induced bodily sway, postural balance ('posturgraphy') is being measured using the stabilometric balance platform (Advanced Medical Technologies Inc., AccuSwayPLUS Balance platform, MA, USA), which measures centre of pressure in anterior-posterior

(y-axis) and medial-lateral (x-axis) directions using four strain gauge load cells at the bottom of the device. Centre of pressure is assessed in eyes open and eyes closed conditions. Postural balance assessment takes < 5 minutes to complete and is repeated 3 times (i.e., baseline, 60-minutes post drug administration, and next-day ~09:30 hours). The psychomotor vigilance test (PVT) is a commonly used reaction time based measure of attention and fatigue related changes in alertness[55] involving a hand-held box with a red-light emitting diode display of a three-digit millisecond counter (PVT-192, Ambulatory Monitoring Inc, Adsley, NY, USA). During the 10-minute task, participants are instructed to respond as quickly as possible to stimuli appearing at variable intervals (ranging 2-10 seconds). The PVT is repeated twice during next-day testing (~09:00 and ~10:00 hours).

Self-report questionnaires

Mood is evaluated using the Profile of Mood States (POMS; 40-items with 5-point Likert scales)[56] questionnaire, a commonly used rating scale of transient and distinct moods. The Drug Effects Questionnaire (DEQ; 7-items with unipolar 100 mm visual analogue scales [VAS])[57] is used to evaluate subjective drug effect; including strength of drug effect, feeling stoned ('high'), liking/disliking the drug effect, feeling sedated, and feeling anxious. The POMS and DEQ are completed at baseline, ~1-hour post drug administration (~21:00), and the next-day (~09:30). The Leeds Sleep Evaluation Questionnaire (LSEQ; 10-items with 100 mm VAS)[58] is a validated measure of the previous night's sleep quality (sleep onset, maintenance, and quality; including behaviour following waking) in comparison to usual. The Richard Campbell Sleep Questionnaire (RCSQ; 5-item with 100 mm VAS)[59] is used to measure perceived sleep depth, sleep latency (time to fall asleep), number of awakenings, and sleep efficiency and quality. The LSEQ and RCSQ are administered upon wake (~06:15 hours).

Subjective and objective sleepiness

Karolinska Sleepiness Scale (KSS) and Karolinska Drowsiness Test (KDT) are used to measure subjective and objective sleepiness, respectively.[60] The KSS is a single-item rating scale assessing current sleepiness, ranging from 1 (*Extremely alert*) to 9 (*Extremely sleepy – fighting sleep)*. KSS scores are typically compared to the objective drowsiness ascertained using the KDT. During the KDT, resting awake EEG is measured while participants focus on a wall marker, with eyes open and closed across 2-minute intervals. The KSS and KDT are administered immediately prior to sleep (~22:00 hours) and upon wake (~06:00 hours).

Polysomnography

Sleep is assessed using polysomnography (Grael polysomnography, Compumedics, USA), a multiparametric tool used for monitoring and diagnosing sleep disorders. Polysomnography consists of 18 channels recording biophysical changes that occur during sleep, including brain activity (via EEG); muscle activity or skeletal muscle activation (electromyography); eye movement (electrooculogram); cardiac function (ECG); respiratory effort using chest and abdominal belts; and digital pulse oximetry. To improve participant comfort/retention, the pulse oximeter, nasal cannula, and thermistor are only used during screening to detect sleep disordered breathing (Visit 2). Polysomnography equipment is fitted by an experienced sleep technician prior to sleep and is removed the next morning once the KDT has been completed. Sleep and associated events are scored according to AASM 2020 criteria in 30 second epochs.[46] Each patient's three polysomnograms are scored by the same sleep technician blinded to treatment allocation. For quantitative EEG analyses, sleep and KDT polysomnography recordings will be processed using a previously validated automated artefact detection program.[61, 62] Power spectral and spindle analyses will be derived from artefact-free epochs in central channels using previously published methodology.[61, 62]

Simulated driving performance

Next-day driving performance is measured at ~08:00 using a fixed-based driving simulator (Hyperdrive, Adelaide, Australia) equipped with standard vehicle controls (described elsewhere[42, 48]) and using custom-built scenario developed using the SCANeR Studio Simulation Engine (version 2022.2 r25, AVSimulation, Paris, France). The 30-minute driving scenario incorporates three independent epochs: (1) a 'car following' drive (~7 minutes duration) that closely resembles one that has previously demonstrated sensitivity to THC-induced impairment;[63] (2) a 'highway' drive (~17 minutes duration); and (3) a novel divided-attention drive (~6 minutes duration), designed to replicate a casual mobile-phone conversation that does not require mental rehearsal.[64] During the divided-attention task, pre-recorded five-word sentences are played every 10 seconds (through a hands-free speaker). Participants immediately indicate whether the sentence was sensical (e.g., "*the truck delivered the package*") or non-sensical (e.g., "*the octopus burned the onions*") in nature then; and at 7-seconds, they are asked to recall the last word in the sentence. The main outcome measure for all epochs of the driving task is SDLP, a widely used measure of lateral vehicular control that is sensitive to drug and alcohol effects. Previous reports show a dose-dependent increase in SDLP with THC (higher represents more erratic driving), particularly in occasional cannabis users.[63, 65] Secondary outcomes for each driving epoch include: (epoch 1) average and standard deviation of car-following headway (i.e., distance relative to car in front) and speed coherence (i.e., correlation between speed of lead and following car); (epochs 2 and 3) average speed, and standard deviation of speed (SDSP). The driving scenarios were programmed by investigator C.I.

Adverse events

Safety outcome measures are described above. Blood pressure is measured four times during each Treatment Session (~17:00, 18:45, 21:00, and 09:30). Adverse event collection occurs via standardised interview at three timepoints (prior to discharge, and 7-hours and 7-days post discharge). Passive adverse event collection occurs throughout the study protocol via open-ended interview and is recorded on paper form. A study physician assesses the severity and causality of each adverse event.

Post-Treatment Session care

Participants leave the research facility at approximately 10:00-11:00 and are contacted by the study co-ordinator that evening (~16:30), and 7-days later, to assess wellbeing and record adverse events (including changes to sleep). Upon study completion, if deemed necessary in the study physician's opinion, a clinical follow-up is arranged for participants on an individual basis.

Data management

Any information obtained for the purpose of this research that could identify participants is treated as confidential and securely stored adhering to guidelines of the Sponsor, HREC, NHMRC National Statement on Ethical Conduct in Human Research (2007) and Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95). All onboarding, consent, and questionnaire data is captured through SPARDAC™ (Single Page Application - Research Data Capture) developed by Wappsystem Pty Ltd and hosted on Amazon Web Services in Sydney, NSW. Participant data is identified by unique codes. The code linking participant data to identity (e.g., name, date of birth, contact details) is stored securely with password protection, and is not accessible via the internet. Paper files are stored in locked storage cabinets on-site. Digital participant data are stored on a secure electronic data system, that is regularly backed up with disaster recovery features. All data will be stored securely for at least 15 years. Only study investigators have access to participant data. Data monitoring occurs on a regular basis by the study investigators. Data integrity is being enforced through a variety of mechanisms including data rules, range checks, and consistency checks against data already stored in the database. Written

documentation of changes are available through electronic logs and audit trails. Study safety is internally monitored and evaluated regularly, with adverse events documented and reported according to Sponsor and HREC requirements. The decision to terminate the study lies with the principal investigators and will be based on the recruitment target and safety data. The PIs and trial coordinator are monitoring data integrity throughout the trial.

Trial management structure

This is a pilot study and does not require an intricate management structure. The advisory committee provide advice or complete discrete tasks (Table 2). The management of day-to-day trial activities is overseen by the principal investigators and the trial coordinator (IL, CH, & BY). This trial does not have an independent data safety monitoring committee because it is a single-site single-night small sample study with a medication that is thought to have a low side-effect profile in a group of patients that are known not to be at high risk of life-threatening events.[66]

Roles and responsibilities

Table 2. Investigator roles and responsibilities

Investigator	**Role**
Dr Camilla Hoyos	Principal Non-Medical Investigator
Professor Brendon Yee	Principal Medical Investigator
Professor Iain McGregor	Advisory committee member (Pharmacologist and Cannabinoid Expert)
Professor Ronald Grunstein	Advisory committee member (Sleep Physician)
Professor Bandana Saini	Advisory committee (Study Pharmacist)
Associate Professor Nathaniel Marshall	Advisory committee (Study Statistician/Epidemiologist)
Associate Professor Christopher Gordon	Advisory committee member (Registered Nurse and Sleep Disorder Expert)
Dr Angela D'Rozario	Advisory committee member (Sleep Neurobiology Expert/Consultant)
Dr Danielle McCartney	Driving Simulator and Cannabinoid Expert
Dr Chris Irwin	Advisory committee member (Driving Simulation Expert)
Dr Anastasia Suraev	Advisory committee member (Cannabinoid and Sleep Expert)
Ms Isobel Lavender	Clinical Trial Coordinator/PhD student

The study investigators have led the design and conduct of this trial, will conduct the analyses, and make all publication decisions.

Statistical analyses

Statistical analyses will be completed using a commercial statistical package (e.g., SPSS, SAS, etc). Descriptive statistics will be described using means and standard deviations or counts and percentages as appropriate. Adverse events and serious adverse events will be tabulated with the total number of incidents by each timepoint. No formal hypothesis testing will be conducted on adverse event and serious adverse event data. Due to insufficient statistical power the KSS will only be described using a median and interquartile range. Continuous outcome data will be analysed using Linear Mixed Models due to expected inter-participant variability with repeated measures and ability to handle missing data. Where variables are collected pre-drug administration (i.e., treatment session 'baseline') for the outcome being analysed, those scores will also be included in the model as a covariate (e.g., posturography, WPT, FTT, DEQ, POMS). Participants will be random factors. Fixed factors will be treatment (placebo, 30 mg CBN, and 300 mg CBN) and the arm (first, second, and third) of the study. Analysis will be by intention to treat. Difference in least squared means inside the treatment main effect will only be investigated for the hypotheses that the two active treatments are superior to placebo. We will not present a p-value comparison for the two active doses. The critical p-values for each of these hypotheses is set at 0.05 (two-tailed). Effect sizes will be calculated as partial eta squared (η_p^2), Cohen's d, and Hedges' *g*, where appropriate. A Statistical Analysis Plan will be finalised before last patient last visit and will be publicly available and/or upon request.[67] We are not undertaking any interim or stratified analyses.

Ethics and dissemination

Ethics and dissemination: Human Research Ethics Committee approval has been granted by Bellberry Limited (2021-08-907). Study findings will be disseminated via scientific peer-reviewed publications, conferences, and media, as applicable.

Access to data

Information will be provided such that participants cannot be identified. Non-identifiable Individual Participant Data (IPD) will become available one year after study completion and will be available upon reasonable request to the principal investigators.

Author contributions

IL, DM, NSM, AS, CI, AD, CG, RRG, BY, ISM, and CMH were involved in methodological design and creation of the study protocol. BY and CMH are the principal investigators (medical and non-medical, respectively) who have overall responsibility for the design, conduct, and decision to submit for publication. NSM is the study epidemiologist and statistician who will design and publish the analysis plan in collaboration with IL. IL is the trial coordinator responsible for collecting trial data. IL drafted the manuscript. Investigator roles and responsibilities are outlined in Table 1. All authors have read and approved the final manuscript.

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Competing interest statement

ISM is the Academic Director of the Lambert Initiative for Cannabinoid Therapeutics, Brain and Mind Centre, University of Sydney. ISM is a consultant for Kinoxis Therapeutics, Psylo Ltd, and Emyria and is an inventor on several patents relating to novel cannabinoid and non-cannabinoid therapeutics unrelated to insomnia. He has received consulting fees from the Medicinal Cannabis Industry Australia (MCIA) and acts as an expert witness in legal cases involving cannabis-related issues. AS has received consulting fees from the Medicinal Cannabis Industry Australia (MCIA) and Haleon Australia. RRG has received discounted investigational products for an unrelated clinical trial from Neurim Pharmaceuticals Inc and received investigational product and matched placebo from Teva Pharmaceutical in unrelated clinical trials. He has received funding for lectures for Pfizer, Teva, Jazz and Eisai in the past 3 years. The other authors have no competing interests to disclose. The Woolcock Institute Sleep and Chronobiology Research Group has received research support from Avadel, Nyxoah, Idorsa, ResMed, BOD Australia, and Philips.

Patient consent for publication

Not required.

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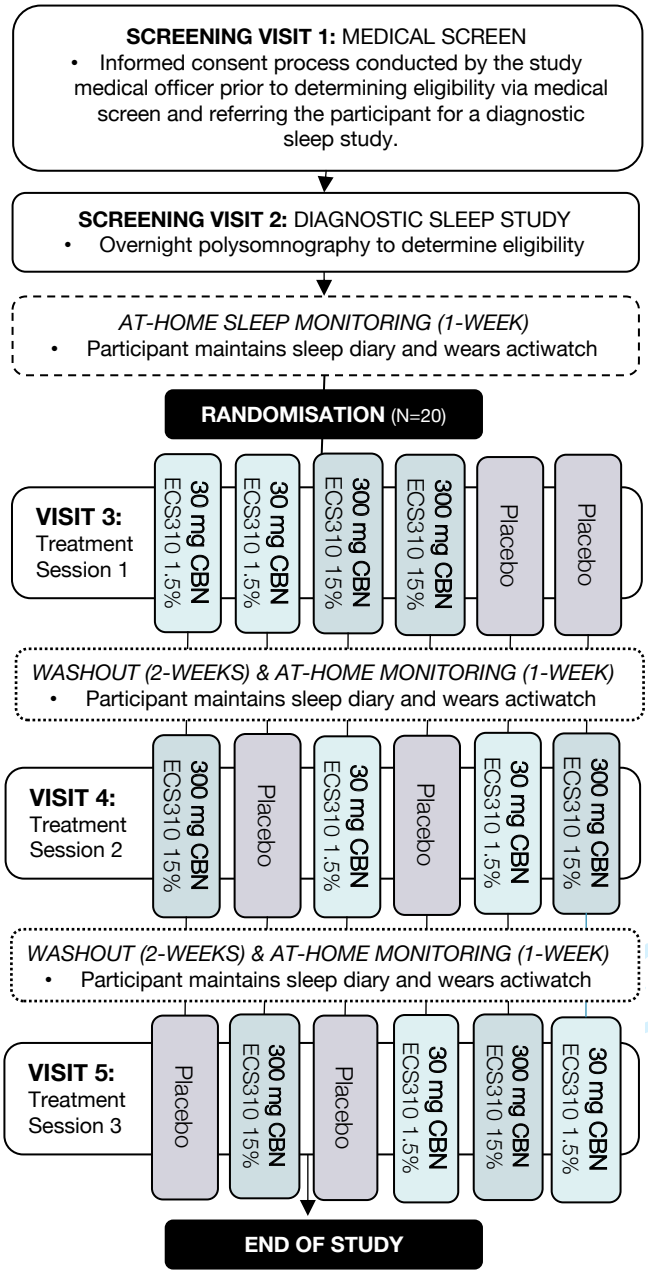
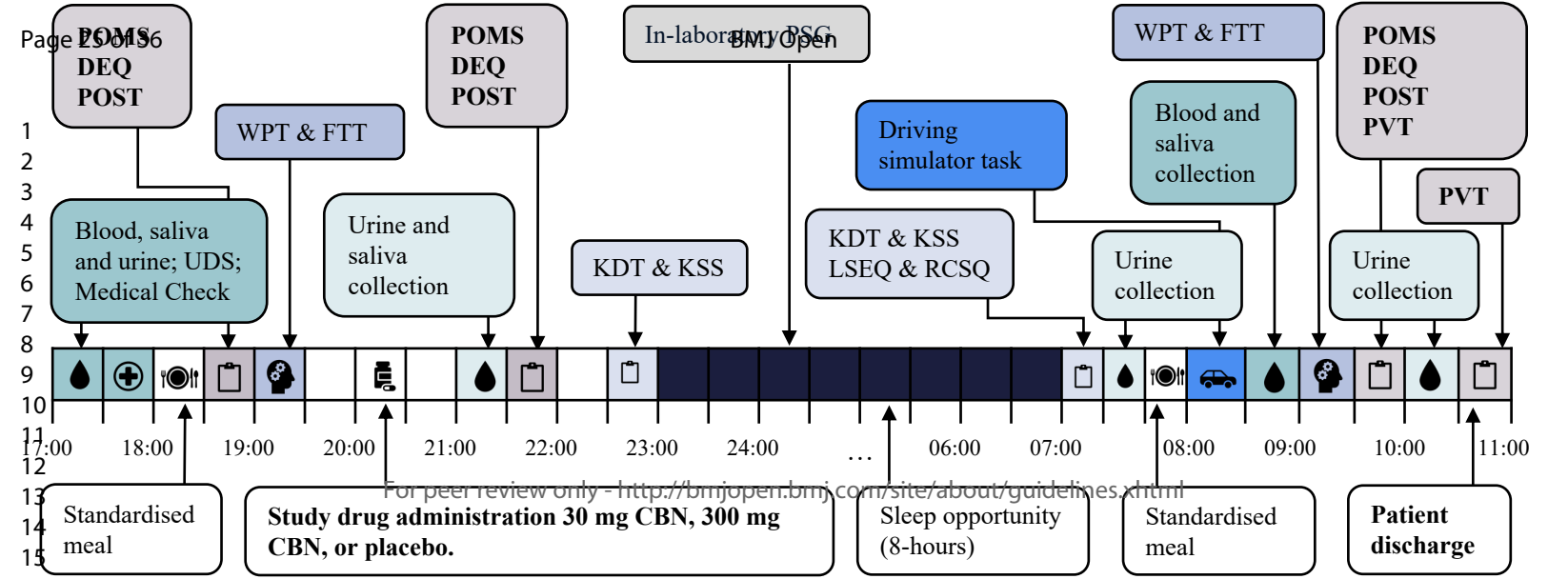

SCREENING VISIT 1: MEDICAL SCREEN
• Informed consent process conducted by the study medical officer prior to determining eligibility via medical screen and referring the participant for a diagnostic sleep study.
SCREENING VISIT 2: DIAGNOSTIC SLEEP STUDY
• Overnight polysomnography to determine eligibility
AT-HOME SLEEP MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
RANDOMISATION (N=20)
VISIT 3: Treatment Session 1
30 mg CBN ECS310 1.5%
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
300 mg CBN ECS310 15%
Placebo
Placebo
WASHOUT (2-WEEKS) & AT-HOME MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
VISIT 4: Treatment Session 2
300 mg CBN ECS310 15%
Placebo
30 mg CBN ECS310 1.5%
Placebo
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
WASHOUT (2-WEEKS) & AT-HOME MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
VISIT 5: Treatment Session 3
Placebo
300 mg CBN ECS310 15%
Placebo
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
30 mg CBN ECS310 1.5%
END OF STUDY



SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1,2,5,7
	2b	All items from the World Health Organization Trial Registration Data Set	In trial registration
Protocol version	3	Date and version identifier	7
Funding	4	Sources and types of financial, material, and other support	1,2,6,18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,6,16-18
	5b	Name and contact information for the trial sponsor	1, 6, and trial registration
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	6-7,18

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16-18
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6-7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-9 (Box 2)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	7-9 (Box 2)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 14
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-9 (Box 2)

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	4-6 (Box 1), trial registry
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 1; Figures 1-2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10-11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10-11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10-11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11, 17-18
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10, 15
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16-17
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15-18
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	17
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	16
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1, 2, 7, 17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	7
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9-10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15-16
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	1, 10, 16-18
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	1, 2, 17
	31b	Authorship eligibility guidelines and any intended use of professional writers	17
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	18; trial registry
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary material

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	13

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

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Protocol version	3	Date and version identifier	7~~5~~
Funding	4	Sources and types of financial, material, and other support	1,2,6,18~~5,16~~
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,6,~~5,14~~-16-18
	5b	Name and contact information for the trial sponsor	1, 6, and trial registration
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	6-7,18~~5,16~~

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	~~14~~-16-~~18~~
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4-~~5~~
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4
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Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6-7~~5~~
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	~~6~~-7-9 (Box 2~~1~~)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9~~7~~
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	~~6~~-7-9 (Box 2~~1~~)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 14~~7, 13~~
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Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	4-6 (Box 1),, trial registry
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 1; Figures 1-2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	97
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	97
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10-118
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10-118
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10-118
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11, 17-188
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	108
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-169-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10, 157
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16-1713-14
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	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	1814
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	1814
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Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15-1813-14
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	1713-14
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	1513-14
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Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1, 2, 7, 175
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	75
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9-107-8
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15-16 13-14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	181,16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	1, 10, 16-1813
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	1, 2, 1714
	31b	Authorship eligibility guidelines and any intended use of professional writers	1716
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	1816; trial registry
Appendices			
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BMJ Open

Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ('CUPID' study): Protocol for a randomised, double-blind, placebo-controlled, crossover, three-arm, proof-of-concept trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-071148.R2
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Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ('CUPID' study): Protocol for a randomised, double-blind, placebo-controlled, crossover, three-arm, proof-of-concept trial.

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Abstract (277/300 words)

Objective: Insomnia is the most prevalent sleep disorder, with few effective pharmacotherapies. Anecdotal reports and recent preclinical research suggest that cannabinol (CBN), a constituent of *Cannabis sativa* derived from delta-9-tetrahydrocannbinol (THC), could be an effective treatment. Despite this, the isolated effects of CBN on sleep have yet to be systematically studied in humans. **Methods:** The present protocol paper describes a randomised, double-blind, placebo-controlled, single-dose, three-arm, crossover, proof-of-concept study which investigates the effects of CBN on sleep and next-day function in 20 participants with clinician-diagnosed insomnia disorder and an Insomnia Severity Index [ISI] Score ≥15. Participants receive a single fixed oral liquid dose of 30 mg CBN, 300 mg CBN, and matched placebo, in random order on three treatment nights; each separated by a two-week washout period. Participants undergo overnight sleep assessment using in-laboratory polysomnography and next-day neurobehavioral function tests. The primary outcome is wake after sleep onset (WASO) minutes. Secondary outcomes include changes to traditional sleep staging, sleep onset latency, and absolute spectral power during non-rapid eye movement (NREM) sleep. Tertiary outcomes include changes to sleep spindles during NREM sleep, arousal indices, absolute spectral power during rapid eye movement (REM) sleep, and subjective sleep quality. Safety-related and exploratory outcomes include changes to next-day simulated driving performance, subjective mood and drug effects, postural sway, alertness and reaction time, overnight memory consolidation, pre and post subjective and objective sleepiness, and plasma, urinary, and salivary cannabinoid concentrations. The study will provide novel preliminary data on CBN efficacy and safety in insomnia disorder, which will inform larger clinical trials. **Ethics and Dissemination:** Human Research Ethics Committee approval has been granted by Bellberry Limited (2021-08-907). Study findings will be disseminated in a peer-reviewed journal and at academic conferences. ClinicalTrials.gov Identifier: NCT05344170.

Key words: Power Spectral Analysis; Resting Wake Electroencephalography; Psychomotor Vigilance Test; Karolinska Drowsiness Test; Karolinska Sleepiness Scale; Leeds Sleep Evaluation Questionnaire; Standard Deviation of Lateral Position; Liquid Chromatography–Mass Spectrometry.

Strengths and limitations of the study

- This study uses a randomised, double-blind, placebo-controlled, crossover design to investigate two active doses of CBN isolate on sleep and neurobehavioral function over ~17-hours using gold-standard objective measures, validated subjective measures, and rigorous design methodology.
- Study participants undergo extensive screening with a sleep physician to confirm insomnia disorder diagnosis, including an overnight diagnostic polysomnogram to rule out other sleep disorders that commonly co-occur with insomnia.
- The study washout period (2-weeks) was chosen to minimise participant burden, as participants cannot undergo insomnia treatment before (3-months) or during (~2 months) the protocol; however, this may lead to carry-over effects between doses as a recent crossover study demonstrated oral cannabinoids can persist in plasma for >4 weeks (1500 mg; single dose).
- The study involves a single-dose, in-laboratory proof-of-concept design and results may, therefore, lack ecological validity (repeated CBN dosing may be necessary to effectively treat insomnia).

Background

Insomnia is a sleep disorder characterised by subjective difficulty falling asleep, maintaining sleep, and/or achieving restorative sleep, where symptoms persist for ≥3-months and are accompanied by daytime sequalae of dysfunction and/or distress.[1] Insomnia disorder is the most prevalent sleep disorder which affects between 10–30% of adults[2] and persists in 37% of cases at 5-year follow-up.[3] Insomnia disorder increases the risk of mental and physical illnesses such as depression [4] and dementia.[5] The economic burden of insomnia disorder is estimated at >$13 billion per annum in Australia.[6]

Insomnia disorder treatments are typically behavioral or pharmacological in nature. Cognitive behavioural therapy for insomnia (CBTi) is the first-line treatment and its efficacy has been confirmed in recent meta-analyses.[7] Nevertheless, significant access barriers to CBTi exist including limited providers, high cost,[8] and delayed perception of subjective benefits.[9] CBTi may also be less effective in individuals with shorter sleep duration (i.e., <6 hours/night).[10] As such, sedative-hypnotics are commonly used in primary care.[7] While effective in the short-term, well-documented side effects include daytime sedation, psychomotor impairment, addiction/dependence, and premature mortality.[11] There is a clear need for safe pharmacological insomnia disorder treatments.

Cannabis sativa is a complex plant containing numerous potentially therapeutic chemical compounds, including over 140 constituent '*cannabinoids*'.[12] As historical legal prohibitions against cannabis are relaxed, cannabinoids are being increasingly used to aid sleep.[13] The most well researched cannabinoids are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD); however, in recent times, *cannabinol* (CBN) has become recognized as a putative 'sleep-enhancing' cannabinoid.[14] CBN is produced via the nonenzymatic oxidation of THC and is therefore present at higher concentrations in aged (rather than 'young') cannabis plant material.[15] This is significant as aged cannabis plant material is anecdotally reported to induce sleep.[16] CBN products are also being sold as sleep aids in unregulated markets.[17] Importantly, a recent narrative review of CBN highlighted insufficient evidence to support sleep-related claims, despite a plausible mechanism of action.

THC and CBN exert some of their physiological effects via interactions with the *endogenous cannabinoid system* (ECS)[12], comprising of signaling molecules termed '*endocannabinoids*' (e.g., anandamide, 2-Arachidonoylglycerol [2-AG]), their receptors, and enzymes.[18, 19] The ECS is involved in regulating a myriad of biological processes including circadian rhythmicity[20]. Systematic administration of anandamide has been shown to promote sleep in rodents and humans [18, 21, 22] via activation of the cannabinoid receptor 1 (CB_1R).[21, 23] As such, the CB_1R is considered a promising therapeutic target.[24] The pharmacological effects of THC occur via partial agonism at CB_1R[25] and can include acute intoxication, psychomotor impairment, sedation, and changes in sleep architecture.[18] CBN also has agonist actions at CB_1R, albeit approximately 10-times less potent than THC[25] which may potentially account for the low levels of intoxication reported in historic CBN studies.[26, 27] Minimal intoxication could render CBN a more practical and safer alternative to THC as a sleep aid.

Two recent clinical studies administered small quantities of CBN in multi-cannabinoid formulations.[28, 29] The first, a crossover randomized controlled trial (RCT) with a one-week washout, investigated two-weeks of an oral liquid containing 20 mg THC, 2 mg CBN, and 1 mg CBD in 20 adults with insomnia disorder. The drug significantly improved insomnia disorder symptoms (Insomnia Severity Index [ISI] -5.1 points, p=0.0001, d=0.94) compared to placebo, with no significant changes to polysomnography (measured on night 14 of dosing).[28] In an interventional open-label

study, current medicinal cannabis users with subjective sleep difficulty reported improved sleep (assessed via an unvalidated subjective survey and a validated non-contact at-home tracking device) after three weeks of nightly administration of an oral capsule containing 10 mg THC and 5 mg CBN.[29] Importantly, the effects of CBN cannot be disentangled from those of THC in these studies. We are not aware of any clinical studies to-date that have tested the isolated effects of CBN on objectively measured human sleep.

Importantly, a recently published conference abstract reported on a preclinical study in which CBN isolate (10, 30, and 100 mg/kg intraperitoneally compared with zolpidem 10 mg/kg as a positive control) increased the proportion of non-rapid eye movement (NREM) sleep and sleep bout duration 4-hours post administration in Long-Evan rats.[30] With the lowest dose, biphasic effects were observed – CBN (10mg/kg) initially decreased rapid eye movement (REM) sleep proportion; however, 4 hours after administration, a significant increase in the percentage of REM sleep occurred. These results suggest CBN could reduce wake after sleep onset (WASO), given such delayed effects on sleep.

Study design and aim

Here we described the protocol for a randomised, double-blinded, placebo-controlled, three-arm, crossover, single-site, proof-of-concept study design to investigate the acute effects of oral CBN on sleep and next-day function in 20 participants with clinician-diagnosed insomnia disorder. The primary study aim is to investigate the effects of CBN (30 and 300 mg) versus placebo on sleep in insomnia disorder. The study evaluates the safety and efficacy of CBN as a pharmacological therapy and generate preliminary data to inform larger clinical trials. Many outcome measures used in insomnia disorder clinical trials are known to be susceptible to placebo effects. A placebo control is scientifically and ethically defensible choice as the effects of CBN on human sleep are unknown and could not be properly elucidated if an active treatment such as zolpidem was the comparator rather than placebo.

Study outcomes

The primary study outcome is WASO measured in minutes using in-laboratory overnight polysomnography, from the first epoch after lights out until the last epoch, scored as any stage of sleep by an experienced polysomnographic technician in accordance with American Academy of Sleep Medicine (AASM) 2020 Sleep Scoring criteria (Version 2.6).[31] Secondary study outcomes include:

1. Traditional sleep staging: Proportion of the sleep opportunity scored at the 5 stages (wake, and N1, N2, N3, and REM sleep) between lights out and lights on, measured using overnight in-laboratory polysomnography, scored by a polysomnography technician in accordance with AASM Sleep Scoring criteria.
2. Sleep Onset Latency (SOL): SOL measured in minutes using in-laboratory polysomnography, calculated from the time of lights out to the first sleep epoch as scored by a polysomnographic technician in accordance with AASM Sleep Scoring criteria.
3. Absolute Electroencephalographic (EEG) Power During NREM Sleep: Spectral power of delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequency ranges between treatment arms. Power spectral analysis will be applied to EEG signals from polysomnography after artefacts are detected and removed.

Tertiary, safety, and exploratory study outcomes are described in Box 1 (see ClinicalTrials.gov Identifier: NCT05344170).

Box 1. *Tertiary, safety, and exploratory study outcomes*

Tertiary outcomes

1. **Sleep Spindles During Non-Rapid Eye Movement (NREM) Sleep**: Sleep spindle and slow oscillation events in NREM sleep from in-laboratory overnight polysomnography. A sleep spindle and slow oscillation detection algorithm will be applied to electroencephalography (EEG) signals from polysomnography after artefacts are detected and removed. Comparisons between each CBN dose versus placebo.
2. **EEG Arousal Index:** Number of cortical arousals captured via the electroencephalogram per hour of sleep scored by the polysomnographic technician on the polysomnogram in accordance with AASM Sleep Scoring criteria. Comparisons between each CBN dose versus placebo.
3. **Absolute EEG Power During Rapid Eye Movement (REM) Sleep:** Spectral power of delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequency ranges between treatment arms. Power spectral analysis will be applied to EEG signals from polysomnography after artefacts are detected and removed. Comparisons between each CBN dose versus placebo.
4. **Next-Day Post Wake Subjective Sleep Evaluation (LSEQ):** RCSQ score. LSEQ score. LSEQ scores range from 0-100, with higher scores indicating better subjective experience. Assessed within 1 h after wake (comparison between each CBN dose versus placebo).
5. **Next-Day Post Wake Subjective Sleep Evaluation (RCSQ):** RCSQ score. RCSQ scores range from 0-100, with higher scores indicating better subjective experience. Assessed within 1 h after wake (comparison between each CBN dose versus placebo).

Safety outcomes

1. **Standard Deviation of Lateral Position (SDLP) During Next-day Post-Wake Simulated Drive:** SDLP ("weaving") is measured across the 'standard', 'car following', and 'divided attention' sub-sections of a ~30 minute simulated driving task. Assessed within 2 h after wake (comparison between each CBN dose versus placebo)
2. **Speed During Next-day Post-Wake Simulated Drive**: Average speed and standard deviation of speed is measured across the 'standard' and 'divided attention' sub-sections of a ~30 minute simulated driving task. Assessed within 2 h after wake (comparison between each CBN dose versus placebo).
3. **Distance Headway During Next-day Post-Wake Simulated Drive**: Average distance headway (i.e., distance between the driver's vehicle and vehicle immediately in front) and standard deviation of distance headway is measured across the 'car following' sub-section of a ~30-minute simulated driving task. Assessed within 2 h after wake at both treatment sessions (comparison between each CBN dose versus placebo).
4. **Subjective Mood Evaluation**: The Abbreviated Profile of Mood States (POMS) consists of 40 items measuring domains of 'tension', 'depressed', 'anger', 'vigour', 'fatigue', and 'concentration'. Participants respond to each item using 5-point Likert scales ranging from 0 (Not at all) to 4 (Extremely). A total mood disturbance score is calculated by summing negative domains and subtracting positive domains. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose versus placebo).
5. **Subjective Drug Effects:** The Drug Effects Questionnaire (DEQ) assesses the extent to which participants feel a drug effects, feel high, like the effects, dislike the effects, want more of the substance, and feel sedated, on self-rating 100mm visual analogue scales. A total mood disturbance score is calculated by summing negative domains and subtracting positive domains. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose versus placebo).
6. **Postural Sway:** Centre-of-pressure (COP) during computerised static posturography. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose versus placebo).
7. **Behavioural Alertness and Reaction Time:** Psychomotor Vigilance Test (PVT) is administered twice the next-day (comparison between each CBN dose versus placebo).

8. **Overnight Verbal Declarative Memory Consolidation**: Word pair recall scores measured using the computerised Word Pairs Task (WPT). Administered pre-drug administration and next-day (comparison between each CBN dose versus placebo).
9. **Overnight Procedural Memory Consolidation**: Motor sequence learning measured using the computerised Finger Tapping Task (FTT). Administered pre-drug administration and next-day (comparison between each CBN dose versus placebo).
10. **Resting Wake EEG Power After Sleep:** Resting wake EEG power during the Karolinska Drowsiness Test (KDT) upon wake: delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequency ranges. Power spectral analysis is applied to EEG signals from polysomnography, after artefacts are detected and removed. Comparison between each CBN dose versus placebo.
11. **Subjective Sleepiness After Sleep:** The Karolinska Sleepiness Scale (KSS) is a 10-item measure of subjective drowsiness. Participants respond to each item using a 9-point Likert scale ranging from 1 (Extremely alert) to 9 (Extremely sleepy). Higher scores are indicative of increased drowsiness. The KSS will be collected in accordance with the KDT protocol but will not be analysed due to insufficient statistical power.

Exploratory outcomes

1. **Resting Wake Electroencephalography (EEG) Power Before Sleep** (Acute Effects of CBN). Resting wake EEG power during the KDT prior to sleep: delta (1-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-15 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequency ranges. Power spectral analysis is applied to EEG signals from polysomnography, after artefacts are detected and removed. Comparison between each CBN dose versus placebo.
2. **Subjective Sleepiness Before Sleep** (Acute Effects): The KSS is a 10-item measure of subjective drowsiness. Participants respond to each item using 9-point Likert scales ranging from 1 (Extremely alert) to 9 (Extremely sleepy). Higher scores are indicative of higher drowsiness. The KSS will be collected in accordance with the KDT protocol but will not be analysed due to insufficient statistical power.
3. **Plasma Cannabinoid Concentrations**: Presence of cannabinoids (CBN, delta-9-tetrahydrocannabinol [THC], and cannabidiol [CBD]) (e.g., 11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD), and endocannabinoids and related molecules (e.g., 2-Arachidonoylglyceroland anandamide) and their metabolites in plasma samples.
4. **Urinary Cannabinoid Concentrations:** Presence of cannabinoids (CBN, THC, and CBD) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD) in urine samples.
5. **Salivary Cannabinoid Concentrations:** Presence of cannabinoids (THC, CBN) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC) in saliva samples.

Safety

The investigational product, CBN, is currently a Schedule 9 'Prohibited Substance' in Australia.[32] The safety profile of CBN can be inferred from human studies conducted between 1973 and 1987. These studies explored oral doses up to 1200 mg CBN with some administering repeated daily doses of CBN for up to 4 weeks.[26, 33-35] There were no notable safety concerns, aberrations or toxicity concerns from these studies, with no adverse changes in parameters such as heart rate, blood pressure, body temperature, respiratory rate, perception, intoxication and postural stability.[26, 33-35] None of these studies measured sleep, sedation, or residual next-day effects of CBN (such as adverse effects on driving performance and cognitive function). These measures are obtained in the current study. CBN is currently being investigated as an analgesic agent (ClinicalTrials.gov Identifier: NCT03675971) and topical treatment for epidermolysis bullosa (ClinicalTrials.gov Identifier: NCT04908215). An RCT of oral CBN isolate (compared with combination CBN/CBD and placebo doses) on the subjective sleep

of healthy adults was retrospectively registered in May 2022 (ClinicalTrials.gov Identifier: NCT05839964). We will monitor and review safety reports from these trials as they become available.

Methods

Participants, interventions, and outcomes

Trial setting

The trial Sponsor and site is the Woolcock Institute of Medical Research, a specialist sleep and respiratory research institute and clinic (Sydney, Australia). The study funder is the Lambert Initiative for Cannabinoid Therapeutics, University of Sydney (Sydney, Australia), a philanthropically funded centre for cannabinoid science. Recruitment commenced in August 2022 and is anticipated to conclude in late 2023. The first participant was enrolled the 24th of August 2022 and the first participant was randomised on the 13th of October 2022. The study has ethical approval from Bellberry Limited Human Research Ethics Committee (HREC; 2021-08-907; Version 2.1, 01-Aug-2022) and is registered on ClinicalTrials.gov (Identifier: NCT05344170; 25-April-2022). The clinical trial protocol is available on request. Significant changes to the study protocol will be documented on ClinicalTrials.gov. The study protocol was developed according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement.[36] The Therapeutics Goods Administration (TGA) has acknowledged the use of CBN in this study through the Clinical Trial Notification (CTN) scheme (CTN Number: CT-2022-CTN-00543-1).

Participant eligibility

Eligible participants are aged between 25-65 years with physician diagnosed insomnia disorder as per the International Classification of Sleep Disorders-3 (ICSD-3)[37] and Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[38] criteria as determined by the study physician (see Box 2). The age range was selected to minimise age-related changes to sleep architecture.[39] As cannabinoids have been shown to improve symptoms (e.g., anxiety, pain) that commonly impair sleep (i.e., causing 'secondary' insomnia disorder),[40] a 'primary' insomnia disorder population is being recruited for this study.

Box 2

Inclusion and exclusion criteria for study participants

Eligible individuals must fulfill the following criteria:

1. Between 25 – 65 years of age
2. Insomnia Severity Index (ISI) score ≥ 15 at eligibility screening
3. Insomnia disorder (symptoms occurring at least 3 times per week and present for longer than 3 months) as determined by the study physician
4. Ability to take oral medication
5. Provision of signed and dated informed consent form
6. Stated willingness to comply with all study procedures and availability for the duration of the study

Individuals who meet any of the following criteria are ineligible to participate in the study:

1. Medical condition or medication that is the cause of the insomnia disorder as determined by the study physician
2. Known hypersensitivity to cannabis or cannabinoid products (including if this becomes evident during the trial)

3. Reported use of cannabis or cannabinoid products within the past 3 months as confirmed by at least one negative urine drug screen (UDS) (or at the study physician's discretion)
4. Sleep apnoea (defined as Apnoea Hypopnea Index [AHI] >15 and Oxygen Desaturation Index [ODI]>10) as confirmed by polysomnography at screening
5. Sleep-related movement disorder as determined by the study physician
6. Delayed or advanced sleep phase syndrome (based on actigraphy and sleep diary) as confirmed during screening
7. Any medical condition that produces an abnormal EEG (i.e., epilepsy, brain injury)
8. Clinically relevant cardiovascular abnormalities as determined by the study physician and a 12-lead electrocardiogram (ECG) at screening
9. Shift work or trans meridian travel (two time zones) within the last month
10. History of major psychiatric disorder in the past 12 months at the study physician's discretion, except clinically managed mild depression and/or anxiety
11. History of suicide attempt or current suicide ideation (score greater than 1 on Q9 of the Patient Health Questionnaire [PHQ-9])[41]
12. Pregnancy or lactating. Female participants are required to complete a urine pregnancy test at screening and treatment sessions and all participants are instructed to use a reliable form of contraception throughout the study duration
13. History of drug or alcohol dependency or abuse within approximately the past 2 years
14. Use of CNS-active drugs (cannabis, amphetamines, cocaine, antidepressants, opioids, benzodiazepines) in the past 3 months as confirmed by a positive urine drug test at screening or at the study physician's discretion
15. Use of medications that may have a clinically significant impact upon the metabolism and excretion of cannabinoids as determined by the study physician (e.g., CYP450 enzyme inducers/inhibitors
16. Excessive caffeine use that in the opinion of the study physician contributes to the participant's insomnia disorder, or the inability to abstain from caffeine use 24 hours prior to each overnight sleep study
17. Inability to refrain from alcohol consumption 24 hours prior to each overnight sleep study
18. Individuals with nicotine dependence (i.e., daily smokers)
19. Medical conditions that result in frequent need to get out of bed (e.g., sleep walking, nocturia)
20. Psychological or behavioural treatment for insomnia disorder, including cognitive behavioural therapy for insomnia, within 3 months before screening (excluding sleep hygiene advice)
21. Occupational or judicially ordered drug screening
22. Has held an unrestricted driving license <1 year
23. Cannot speak English fluently

Patient and Public Involvement

Patients and/or the public were not involved in the design, conduct, reporting, nor dissemination plans of this research.

Intervention

Study doses

Participants receive a single fixed 2 mL oral liquid dose of: (1) 30 mg CBN ('ECS310' 1.5% containing 15 mg/mL CBN); (2) 300 mg CBN ('ECS310' 15% containing 150 mg/mL CBN); and (3) matched placebo. Treatments are administered across three separate overnight Treatment Sessions in randomised order two-hours prior to habitual lights out. The lower dose was selected to match the CBN products

available in the United States and supported by customer testimonies.[17] The higher dose was informed by a preclinical study of CBN isolate and sleep.[30]

Investigational product

The investigational drug ('ECS310') is a liquid formulation of CBN suspended in medium chain triglyceride (MCT) oil. The placebo contains MCT oil only. The maximum detectable THC content of the active investigational products is ≤ 150 µg. Neither the study drug nor matched placebo contain any other cannabinoids or terpenes/terpenoids. The study drug and matched placebo are expected to be identical in their visual appearance, taste, and smell. The MCT oil contains paprika oleoresin colorant to ensure the double-blind is maintained and prevent oxidation. The Sponsor purchased the investigational product from BOD Australia (Sydney, Australia), who sourced investigational products from Medropharm (Zürich, Switzerland). Stability studies supporting the shelf life applied to the drug product were conducted in accordance with the International Conference on Harmonization (ICH) Guideline Q1A Stability Testing of new Drug Substances and Products and are ongoing. The CBN solution stability is 24 months at 15-25°C, 60% humidity, and away from sunlight.

Recruitment and retention

We aimed to recruit 20 participants over an 18-month period. The sample size was selected with consideration for practical factors such as time, cost, and resource allocation (rather than using formal statistical techniques) as it is pilot in nature. Participants are recruited from social media, the Woolcock Institute Volunteer Database, and locally displayed physical study advertisements. Participants are reimbursed a fixed amount equivalent to that used in previous clinical trials with similar participant involvement and loss of working hours.[42] To encourage retention, participants receive reimbursement upon completion of the final Treatment Session. Study advertisements include an embedded link and QR code to the study website (described below).

Eligibility screening

Eligibility assessment is performed in three steps undertaken by the study team at the Woolcock Institute of Medical Research (see Figure 1 *Study Flow Diagram*):

1. *On-line and telephone pre-screening*. Interested individuals are directed to the study webpage. They provide electronic consent before completing an online pre-screening questionnaire to assess initial eligibility (e.g., age, location, ISI score, transmeridian travel, shift work). Potentially suitable individuals can enter their contact details and are provided the Participant Information Statement so that the trial coordinator may conduct a brief telephone interview before scheduling screening appointments. Individuals deemed unsuitable are provided with support resources.
2. *Screening visit 1*. Willing and potentially suitable participants attend an on-site medical screening. The study physician obtains written informed consent before conducting a screening interview to compile medical history, assess suitability against study eligibility criteria (Box 2), and diagnose insomnia disorder (ICSD-3[37] and DSM-V[38]). The following validated self-report questionnaires are used to evaluate eligibility: ISI,[43] a measure of insomnia disorder symptom severity over the past two-weeks; Epworth Sleepiness Scale (ESS),[44] a measure of sleep propensity in daily life; Hospital Anxiety and Depression Scale (HADS),[45] a measure of anxiety and depression; and, the Patient Health Questionnaire-9 (PHQ-9),[41] an instrument for screening and monitoring the severity of depression and suicidal ideation. An electrocardiogram (ECG) and urinary screen (pregnancy and recent alcohol, cannabis, cocaine, benzodiazepines, opiates, or amphetamines, use) are also conducted. Participants must agree to

the study protocol, including use of contraception and refraining from driving until the day after Treatment Session discharge.

3. *Screening visit 2*. Participants are required to complete a clinical overnight polysomnography to rule out the presence of other sleep disorders (obstructive sleep apnoea or sleep-related movement disorder, see Box 2). Diagnostic polysomnography results within the previous 12-months may be used where weight has not changed substantially in that time (at discretion of the study physician). Diagnostic sleep studies are scored by an experienced sleep technician[46] and reviewed by the study physician.

Insert Figure 1 here

Figure 1. *Study flow diagram*

Randomisation, allocation concealment, and blinding

Participants are allocated to one of six possible treatment orders in a 1:1:1:1:1:1 ratio using a pre-populated randomisation schedule generated by the study epidemiologist (NSM). The six-orders constitute a balanced Latin square. Sequences were computer-generated and are stored in a password-protected system inaccessible to blinded study personnel (centralised computerised randomisation). Allocation concealment is managed by the unblinded study epidemiologist and an independent staff member who do not have any contact with participants, nor involvement in day-to-day trial activities. A study physician may request to be unblinded in the event of a medical emergency. Participants who are enrolled in the study, but not yet randomised, are assigned a unique screening number. Randomisation occurs at the outset of the first Treatment Session assuming participant eligibility persists. Working under the study physician, the trial coordinator records the decision to randomise the patient. The study database then issues an irrevocable randomisation number for that participant. Investigational product is stored in opaque sequentially numbered containers labelled with the patient randomisation and Treatment Session number to maintain blinding of all patients, staff, and outcome assessors. The drug manufacturer packaged and labelled the investigational product according to the randomisation list generated by the study epidemiologist.

Pre-Treatment Session procedures

Participants are asked to maintain a regular sleep-wake schedule (i.e., lights out between 22-23:00 each evening) for one week prior to each Treatment Session. Participants wear a wrist-worn actigraphy monitor (GENEActive, Activinsights, United Kingdom) and complete a daily sleep diary (Karolinska Sleep Diary [KSS][47] modified to capture additional information, such as naps, alcohol/caffeine consumption, and GENEactiv removal times) throughout this period. If the sleep schedule is significantly irregular during the 7-night leadup (e.g., > 2 hours on > 2 nights), the principal medical and non-medical investigators may reschedule the visit. Participants are required to abstain from consuming cannabis, cannabinoids, and other CNS-active drugs throughout the entire study; and avoid alcohol and caffeine 24 hours prior to Treatment Sessions. Urinary drug, alcohol (DrugCheck NxStep OnSite, USA), and pregnancy (SureStep, Germany) screening occurs at the outset of each Treatment Session.

Treatment Session procedures

Participants arrive at the Woolcock Sleep Clinic in the early evening (~17:00-18:00 depending on habitual lights out), where they remain until late morning (~10-11:00) the following day, as per the Treatment Session schedule in Figure 2. Standardised meals are provided. All Treatment Sessions are separated by a washout period of ≥ 2-weeks to minimise carry-over effects between doses.[48]

Study drug administration

After a brief medical examination, the study physician prescribes and dispenses the study drug, drawing a fixed 2 mL volume of liquid into a pre-labelled amber plastic syringe. Participants self-administer the randomly allocated study drug 2-hours prior to lights out. The trial coordinator observes drug administration to ensure accuracy of dosing. The dose is documented in participant records and accountability logs. Timing of administration has been informed by delayed onset of effects observed with oral dosing of other cannabinoids[49] and delayed effects observed in a preclinical study of CBN.[30]

Data collection

Study measures are described below (see Table 1).

Table 1. *A summary of assessments completed throughout the investigation.*

Measure (ordered first to last)	Screening	Treatment Session 1-3		
		At-home sleep monitoring	Night	Day
Informed consent	●			
Medical examination	●		●	
Baseline questionnaires (ISI, ESS, HADS & PHQ-9)	●			
Electrocardiogram	●			
Urinary drug and alcohol screen	●		●	
Pregnancy test	●		●	
Overnight polysomnography (clinical EEG)	●		●	
Actigraphy		●		
Sleep diary (KSD)		●		
Oral fluid drug screen (Securetech DrugWipe)			●	●
Quantisal saliva collection			●	●
Blood plasma collection			●	●
Urine collection			●	●
Mood (POMS)			●	●
Drug effects (DEQ)			●	●
Balance (posturography)			●	●
Memory (FTT & WPT)			●	●
KDT with EEG			●	●
Sleep questionnaires (LSEQ, RCSQ, & KSS)				●
Simulated driving performance				●
PVT				●

Note. DEQ=Drug Effects Questionnaire; EEG=electroencephalography; ESS=Epworth Sleepiness Scale; FTT=Finger Tapping Task; HADS=Hospital Anxiety Depression Scale; ISI=Insomnia Severity Index; KSD=Karolinska Sleep Diary; KSS, Karolinska Sleepiness Scale; LSEQ= Leeds Sleep Evaluation Questionnaire; PHQ-9=Patient Health Questionnaire-9; POMS=Profile of Mood States; PSG=polysomnography; PVT=Psychomotor Vigilance Task; RCSQ=Richard Campbell Sleep Questionnaire; WPT=Word Pairs Task.

Insert Figure 2 here

Figure 2. *Schedule of events during each Treatment Session.*
Note. DEQ=Drug Effects Questionnaire; EEG=electroencephalography; FTT=Finger Tapping Task; ISI=Insomnia Severity Index; KSD=Karolinska Sleep Diary; KSS=Karolinska Sleepiness Scale; LSEQ= Leeds Sleep Evaluation Questionnaire; POMS=Profile of Mood States; POST=Posturography; PSG=polysomnography; PVT=Psychomotor Vigilance Task; RCSQ=Richard Campbell Sleep Questionnaire; UDS=Urinary Drug Screen; WPT=Word Pairs Task.

Blood collection and plasma cannabinoid levels

Venous blood samples are taken at baseline (~17:00) and approximately 13-hours post administration (~08:45). Samples are drawn into EDTA vacutainers (Becton, Dickinson and Company, New Jersey, USA) and centrifuged (2500×g for 15 min at 4°C). Supernatant plasma is aliquoted into 1.5 mL Eppendorf Tubes and stored at -80ºC in the Woolcock Institute of Medical Research until study completion when samples will be transferred to the Lambert Initiative Laboratory. Samples will undergo liquid chromatography–mass spectrometry (LC–MS/MS) analysis, using previously published methodology.[50-52] Plasma will be analysed for cannabinoids (e.g., CBN, THC, CBD), their metabolites (e.g., 11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD), and endocannabinoids and related molecules (e.g., 2-AG and anandamide). THC and CBD and their metabolites are being tested to verify abstinence from common cannabinoids, notwithstanding the trace quantities of THC that the investigational product may contain (≤ 150 µg/2ml).

Urine collection and urinary cannabinoid levels

Urine collection occurs at baseline (~17:00), and approximately 90-minutes (~21:30), 11-hours (~07:00), and 14-hours (~10:00) post drug administration. Urine (~6 mL) is aliquoted into 1.5 mL Eppendorf Tubes and stored at -80ºC before being transferred to the Lambert Initiative Laboratory for quantification of cannabinoids (primarily THC, CBD and CBN) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD), and endocannabinoids at study completion. LC–MS/MS analyses will be conducted using our previously described methods .[50, 51] It is important to conduct urinary analyses to further characterise the pharmacokinetic profile of CBN, which is currently not well described.

Salivary drug testing

Oral fluid testing occurs at baseline (~17:00), and at approximately 75-minutes (~21:15) and 13-hours (~08:55) post drug administration using the DrugWipe® 5S (DW5s; Securetec, Neubiberg, Germany) and Quantisal™ collection (Immunalysis, Pomona, CA) devices. The DW5s collects oral fluid (approximately 10–20 µL) from the tongue. The DW5s is commonly used in routine roadside drug tests; however, demonstrates variable sensitivity (22-89%), specificity (50-100%), and accuracy (53-94%).[53] While the investigational product contains negligible quantities of THC, similarities in chemical structure between CBN and THC may conceivably lead to false-positive test results that would be a concern should CBN become widely used by patients.[54] As such, the Quantisal™ device is used to collect oral fluid from participants (~1 mL) which will be used in LC–MS/MS quantification of cannabinoid (CBN and THC) and metabolite concentrations. Samples are placed in a stabilising buffer and stored at 4ºC before being transferred to the Lambert Initiative analytical chemistry laboratory for analysis. Analyses occur within three-months of collection.

Cognitive and psychomotor performance

Overnight declarative and procedural memory consolidation is assessed at each Treatment Session using the Word Pairs Task [WPT] and Finger Tapping Task [FTT], respectively. For both tasks, memory encoding during a learning phase occurs prior to drug administration (~19:15-20:00) with next-day retest (~09:00). As THC can acutely impair cognitive function and memory, it is necessary to examine CBN effects in this regard. Contrary to oral dosing studies, in one study, CBN reportedly caused a mild intoxication when administered intravenously at high doses (1.2mg/min)[27]. To investigate possible intoxication-induced bodily sway, postural balance ('posturgraphy') is being measured using the stabilometric balance platform (Advanced Medical Technologies Inc., AccuSwayPLUS Balance platform, MA, USA), which measures centre of pressure in anterior-posterior

(y-axis) and medial-lateral (x-axis) directions using four strain gauge load cells at the bottom of the device. Centre of pressure is assessed in eyes open and eyes closed conditions. Postural balance assessment takes < 5 minutes to complete and is repeated 3 times (i.e., baseline, 60-minutes post drug administration, and next-day ~09:30 hours). The psychomotor vigilance test (PVT) is a commonly used reaction time based measure of attention and fatigue related changes in alertness[55] involving a hand-held box with a red-light emitting diode display of a three-digit millisecond counter (PVT-192, Ambulatory Monitoring Inc, Adsley, NY, USA). During the 10-minute task, participants are instructed to respond as quickly as possible to stimuli appearing at variable intervals (ranging 2-10 seconds). The PVT is repeated twice during next-day testing (~09:00 and ~10:00 hours).

Self-report questionnaires

Mood is evaluated using the Profile of Mood States (POMS; 40-items with 5-point Likert scales)[56] questionnaire, a commonly used rating scale of transient and distinct moods. The Drug Effects Questionnaire (DEQ; 7-items with unipolar 100 mm visual analogue scales [VAS])[57] is used to evaluate subjective drug effect; including strength of drug effect, feeling stoned ('high'), liking/disliking the drug effect, feeling sedated, and feeling anxious. The POMS and DEQ are completed at baseline, ~1-hour post drug administration (~21:00), and the next-day (~09:30). The Leeds Sleep Evaluation Questionnaire (LSEQ; 10-items with 100 mm VAS)[58] is a validated measure of the previous night's sleep quality (sleep onset, maintenance, and quality; including behaviour following waking) in comparison to usual. The Richard Campbell Sleep Questionnaire (RCSQ; 5-item with 100 mm VAS)[59] is used to measure perceived sleep depth, sleep latency (time to fall asleep), number of awakenings, and sleep efficiency and quality. The LSEQ and RCSQ are administered upon wake (~06:15 hours).

Subjective and objective sleepiness

Karolinska Sleepiness Scale (KSS) and Karolinska Drowsiness Test (KDT) are used to measure subjective and objective sleepiness, respectively.[60] The KSS is a single-item rating scale assessing current sleepiness, ranging from 1 (*Extremely alert*) to 9 (*Extremely sleepy – fighting sleep)*. KSS scores are typically compared to the objective drowsiness ascertained using the KDT. During the KDT, resting awake EEG is measured while participants focus on a wall marker, with eyes open and closed across 2-minute intervals. The KSS and KDT are administered immediately prior to sleep (~22:00 hours) and upon wake (~06:00 hours).

Polysomnography

Sleep is assessed using polysomnography (Grael polysomnography, Compumedics, USA), a multiparametric tool used for monitoring and diagnosing sleep disorders. Polysomnography consists of 18 channels recording biophysical changes that occur during sleep, including brain activity (via EEG); muscle activity or skeletal muscle activation (electromyography); eye movement (electrooculogram); cardiac function (ECG); respiratory effort using chest and abdominal belts; and digital pulse oximetry. To improve participant comfort/retention, the pulse oximeter, nasal cannula, and thermistor are only used during screening to detect sleep disordered breathing (Visit 2). Polysomnography equipment is fitted by an experienced sleep technician prior to sleep and is removed the next morning once the KDT has been completed. Sleep and associated events are scored according to AASM 2020 criteria in 30 second epochs.[46] Each patient's three polysomnograms are scored by the same sleep technician blinded to treatment allocation. For quantitative EEG analyses, sleep and KDT polysomnography recordings will be processed using a previously validated automated artefact detection program.[61, 62] Power spectral and spindle analyses will be derived from artefact-free epochs in central channels using previously published methodology.[61, 62]

Simulated driving performance

Next-day driving performance is measured at ~08:00 using a fixed-based driving simulator (Hyperdrive, Adelaide, Australia) equipped with standard vehicle controls (described elsewhere[42, 48]) and using custom-built scenario developed using the SCANeR Studio Simulation Engine (version 2022.2 r25, AVSimulation, Paris, France). The 30-minute driving scenario incorporates three independent epochs: (1) a 'car following' drive (~7 minutes duration) that closely resembles one that has previously demonstrated sensitivity to THC-induced impairment;[63] (2) a 'highway' drive (~17 minutes duration); and (3) a novel divided-attention drive (~6 minutes duration), designed to replicate a casual mobile-phone conversation that does not require mental rehearsal.[64] During the divided-attention task, pre-recorded five-word sentences are played every 10 seconds (through a hands-free speaker). Participants immediately indicate whether the sentence was sensical (e.g., "*the truck delivered the package*") or non-sensical (e.g., "*the octopus burned the onions*") in nature then; and at 7-seconds, they are asked to recall the last word in the sentence. The main outcome measure for all epochs of the driving task is SDLP, a widely used measure of lateral vehicular control that is sensitive to drug and alcohol effects. Previous reports show a dose-dependent increase in SDLP with THC (higher represents more erratic driving), particularly in occasional cannabis users.[63, 65] Secondary outcomes for each driving epoch include: (epoch 1) average and standard deviation of car-following headway (i.e., distance relative to car in front) and speed coherence (i.e., correlation between speed of lead and following car); (epochs 2 and 3) average speed, and standard deviation of speed (SDSP). The driving scenarios were programmed by investigator C.I.

Adverse events

Safety outcome measures are described above. Blood pressure is measured four times during each Treatment Session (~17:00, 18:45, 21:00, and 09:30). Adverse event collection occurs via standardised interview at three timepoints (prior to discharge, and 7-hours and 7-days post discharge). Passive adverse event collection occurs throughout the study protocol via open-ended interview and is recorded on paper form. A study physician assesses the severity and causality of each adverse event.

Post-Treatment Session care

Participants leave the research facility at approximately 10:00-11:00 and are contacted by the study co-ordinator that evening (~16:30), and 7-days later, to assess wellbeing and record adverse events (including changes to sleep). Upon study completion, if deemed necessary in the study physician's opinion, a clinical follow-up is arranged for participants on an individual basis.

Data management

Any information obtained for the purpose of this research that could identify participants is treated as confidential and securely stored adhering to guidelines of the Sponsor, HREC, NHMRC National Statement on Ethical Conduct in Human Research (2007) and Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95). All onboarding, consent, and questionnaire data is captured through SPARDAC™ (Single Page Application - Research Data Capture) developed by Wappsystem Pty Ltd and hosted on Amazon Web Services in Sydney, NSW. Participant data is identified by unique codes. The code linking participant data to identity (e.g., name, date of birth, contact details) is stored securely with password protection, and is not accessible via the internet. Paper files are stored in locked storage cabinets on-site. Digital participant data are stored on a secure electronic data system, that is regularly backed up with disaster recovery features. All data will be stored securely for at least 15 years. Only study investigators have access to participant data. Data monitoring occurs on a regular basis by the study investigators. Data integrity is being enforced through a variety of mechanisms including data rules, range checks, and consistency checks against data already stored in the database. Written

documentation of changes are available through electronic logs and audit trails. Study safety is internally monitored and evaluated regularly, with adverse events documented and reported according to Sponsor and HREC requirements. The decision to terminate the study lies with the principal investigators and will be based on the recruitment target and safety data. The PIs and trial coordinator are monitoring data integrity throughout the trial.

Trial management structure

This is a pilot study and does not require an intricate management structure. The advisory committee provide advice or complete discrete tasks (Table 2). The management of day-to-day trial activities is overseen by the principal investigators and the trial coordinator (IL, CH, & BY). This trial does not have an independent data safety monitoring committee because it is a single-site single-night small sample study with a medication that is thought to have a low side-effect profile in a group of patients that are known not to be at high risk of life-threatening events.[66]

Roles and responsibilities

Table 2. Investigator roles and responsibilities

Investigator	**Role**
Dr Camilla Hoyos	Principal Non-Medical Investigator
Professor Brendon Yee	Principal Medical Investigator
Professor Iain McGregor	Advisory committee member (Pharmacologist and Cannabinoid Expert)
Professor Ronald Grunstein	Advisory committee member (Sleep Physician)
Professor Bandana Saini	Advisory committee (Study Pharmacist)
Associate Professor Nathaniel Marshall	Advisory committee (Study Statistician/Epidemiologist)
Associate Professor Christopher Gordon	Advisory committee member (Registered Nurse and Sleep Disorder Expert)
Dr Angela D'Rozario	Advisory committee member (Sleep Neurobiology Expert/Consultant)
Dr Danielle McCartney	Driving Simulator and Cannabinoid Expert
Dr Chris Irwin	Advisory committee member (Driving Simulation Expert)
Dr Anastasia Suraev	Advisory committee member (Cannabinoid and Sleep Expert)
Ms Isobel Lavender	Clinical Trial Coordinator/PhD student

The study investigators have led the design and conduct of this trial, will conduct the analyses, and make all publication decisions.

Statistical analyses

Statistical analyses will be completed using a commercial statistical package (e.g., SPSS, SAS, etc). Descriptive statistics will be described using means and standard deviations or counts and percentages as appropriate. Adverse events and serious adverse events will be tabulated with the total number of incidents by each timepoint. No formal hypothesis testing will be conducted on adverse event and serious adverse event data. Due to insufficient statistical power the KSS will only be described using a median and interquartile range. Continuous outcome data will be analysed using Linear Mixed Models due to expected inter-participant variability with repeated measures and ability to handle missing data. Where variables are collected pre-drug administration (i.e., treatment session 'baseline') for the outcome being analysed, those scores will also be included in the model as a covariate (e.g., posturography, WPT, FTT, DEQ, POMS). Participants will be random factors. Fixed factors will be treatment (placebo, 30 mg CBN, and 300 mg CBN) and the arm (first, second, and third) of the study. Analysis will be by intention to treat. Difference in least squared means inside the treatment main effect will only be investigated for the hypotheses that the two active treatments are superior to placebo. We will not present a p-value comparison for the two active doses. The critical p-values for each of these hypotheses is set at 0.05 (two-tailed). Effect sizes will be calculated as partial eta squared (η_p^2), Cohen's d, and Hedges' *g*, where appropriate. A Statistical Analysis Plan will be finalised before last patient last visit and will be publicly available (e.g., Clinical Trial Registry) and/or upon request.[67] We are not undertaking any interim or stratified analyses.

Ethics and dissemination

Ethics and dissemination: Human Research Ethics Committee approval has been granted by Bellberry Limited (2021-08-907). Study findings will be disseminated via scientific peer-reviewed publications, conferences, and media, as applicable.

Access to data

Information will be provided such that participants cannot be identified. Non-identifiable Individual Participant Data (IPD) will become available one year after study completion and will be available upon reasonable request to the principal investigators.

Author contributions

IL, DM, NSM, AS, CI, AD, CG, RRG, BY, ISM, and CMH were involved in methodological design and creation of the study protocol. BY and CMH are the principal investigators (medical and non-medical, respectively) who have overall responsibility for the design, conduct, and decision to submit for publication. NSM is the study epidemiologist and statistician who will design and publish the analysis plan in collaboration with IL. IL is the trial coordinator responsible for collecting trial data. IL drafted the manuscript. Investigator roles and responsibilities are outlined in Table 1. All authors have read and approved the final manuscript.

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Competing interest statement

ISM is the Academic Director of the Lambert Initiative for Cannabinoid Therapeutics, Brain and Mind Centre, University of Sydney. ISM is a consultant for Kinoxis Therapeutics, Psylo Ltd, and Emyria and is an inventor on several patents relating to novel cannabinoid and non-cannabinoid therapeutics unrelated to insomnia. He has received consulting fees from the Medicinal Cannabis Industry Australia (MCIA) and acts as an expert witness in legal cases involving cannabis-related issues. AS has received consulting fees from the Medicinal Cannabis Industry Australia (MCIA) and Haleon Australia. RRG has received discounted investigational products for an unrelated clinical trial from Neurim Pharmaceuticals Inc and received investigational product and matched placebo from Teva Pharmaceutical in unrelated clinical trials. He has received funding for lectures for Pfizer, Teva, Jazz and Eisai in the past 3 years. The other authors have no competing interests to disclose. The Woolcock Institute Sleep and Chronobiology Research Group has received research support from Avadel, Nyxoah, Idorsa, ResMed, BOD Australia, and Philips.

Patient consent for publication

Not required.

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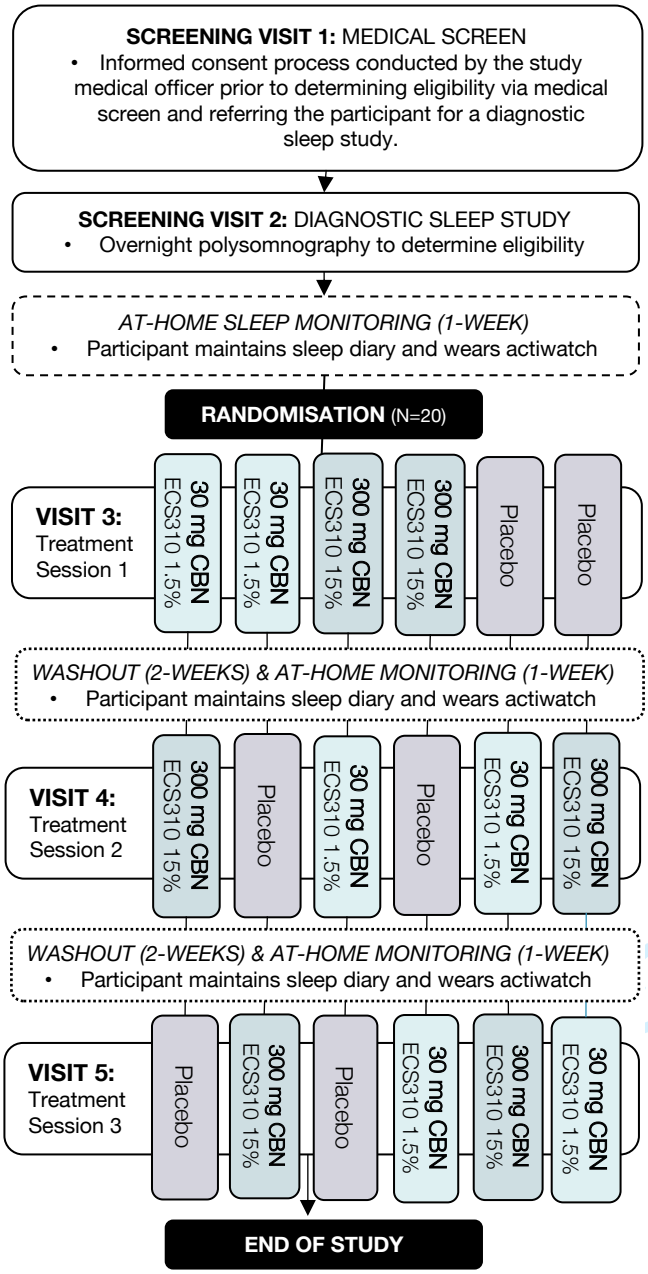
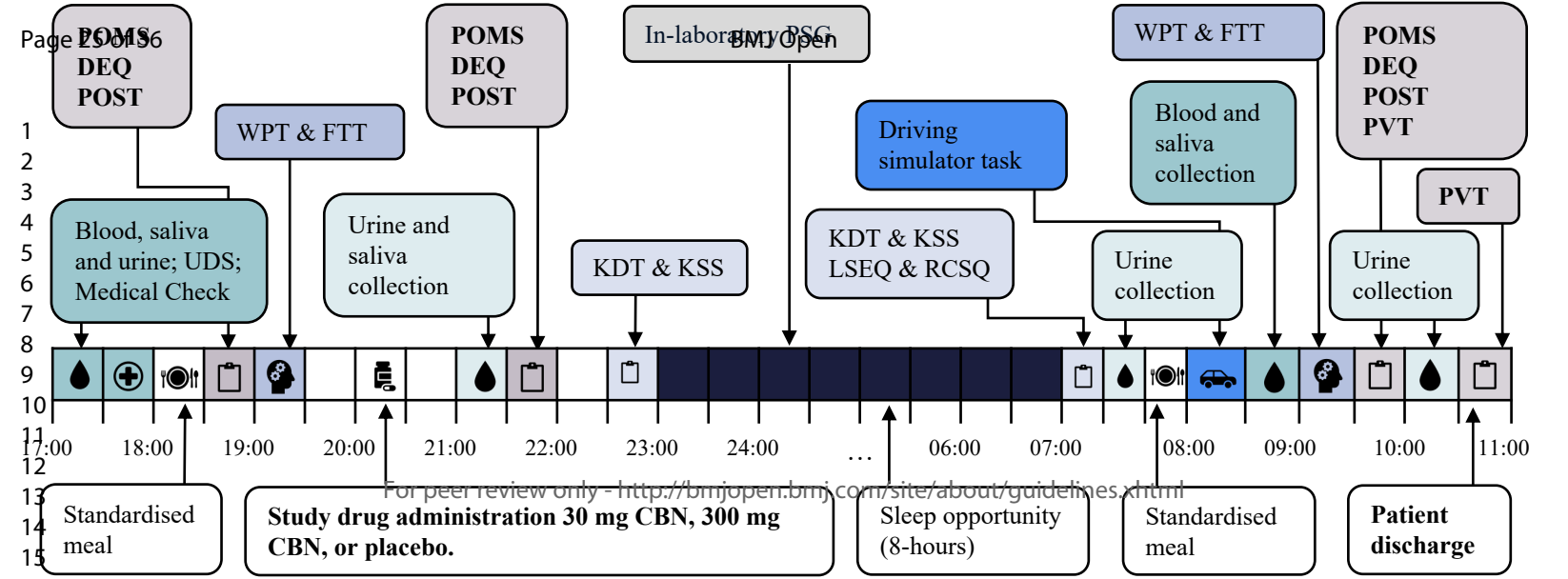

SCREENING VISIT 1: MEDICAL SCREEN
• Informed consent process conducted by the study medical officer prior to determining eligibility via medical screen and referring the participant for a diagnostic sleep study.
SCREENING VISIT 2: DIAGNOSTIC SLEEP STUDY
• Overnight polysomnography to determine eligibility
AT-HOME SLEEP MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
RANDOMISATION (N=20)
VISIT 3: Treatment Session 1
30 mg CBN ECS310 1.5%
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
300 mg CBN ECS310 15%
Placebo
Placebo
WASHOUT (2-WEEKS) & AT-HOME MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
VISIT 4: Treatment Session 2
300 mg CBN ECS310 15%
Placebo
30 mg CBN ECS310 1.5%
Placebo
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
WASHOUT (2-WEEKS) & AT-HOME MONITORING (1-WEEK)
• Participant maintains sleep diary and wears actiwatch
VISIT 5: Treatment Session 3
Placebo
300 mg CBN ECS310 15%
Placebo
30 mg CBN ECS310 1.5%
300 mg CBN ECS310 15%
30 mg CBN ECS310 1.5%
END OF STUDY



SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1,2,5,7
	2b	All items from the World Health Organization Trial Registration Data Set	In trial registration
Protocol version	3	Date and version identifier	7
Funding	4	Sources and types of financial, material, and other support	1,2,6,18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,6,16-18
	5b	Name and contact information for the trial sponsor	1, 6, and trial registration
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	6-7,18

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16-18
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6-7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-9 (Box 2)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	7-9 (Box 2)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 14
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-9 (Box 2)

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	4-6 (Box 1), trial registry
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 1; Figures 1-2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10-11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10-11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10-11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11, 17-18
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10, 15
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16-17
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15-18
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	17
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	16
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1, 2, 7, 17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	7
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9-10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15-16
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
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Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	1, 2, 17
	31b	Authorship eligibility guidelines and any intended use of professional writers	17
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	18; trial registry
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary material

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	13

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.

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	5b	Name and contact information for the trial sponsor	1, 6, and trial registration
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	6-7,18~~5,16~~

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	~~14~~-16-~~18~~
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	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	~~6~~-7-9 (Box 2~~1~~)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 14~~7, 13~~
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Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10-118
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Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11, 17-188
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	108
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-169-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10, 157
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	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15-16 13-14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	181,16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	1, 10, 16-1813
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	31b	Authorship eligibility guidelines and any intended use of professional writers	1716
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	1816; trial registry
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