PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	The effects of cannabidiol on psychosocial stress, situational anxiety and nausea in a virtual reality environment: A protocol for a single-centre randomised clinical trial
AUTHORS	Bawa, Zeeta; McCartney, Danielle; Bedoya-Pérez, Miguel; Lau, Namson; Fox, Richard; MacDougall, Hamish; McGregor, Iain

VERSION 1 – REVIEW

REVIEWER	Kirkland, Anna E Medical University of South Carolina Department of Psychiatry and Behavioral Sciences
REVIEW RETURNED	06-Feb-2024

GENERAL COMMENTS	This is an interesting protocol that will help to provide clarification on the use of cannabidiol (CBD) for non-clinical levels of anxiety, stress, and nausea in healthy individuals. The results have implications for the use of low doses of CBD inside and outside of the clinical setting. Overall, the protocol is very detailed, easy to follow, and rooted in previous research. The use of virtual reality (VR) builds on previous work using CBD for acute stress (e.g., public speaking) and allows for examination of other types of stressful/motion-related tasks. The included figures and tables are great additions. I also appreciated the language used when talking about the "therapeutic potential" of CBD since it did not overstate the findings, but rather indicated that CBD could be useful for a variety of symptoms. There are a couple things that could enhance the protocol: 1. The introduction section of the abstract reads as though it is setting up a protocol focused on epilepsy, anxiety, and/or psychosis (lines 29-30) rather the actual outcomes which are brought up in the methods. 2. Introduction (lines 115-117) – it would be nice to have a little bit more information about the "constituents that enhance the oral bioavailability of CBD" to preface the materials section. 3. Exclusion criterion K: could you provide some examples or definition for "uncontrolled"? 4. What are the consenting procedures for the Screening Questionnaire? 5. Including more details about the caloric and macronutrient composition of the "Up & Go Liquid Breakfast" would be helpful. 6. REDCap is defined for the first time after it has been used several times (line 508).

REVIEWER	Parker, Linda
REVIEW RETURNED	07-Feb-2024

GENERAL COMMENTS	The authors propose to use a randomised, double-blind, placebo-controlled, parallel-group, clinical trial comparing the efficacy of low-dose CBD versus placebo to reduce anxiety nausea and vomiting induced by VR tasks in humans. This study will evaluate lower doses (150 mg) than are typically employed in human studies (300-600 mg). TPM will be the vehicle, which has been shown to increase the bioavailability of lipid-soluble substances. The bioavailability of CBD will further be increased by providing a caloric beverage at the same time. The authors should specify whether the participants will be required to drink the entire serving for consistency across participants. Sample size was appropriately determined by a power analysis. To provide a stronger rationale for the choice of lower doses of CBD, the authors should provide a discussion of the dose-dependent effects previously described in the preclinical work on shrew vomiting and rat gaping. In the preclinical animal studies lower doses of CBD (1-10 mg/kg ip or sc) but not higher doses (20-40 mg/kg, ip or sc) effectively reduce toxin-induced nausea and vomiting. Such a review will aid in strengthening the rationale for the choice of dose to investigate.

VERSION 1 – AUTHOR RESPONSE

Section 2: Responses to Reviewer 1

Reviewer 1: "This is an interesting protocol that will help to provide clarification on the use of cannabidiol (CBD) for non-clinical levels of anxiety, stress, and nausea in healthy individuals. The results have implications for the use of low doses of CBD inside and outside of the clinical setting. Overall, the protocol is very detailed, easy to follow, and rooted in previous research. The use of virtual reality (VR) builds on previous work using CBD for acute stress (e.g., public speaking) and allows for examination of other types of stressful/motion-related tasks. The included figures and tables are great additions. I also appreciated the language used when talking about the "therapeutic potential" of CBD since it did not overstate the findings, but rather indicated that CBD could be useful for a variety of symptoms. There are a couple things that could enhance the protocol:"

- **Comment 1:** The introduction section of the abstract reads as though it is setting up a protocol focused on epilepsy, anxiety, and/or psychosis (lines 29-30) rather the actual outcomes which are brought up in the methods.

  Thank you for your feedback. We have updated abstract introduction to minimise the focus on epilepsy and psychosis. The updated text reads as follows:

  Page 2, line: 28-32

  "Introduction: The non-intoxicating plant-derived cannabinoid, cannabidiol (CBD), has demonstrated therapeutic potential in a number of clinical conditions. Most successful clinical

trials have utilised relatively high (≥ 300 mg) oral doses of CBD. Relatively few studies have investigated the efficacy of lower (<300 mg) oral doses, typical of those available in over-the-counter CBD products."

- **Comment 2:** Introduction (lines 115-117) – it would be nice to have a little bit more information about the "constituents that enhance the oral bioavailability of CBD" to preface the materials section.

  We have updated the introduction section to include further detail about the use of

  Tocopheryl Phosphate Mixture' to preface the 'Intervention' section. The updated text reads as follows:

  Page 4, line: 112-117

  "It should be noted that the oral bioavailability of CBD is limited (~13-19%) but may be enhanced by certain lipid-rich formulations or by administration with fatty foods [25]. The current study will utilise a proprietary blend of tocopherol phosphates (so-called 'Tocopheryl Phosphate Mixture' (TPM®)), which has been shown to increase the bioavailability of lipid-soluble substances [26, 27]."

  AND

  Page 8, line: 222-226

  "2.3.1 Intervention

  The investigational product (Avecho Biotechnology Limited, Victoria, Australia) is an oil-based, soft-gel capsule. Each gel capsule contains 75 mg of pure, synthetic (-)-CBD enantiomer and 75 mg of TPM® in medium chain triglyceride (MCT) oil. The capsules do not contain any other cannabinoids or cannabis constituents."

- **Comment 3:** Exclusion criterion K: could you provide some examples or definition for "uncontrolled"?

  We have provided further clarification to exclusion criterion k. The updated text reads as follows:

  Page 7, line: 196-197

  "k. A chronic medical condition (mental or physical) that is uncontrolled i.e., has been either newly diagnosed, or previously diagnosed and remains symptomatic."

- **Comment 4:** What are the consenting procedures for the Screening Questionnaire? We have provided further details about the consenting procedures for the Screening Questionnaire. The updated text reads as follows:

  Page 9, line: 259-264

  "Willing volunteers will complete a comprehensive online Screening Questionnaire in REDCap™ (~20 minutes). Volunteers are required to complete a compulsory online declaration tick-box at the start of the questionnaire consenting to the use of the information they provide to evaluate their eligibility. The questionnaire will assess their eligibility to participate…"

- **Comment 5:** Including more details about the caloric and macronutrient composition of the "Up & Go Liquid Breakfast" would be helpful.

  We have provided further details about the caloric and macronutrient composition of the "Up & Go Liquid Breakfast". The updated text reads as follows:

  Page 13, line: 323-328

  "After this, the VR headset and Equivital EQ02+ LifeMonitor belt will be temporarily removed, the treatment administered, and the participant given a compulsory standardised caloric beverage to consume; specifically, 500 mL of "Up & Go Liquid Breakfast" (Sanitarium, Berkeley Vale NSW, Australia) containing approximately 1,640 kilojoules, 16.8g of protein, 8.6g of fat and 57g of carbohydrates."

- **Comment 6:** REDCap is defined for the first time after it has been used several times (line 508).

  We have provided the definition of REDCap on first mention and removed its definition later on in the protocol. The updated text reads as follows:

  Page 9, line: 259-261

  "A Study Flowchart is presented in Figure 1. Willing volunteers will complete a comprehensive online Screening Questionnaire using the 'Research Electronic Data Capture' (REDCap™) web-based system (~20 minutes)."

  AND

Page 19, line: 522-524

"Clinical trial data will be collected and managed using the REDCap™, a secure, online program supported by the University of Sydney…"

Section 3: Responses to Reviewer 2

Reviewer 2: "The authors propose to use a randomised, double-blind, placebo-controlled, parallel- [illegible] will be the vehicle, which has been shown to increase the bioavailability of lipid-soluble substances. The

bioavailability of CBD will further be increased by providing a caloric beverage at the same time. The authors should specify whether the participants will be required to drink the entire serving for consistency across participants. Sample size was appropriately determined by a power analysis."

- **Comment 1:** The authors should specify whether the participants will be required to drink the entire serving for consistency across participants.

  Thank you for your feedback. All participants are required to consume the entire serving of the caloric beverage and these details have been included the manuscript. The updated text reads as follows:

  Page 13, line: 325-330

  "… and the participant given a compulsory standardised caloric beverage to consume; specifically, 500 mL of "Up & Go Liquid Breakfast" (Sanitarium, Berkeley Vale NSW, Australia) containing approximately 1,640 kilojoules, 16.8g of protein, 8.6g of fat and 57g of carbohydrates. For consistency, all participants are required to consume this beverage in its entirety, which aims to potentiate the absorption of CBD in the gastrointestinal tract [6, 48-50] and provide participants with sustenance during the test session."

- **Comment 2:** To provide a stronger rationale for the choice of lower doses of CBD, the authors should provide a discussion of the dose-dependent effects previously described in the preclinical work on shrew vomiting and rat gaping. In the preclinical animal studies lower doses of CBD (1-10 mg/kg ip or sc) but not higher doses (20-40 mg/kg, ip or sc) effectively reduce toxin-induced nausea and vomiting. Such a review will aid in strengthening the rationale for the choice of dose to investigate.

  Thank you for your feedback. These details have been included in the introduction of the manuscript. The updated text reads as follows:

  Page 5, line 126-131

"CBD has shown anti-nausea and anti-emetic effects in preclinical studies involving laboratory animals [35-37]. Interestingly, two of these studies demonstrated that CBD administered intraperitoneally at low doses (2.5 - 10 mg/kg) but not higher doses (25 and 40 mg/kg) reduced toxin-induced vomiting in house musk shrews [35, 36]. CBD also reduced vomiting in human studies when used in combination with Δ^9-tetrahydrocannabinol to treat chemotherapy-induced nausea and vomiting [38]."

VERSION 2 – REVIEW

REVIEWER	Kirkland, Anna E Medical University of South Carolina Department of Psychiatry and Behavioral Sciences
REVIEW RETURNED	08-Mar-2024

GENERAL COMMENTS	Thank you for addressing my comments. No further edits!

REVIEWER	Parker, Linda
REVIEW RETURNED	22-Feb-2024

GENERAL COMMENTS	The author has addressed my comments.