Study Title

Double Blind Clinical Evaluation on the combination of Gamma-Aminobutyric Acid Tartrate 100mg, Glutamic Acid 100mg, Dibasic Calcium Phosphate 50mg, Thiamine Nitrate 25mg, Pyridoxine Hydrochloride 10mg and Cyanocobalamin 5mcg compared to Ginger Extract as adjuvant therapy in symptomatic control of chronic vertigo motion sickness

1. BACKGROUND:

Motion sickness is a chronic condition characterized by vestibular changes in response to stimuli caused either by movement or movement perception triggered by car, train, ship or aircraft transportation, amusement park rides, virtual reality and simulators, walking, exercising, as well as under the absence of gravity in space. It can also be triggered by movies and television shows that exhibit glowing colors, movement, and focus shifting.

The studied combination drug is composed, among other substances, by **GABA** (gamma-aminobutyric acid), the main inhibitory neurotransmitter of the central nervous system. The former lowers anxiety symptoms. Its precursor, glutamic acid, is the amino acid found in greater concentration under free form in the CNS; it is closely related to brain metabolism and, in contrast to GABA, it is the main excitatory neurotransmitter; glutamic acid also has anesthetic and anxyolytic therapeutic properties. **Thiamine or vitamin B1** is a cofactor in the synthesis of acetylcholine, which plays a central role in the initiation and propagation of neural impulse in the CNS as well as it does in the skeletal and myocardial muscles. **Pyridoxine or vitamin B6** plays a role in the synthesis of neurotransmitters such as dopamine as well as in the metabolism of tryptophan, resulting in an increase in serotonin so providing well-being and anxiety relief. Pyridoxine is essential for the proper functioning of the CNS (including at nausea and vomiting center) and immune system. **Cyancobalamin or Vitamin B12** plays a role in the formation of red blood cells, in the growth and repair of nerve fibers as well as in pain relief. Importantly it also improves blood flow in the brain, with secondary vertigo improvement (Goodman & Gilman's, 2018; Gomes, Coutinho, Geller 2018). The combined use of these substances provides relief of vertigo due to motion sickness. ***Zingiber officinale*** is a species of the Zingiberacaea family, widely used in traditional and herbal medicine for the treatment of various clinical conditions such as vertigo.

Keywords: Kinetosis, Gamma-aminobutyric acid tartrate, glutamic acid, calcium phosphate dibasic, thiamine nitrate, pyridoxine hydrochloride, cyanocobalamin, *Zingiber officinale*, Motion Sickness Assessment Questionnaire

2. HYPOTHESIS:

Oral administration of the combination gamma-aminobutyric acid tartrate 100mg, glutamic acid 100mg, dibasic calcium phosphate 50mg, thiamine nitrate 25mg, pyridoxine hydrochloride 10mg and cyanocobalamin 5mcg or *Zingiber officinale* 160mg (8mg Gingerols) results in symptomatic improvement of vertigo due to motion sickness, according to pre- and post-treatment MSAQ statements.

3. OBJECTIVES:

3.1 Primary objective: To evaluate the efficacy and safety of the combination gamma-aminobutyric acid tartrate 100mg, glutamic acid 100mg, dibasic calcium phosphate 50mg, thiamine nitrate 25mg, pyridoxine hydrochloride 10mg and cyanocobalamin 5mcg versus *Zingiber officinale* 160mg dry extract (8mg gingerols) as adjuvant therapy in symptomatic control of chronic vertigo motion sickness.

3.2 Secondary objective: To assess pre- and post-therapeutic efficacy according to MSAQ scores and sub-scores, as well as tolerability of the association.

4. STUDY LOCATION: UNIFESO.

5. PROPOSED METHODOLOGY:

5.1 Study design and population:

Patients sample for this study was calculated using the Power & Precision software version 4.0, based on the null hypothesis that the proportion of patients who might show clinical improvement would be identical in both groups. Approximately 334 patients are expected to be included, with 167 receiving the combination and 167 receiving *Z. officinale* dry extract. The level for statistical significance (alpha) was set at 0.050, and the test is two-tailed. With a sample size of 167 patients per group, the study has a power of 36.4% to generate a statistically significant result, with a proportional difference between groups estimated as 0.05. This will be a double-blind randomized study in patients with chronic vertigo-motion sickness. The treatment will have a total duration of 7 days.

After undersigning the Informed Consent Form, participants will be evaluated for inclusion and non-inclusion criteria. It is estimated that 451 recruited participants will be necessary to reach 334 evaluable participants. A sequential number from 001 will be assigned to each participant included in the study, in order to preserve patients' identity.

INCLUSION CRITERIA	**NON-INCLUSION CRITERIA**
To qualify for inclusion in this study, the participant must meet all of the criteria listed below: • Participant of both sexes aged between 18 and 65 years • Clinical presentation of motion sickness • Female participant of reproductive age agrees to use contraception during the study period • Participant read, understood, undersigned and dated the free and informed consent form	Participants will be excluded from the study if they present: • Hypersensitivity to any component of the study drug • Gallstone history • History of gastric irritation (according to product package insert) • Arterial blood pressure >145/100mmHg (according to product package insert) • Concomitant use of other medications for the treatment of motion sickness

Four assessments will be carried out for vertigo - MSAQ (motion sickness assessment questionnaire), consisting of a pre-treatment assessment and three assessments during treatment, for both study groups. The primary variable in this study will be the MSAQ answered by the study subjects. The MSAQ consists of 16 questions, answered on a scale from one to nine points, which assesses the gastrointestinal, central and peripheral nervous system and soporous symptoms related to motion sickness. Secondary variables include the Investigating Physician's Assessment (10-point scale), willingness to continue treatment, physical examination results and vital signs collected during face-to-face visits to the study center, and the occurrence, duration, and severity of any adverse events which might have occurred during the treatment period.

The Clinical Research Form will be filled out, stored, coded, and the data will be analyzed using the GraphPad Prism program, version 5.0. Frequency tables will be generated, central tendency measurements will be taken (mean, median, mode) and the percentages will be

calculated using the number of analyzed values as the denominator. Whenever relevant, confidence intervals of proportions will be calculated.

4. Study Procedures

4.1. Visit 1 / Pre-Treatment: will be held at the study center. After informed consent signature and inclusion and non-inclusion criteria assessment, an evaluation will be carried out covering:

- Date of birth, gender, ethnicity, medical history, history of motion sickness, acute and chronic illnesses, concomitant medications
- Physical examination [height (cm), weight (kg), BMI (kg/cm^2), arterial blood pressure (mmHg), heart rate (bpm), respiratory rate (ipm)]
- Global Evaluation of the Investigating Physician (10-point graduated scale)
- Routine laboratory tests
- Imaging exams (MRI, CT, EEG) at attending physician's discretion.

4.2 Study Medicine and MSAQ

During Visit 1, participants will be given the study medicine to be used during the treatment period. Each participant will receive a total of 5 tablets - three tablets for the expected evaluations, and two extra tablets in case the former are lost. MSAQ will be fulfilled before and after treatment. Then participants will receive the printed Four-Way MSAQ. The former will be asked to fulfill MSAQ at four different times (Travel 1, Travel 2, Travel 3 and Travel 4).

The first MSAQ form will be fulfilled after a ride of at least 15 minutes, with no use of the study drug (Travel 1). Before Travels 2, 3, and 4, the participant will be instructed to take one tablet 30 minutes before them and to answer the questionnaire at its end. In addition to MSAQ other data are: travel date and duration (standard end time), type of transport (ferry, train, subway, car, bus), and time of completion of the questionnaire.

4.3 Visit 2 / Post-Treatment: it will occur either at the end of the treatment period or if the study participant is withdrawn. During this visit, participants will return to the study center, where the study material (study medicine and MSAQ) will be collected and the following procedures performed:

- Physical examination [weight (kg), BMI (kg/cm^2), blood pressure (mmHg), heart rate (bpm), respiratory rate (pmi)]
- Evaluation of the use of concomitant medications
- Global Assessment of the Research Physician (graded scale of 10 points)
- Efficacy and general tolerability assessed by the Medical Investigator
- Routine laboratory tests
- Imaging tests (MRI, CT, EEG) at medical discretion.

Risks	Benefits
The risks to research participants are the adverse effects associated with the study drug, according to the product package leaflet: mild gastrointestinal disorders (bloating, stomach pressure), and headache. Occasionally heartburn, contact dermatitis, hypotension and, at very high dosages, dyspeptic symptoms.	Immediate benefit is symptomatic chronic kinetosis improvement. Secondary benefit is knowledge increase on the use of the combination gamma-aminobutyric acid tartrate 100mg, glutamic acid 100mg, dibasic calcium phosphate 50mg, thiamine nitrate 25mg, pyridoxine hydrochloride 10mg and cyanocobalamin 5mcg as well as *Zingiber officinale* dry extract 160mg (8mg gingerols) in the management of vertigo, nausea, and anxiety associated with kinetosis.

5. OUTCOMES

5.1 Primary outcome

The primary outcome will be the percentage of participants showing improvement >2 points in the MSAQ of Travel 2, Travel 3 and Travel 4 in relation to the pre-treatment score (Travel 1).

5.2 Secondary outcomes

The secondary outcomes include:

- The percentage of participants showing improvement in the subscores of the MSAQ of Travel 2, Travel 3 and Travel 4 in relation to the pre-treatment score (Travel 1).
- The percentage of participants who presented adverse events related to the study medication.
- Average values of pre- and post-treatment according to Physician Evaluation scores.

6. Schedule

Activity	Date
Literature review	May -July 2021
Design of the project	July 2021
Research Ethics Committee	August 2021
Data collection	October 2021 - October 2022
Data analysis	November 2022
Final report	December 2022
Submission for publication in a journal	January 2023 - February 2023

7. Ethical Issues

Participants who agree to participate in the study will undersign the Free and Informed Consent Form (Annex) in accordance with resolution 466/12 of National Health Council. Main investigator and his/her associates are aware of and will comply with the requirements of Resolution 466/12 of the National Health Council and its supplements and the Code of Medical Ethics of 1988 (Article 130). The former ones as well as the rest of the health team are commited in maintaining the privacy and confidentiality of the data from the participants'medical records, fully preserving the their anonimity. The data collected in the medical records will be used solely for the purpose informed in this protocol. Similarly, the variables generated by the study can only be used for the project purposes .

8. Budget

The study will be sponsored by the Gross Pharmaceuticals under the form of medicines supply, administrative support material, copying, printing, laboratory and imaging tests, transportation, published articles and books as well as statistical support.

9. Researchers

Main Investigator: Prof. Carlos Pereira Nunes

Associate Investigator: Prof. Claudio Rodrigues

Annex. Free and Informed Consent Term

FREE AND INFORMED CONSENT TERM FOR PATIENTS DIAGNOSED WITH KINETOSIS

Research Project "Double Blind Clinical Evaluation on the combination of Gamma-Aminobutyric Acid Tartrate 100mg, Glutamic Acid 100mg, Dibasic Calcium Phosphate 50mg, Thiamine Nitrate 25mg, Pyridoxine Hydrochloride 10mg and Cyanocobalamin 5mcg compared to Ginger Extract as adjuvant therapy in symptomatic control of chronic vertigo motion sickness ".

Study Site:

Main Investigator:

Patient Data:

- Full Name: _____
- Date of Birth: (day/month/year) ____/____/_____
- Phone: _____

Attending Physician: _____

AUTHORIZATION

You are being invited to participate in a research project on kinetosis (motion sickness). This disease correspond to a set of symptoms of malaise associated with movement, especially during travel in cars, trains, buses and ferries. During this research, you will be asked to answer a questionnaire evaluating the presence and severity of kinetosis symptoms before and after treatment with ginger extract or a combination of gamma-aminobutyric acid, glutamic acid calcium, thiamine, pyridoxine and cyanocobalamin. First, you will take a journey of at least 15 minutes without the treatment and answer the questionnaire afterwards. Then, you will start taking the study tablet every 8 hours (3 times a day) for 7 days making your routine trips of 15 minutes duration minimun and after each travel you will answer the questionnaire again. This questionnaire should be completed on three different occasions, that means, after three trips. You will also be asked to write down the date of each trip and the time of travel.

I understand that I will have no compensations in exchange of my participation, as well as any family member,son, daughter, or anyone else.

I had the opportunity to ask questions about the procedures of this clinical investigation and to reflect on the answers I have been given.

I understood that participation in this clinical investigation is voluntary, that I can quit anytime, and that leaving this investigation would not compromise my clinical follow-up. I know that the doctor responsible for the study is Prof. Carlos Nunes and that I can ask him questions in the clinic or by phone.

I authorize the use of the information on me contained in my medical chart, which will be used only for the purposes of this clinical research, will not be used in other studies without a new authorization, and which will be kept confidential (secret) regarding my identification.

I, _____ agree to participate in this clinical investigation.

I confirm that I have explained the nature of this investigation to the patient.

Physician Signature: _____

Witness Signature: _____

Signature Date: ____/____

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