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Lipid-based formulations to increase cannabidiol bioavailability: *In vitro* digestion tests, pre-clinical assessment and clinical trial

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ABSTRACT

Herein, medium-chain triglycerides (MCT), glyceryl monolinoleate (GML), and a self-emulsifying drug delivery system (SEDDS) for cannabidiol (CBD) delivery were compared using *in vitro* and *in vivo* (mouse and human) studies. *In vitro* digestion tests showed that SEDDS yielded the highest CBD recovery in the aqueous phase (86 ± 2%), followed by GML (13 ± 2%) and MCT (5.6% ± 0.8%). *In vivo* tests (mouse) revealed that SEDDS promoted the highest CBD exposure, exhibiting an area under the plasma concentration-time curve ($AUC_{0\text{-}6h}$) 1.48 times greater than GML and 3.97 times greater than that of the MCT formulation. A single-dose, open-label, crossover study performed in 11 volunteers showed that SEDDS increased CBD $AUC_{0\text{-}12h}$ by 1.12 and 1.48 times in relation to GML and MCT, respectively. The *in vitro–in vivo* correlation was r^2 0.75 for mice and r^2 0.66 for humans. The AUC correlation between mice and humans was 0.98. Collectively, these results indicate that the lipid profile substantially influences CBD delivery and highlights the potential of the SEDDS and GML formulations as candidate solutions for increasing CBD AUC and bioavailability.

1. Introduction

In recent years, we have witnessed the popularization of medical cannabis and the recognition of its therapeutic potential by the scientific community worldwide (Tran and Kavuluru, 2020; Colizzi et al., 2020; Abrams, 2018; Hutchison et al., 2019). The growing interest in cannabinoids has fostered scientific research, contributing to a better understanding of the endocannabinoid system (Jonsson et al., 2006; Crocq, 2020; Morales and Jagerovic, 2020). Regulatory advances have allowed market development, and numerous patients have been able to access a wide range of cannabis products, while important clinical trials have established a new avenue for cannabinoids as medicines (Giacoppo et al., 2017; Devinsky et al., 2018; Schlag, 2020). Despite these advances, there is still insufficient data regarding the oral delivery of cannabinoids and the factors that affect their pharmacokinetics, particularly for standardized formulations containing cannabis extracts.

Poor oral bioavailability owing to CBD lipophilicity and marked liver first-pass metabolism are two of the most important challenges that need to be addressed in the oral delivery of cannabinoid-derived products (Feeney et al., 2016). Cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) are permeable and highly lipophilic class II molecules according to the Biopharmaceutics Classification System (BCS) (Cherniakov et al., 2017). Therefore, class II BCS are good candidates for lipid-based formulations (LBF), and, currently, most cannabinoid market products utilize triglycerides as vehicles.

LBF is classified into four types according to Pouton (Pouton, 2000). Type I formulations are composed of carrier lipids and an active compound, require digestion and enzymatic action to form amphiphilic products and micelles for subsequent absorption, and accordingly exhibit slow absorption and variable bioavailability. Type II formulations possess an additional lipophilic surfactant, typically used to improve drug solubility in oil and promote emulsification with

Abbreviations: **11-OH-THC**, 11-hydroxy-9- tetrahydrocannabinol; **AUC**, area under the concentration-time curve; **$AUC_{0\text{-}6h}$**, area under the concentration-time curve from the study in mice; **$AUC_{0\text{-}12h}$**, area under the concentration-time curve from the study in humans; **BCS**, Biopharmaceutics Classification System; **BMI**, body mass index; **$CaCl_2{\cdot}H_2O$**, calcium chloride; **CBD**, cannabidiol; **C_{max}**, Maximum plasma concentration; **CYP**, cytochrome P450; **DAD**, diode-array detector; **GML**, glyceryl monolinoleate; **IVIVC**, *in vitro–in vivo* correlation; **LBF**, lipid-based formulations; **LoQ**, limit of quantification; **MCT**, medium-chain triglyceride; **NaCl**, sodium chloride; **NaOH**, sodium hydroxide; **NaTDC**, sodium taurodeoxycholate hydroxide; **SEDDS**, self-emulsifying drug delivery system; **SEM**, standard error of the mean; **THC**, Δ^9-Tetrahydrocannabinol; **T_{max}**, time to reach the maximum concentration; **UGT**, uridine 5′-diphospho-glucuronosyltransferase; **UPLC**, ultra-performance liquid chromatographer.

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gastrointestinal fluids. Type III formulations have a hydrophilic surfactant and co-solvent, in addition to oil and the active compounds, and can be further divided into Type IIIA and Type IIIB (higher hydrophilicity). Type IV LBFs are composed of surfactants and cosolvents without lipids, and consequently, exhibit the highest hydrophilicity. Notably, the susceptibility to digestion decreased as the proportion of the surfactant increased. Type II, III, and IV can self-emulsify in contact with the gastrointestinal fluid and form micelles; therefore, these formulations are classified as self-emulsifying drug delivery systems (SEDDS) and are generally filled into soft or hard gelatin capsules (Pouton, 2000).

In SEDDS formulations, surfactants and cosolvents promote rapid micelle formation, potentially increasing the rate of absorption and bioavailability (Yáñez et al., 2011). Several BCS Class II and IV drugs have been successfully formulated using this technology and are currently marketed. The main advantages of SEDDS include the promotion of bioavailability and faster onset of action, dose and interindividual variability reduction in relation to conventional dosage forms. Possible disadvantages are the necessity of large quantities of surfactants that can irritate in the gastrointestinal tract, potential drug leakage from capsules, stability issues such as migration of co-solvents and precipitation of drugs during storage, and poor correlation between *in vitro* and *in vivo* tests (Czajkowska-Kośnik et al., 2015).

The type and length of the lipid chain, degree of saturation, and digestibility of the oily excipients may influence the oral absorption and bioavailability of lipophilic drugs (Izgelov et al., 2020). Short- and medium-chain triglycerides (MCT) reach the systemic circulation through the portal system, while long-chain triglycerides are absorbed via the intestinal lymphatic system, bypassing the liver first-pass metabolism. Lipophilic drugs with log P > 5 and solubility higher than 50 mg/g in the long-chain triglyceride carrier can be absorbed by intestinal lymph capillaries by associating with lipoproteins called chylomicrons. The associated drug-lipoproteins are transported from the mesenteric to the thoracic lymphatic duct and then directly into the bloodstream, avoiding the first-pass liver metabolism (Izgelov et al., 2020; Porter et al., 2007; McClements, 2020).

The use of long-chain lipids has proven valuable for increasing the bioavailability of lipophilic drugs, such as cannabinoids (Zgair et al., 2017; Zgair et al., 2016). For example, Zgair et al. (2016) have demonstrated the ability of long-chain lipids to increase CBD and THC bioavailability by approximately three-fold when compared with the lipid-free formulation administered to rats; this increase was related to intestinal lymphatic absorption. Lipids with less susceptibility to digestion and a higher degree of saturation have faster absorption and higher peak concentration owing to a faster onset of chylomicron synthesis and subsequent lymphatic absorption (Cheema et al., 1987; Porter and Charman, 2001).

Despite this evidence, the influence of lipid characteristics on the pharmacokinetic profile of cannabinoids has not been extensively investigated. Thus, the potential of partially hydrolyzed long-chain triglycerides (GML) and SEDDS on enhanced oral bioavailability of CBD were investigated. The main objective of the present study was to increase the bioavailability of CBD to improve treatment efficacy and reduce the dose, cost, and side effects. *In vitro* digestion tests with MCT, GML, and SEDDS were performed and, the impact of these formulations on the CBD pharmacokinetic profile was assessed using *in vivo* tests in animals and humans. Finally, the potential correlation between *in vitro* and *in vivo* responses was investigated.

2. Material and methods

2.1. Chemicals

Glycerol monolinoleate (Maisine® CC) and polyoxyl 40 castor oil (Kolliphor® RH 40) were kindly gifted by Gattefossé (Saint-Priest, France) and BASF (Ludwigshafen, Germany), respectively. MCT was kindly donated by Concepta (São Paulo, Brazil), and the orange flavor agent and neotame were donated by SweetMix (Sorocaba, Brazil). Polyethylene glycol 400, tris-maleate, calcium chloride ($CaCl_2{\cdot}H_2O$), sodium chloride (NaCl), sodium hydroxide (NaOH), ethanol (99.9%), and formic acid (85%) were purchased from Synth (Diadema, Brazil). Sodium taurodeoxycholate hydroxide > 95% (NaTDC), 4-bromophenylboronic acid, and porcine pancreatin extract (8 × USP specifications activity) were all obtained from Sigma-Aldrich (Saint Louis, USA), acetonitrile (99.99%) and methanol (99.99%) were purchased from J.T. Baker (Phillipsburg, USA), chloroform was purchased from Merck (Darmstadt, Germany), and phosphatidylcholine (PC) was obtained from Lipoid (Ludwigshafen, Germany). CBD (1.0 mg/mL in methanol) and 11-hydroxy-9-tetrahydrocannabinol (11-OH-THC, 1.0 mg/mL in methanol) standards were purchased from LGC (Luckenwalde, Germany). The internal standards CBD-D_3 and 11-OH-THC-D_3 (0.1 mg/mL in methanol) were obtained from Cerilliant (Round Rock, USA).

2.2. Preparation of test formulations

The extract used to formulate the testing products was obtained by supercritical fluid extraction from the aerial parts of *Cannabis sativa* L. (Bedrolite®, Bedrocan, Netherlands). The extraction process was performed in a pilot-scale equipment (Thar Technologies, model SFE-2 × 5LF-2-FMC, Pittsburg, USA) equipped with two 5.15 L extraction vessels and three 1 L separators displayed in series. Only one extractor was employed, which was filled with the aerial parts of *C. sativa.* Carbon dioxide (CO_2) was used as the extracting solvent, and operational conditions were adjusted to reach a CO_2 density of 787 kg/m^3; this process was performed for 120 min. The extract afforded CBD and THC contents at 73.7% and 1.7%, respectively. For both *in vitro* and *in vivo* animal studies, test formulations were prepared to contain a final CBD concentration of 100 mg/mL. For the human *in vivo* study, the administered preparations were formulated to contain a CBD concentration of 40 mg/mL to achieve a THC concentration < 0.2% to comply with local regulations.

The oily excipients were selected according to the composition and size of the triglyceride chain. MCT is composed of caprylic (C8) and capric (C10) fatty acids and is a common carrier for CBD products; therefore, it was used as a reference. GML is obtained by an alcoholysis reaction between glycerol and corn oil and is composed of a mixture of mono, di and triglycerides of oleic (C18:1) and linoleic acids (C18:2).

For SEDDS, polyoxyl 40 castor oil was chosen as the emulsifier and polyethylene glycol 400 as the co-emulsifier owing to its long-term use in pharmaceutical products and safety profile. For generating the SEDDS, the extract was added to GML, and polyoxyl 40 castor oil, followed by polyethylene glycol 400 and the sweetener. The components were mixed under agitation at 60 °C for 30 min. Then, the flavoring agent was added at < 30 °C and then mixed for a further 15 min. The content of each component was selected according to the best micelle size and distribution after preliminary tests with different emulsifier proportions.

MCT and GML formulations were prepared by adding the extract and the sweetener to the oily vehicle and mixing under agitation at 60 °C for 30 min. The flavoring agent was added at < 30 °C and mixed for a further 15 min. The components of the tested formulations are presented in Table 1, which consist of Type I lipid oral solutions prepared with different oils and Type III novel SEDDS formulation containing a low concentration of surfactants and a partially hydrolyzed oil as a vehicle.

2.3. Characterization of test formulations

The SEDDS were characterized by micelle size, polydispersity index, and zeta potential measured by dynamic light scattering (Litesizer 500®, Anton Paar, Graz, Austria) at 25 °C. Sample dilution at a ratio of 1:500 was performed in deionized water for particle size analyses and in 1 mM KCl for zeta potential analysis. Samples were diluted immediately before reading and all analyses were performed in triplicates. The zeta

Table 1
Test formulations designed for samples containing CBD 100 mg/mL for *in vitro* and *in vivo* study in animals and CBD 40 mg/mL for *in vivo* study in humans.

Component	CBD 100 mg/mL Quantity (%, w/w)			CBD 40 mg/mL Quantity (%,w/w)		
	MCT	GML	SEDDS	MCT	GML	SEDDS
Cannabis sativa L. extract	13.56	13.56	13.56	5.42	5.42	5.42
Glyceryl monolinoleate	–	86.42	48,42	–	94.56	56,55
Polyoxyl castor oil	–	–	30	–	–	30
Polyethylene glycol 400	–	–	8	–	–	8
Medium chain triglyceride	86.42	–	–	94.56	–	–
Sweetener	0.01	0.01	0.01	0.01	0.01	0.01
Flavoring	0.01	0.01	0.01	0.01	0.01	0.01

potential was determined based on the electrophoretic mobility of particles.

SEDDS containing 100 mg CBD/mL resulted in a hydrodynamic diameter of 148 nm, with a polydispersity index of 23.7% and zeta potential (V) of −9.5, whereas the SEDDS formulation containing 40 mg CBD/mL resulted in a hydrodynamic diameter of 194 nm, polydispersity index of 25.8% and zeta potential (V) of −6.9. The SEDDS was quickly dispersed in water (<30 s), forming a uniform and stable yellowish dispersion.

2.4. In vitro study

2.4.1. Digestion experiments

The *in vitro* digestion study was performed as described by Williams et al. (Williams et al., 2012). The digestion buffer (pH 6.5) contained 2 mM Tris–maleate, 1.4 mM $CaCl_2{\cdot}H_2O$, and 150 mM NaCl and was supplemented with 3 mM NaTDC and 0.75 mM PC. Pancreatin from porcine pancreas containing pancreatic lipolytic enzymes was prepared by vortex mixing 1.0 g of pancreatin powder in 5 mL digestion buffer free of bile salt and phospholipid adjusting the pH to 6.5. The pancreatin suspension was centrifuged (4000 rpm for 15 min) before use and prepared fresh before each test.

In *in vitro* experiments, 1.0 g of the formulation (in triplicate) was weighed directly in the reaction vessel containing 36 mL of digestion buffer pre-heated at 37 °C and dispersed for 10 min. The pH was adjusted to 6.5 using 0.6 M NaOH. Digestion was initiated by adding 4 mL pancreatin extract. The NaOH titration solution (0.6 M) was added to the reaction vessel to maintain a constant pH of 6.5 during digestion. After the digestion period (30 min), 1 mL of the sample (in triplicate) was transferred to microtubes previously tared, and 5 μL of lipolysis inhibitor (1 M 4-bromophenylboronic acid in methanol) was added to arrest digestion. Samples were then separated by centrifugation (1400 rpm for 30 min) into three phases: a floating oily phase, a dispersed aqueous colloidal phase, and a precipitated pellet phase. To recover the phases, the supernatant (aqueous + oily phases) of the centrifuged solution was transferred to a second tared microtube and the mass of the pellet phase remaining in the first microtube was recorded by weight difference. To separate the phases, the aqueous phase was removed with a needle coupled to a syringe and transferred to a third microtube previously tared. Finally, the oily and aqueous phases were recorded by weight difference from the second and third microtubes, respectively. The recovered aqueous phase was diluted directly in ethanol prior to analysis. The pellet and oily phases were transferred to 5 mL volumetric flasks, and the volume was made up with chloroform–methanol (2:1, v/v) prior to subsequent dilution in ethanol.

2.4.2. CBD quantification

Samples obtained in the digestion experiments were characterized for their CBD content in the ultra-performance liquid chromatography (UPLC) system coupled to a diode-array detector (DAD) (Waters, Acquity®, Pittsburg, USA). The quantification method was performed according to the United Nations Office on Drugs and Crime (UNODC) guide with some modifications to suit the operating conditions of the UPLC system. The method was successfully verified prior to use in the present study by analyzing known reference materials. Compound separation was achieved using a C18 column (Kinetex, 150 mm × 2.6 mm, 2.1 μm; Phenomenex, Torrance, USA). The mobile phases were water (A) and acetonitrile (B), both acidified with 0.1% formic acid, performed at the following gradient: 0 min, 34% A; 8 min, 34% A; 12 min, 5% A; 13 min, 5% A; 17 min, 34% A, 18 min, 34% A. The flow rate was maintained at 0.5 mL/min. Samples and column temperatures were maintained at 8 °C and 30 °C, respectively. The injection volume was 1.5 μL, and data acquisition was performed at 220 nm using Empower ® 3 (Waters, Pittsburg, USA). The compounds were quantified using external calibration curves of CBD with linearity between 2 and 60 μg/mL ($r > 0.999$). The method was validated in terms of accuracy, precision, specificity, linearity and robustness. The limit of quantification (LoQ) was 2 μg/mL.

2.5. In vivo study

2.5.1. Animals and surgery

The *in vivo* study was performed at the Center for Innovation and Preclinical Trials (CIEnP), a Brazilian non-profit institution dedicated to providing non-clinical assays for evaluating efficacy and safety for developing pharmaceutical and cosmetic products. Male mice (*Mus musculus*) between 8 and 12 weeks were maintained under a 12 h light/dark cycle in a room at 21 ± 2 °C and relative moisture of 45 – 70%, with *ad libitum* access to food (Nuvital CR1®, Quimtia, Colombo, Brazil) and water. The experimental procedures followed ethical principles of animal experimentation adopted by the National Council for the Control of Animal Experimentation (CONCEA). The experimental protocol used in this study was reviewed and approved by the CIEnP Animal Use Ethics Committee (CEUA) (number 264/00).

2.5.2. Experimental protocol

Male CD1 mice (n = 3) were administered a single dose (100 mg of CBD/kg) of MCT, GML, or SEDDS formulations. Oral administration was performed by gavage, and blood samples were collected via the mandibular vein for the first two points and via terminal cardiac punch at the final time point. The animals were fasted for a maximum of 4 h before study initiation and up to 2 h after administering the test substances. Blood samples were collected after 0.25, 0.50, 1, 2, 4, and 6 h after administration. To determine the experimental groups, the animals were divided into three 3 groups containing six animals per group, ensuring that the age and weight variations do not exceed ± 20% average weight. Each animal was submitted to a maximum of three collection times.

2.5.3. Assay and CBD quantification

The blood samples containing sodic heparin were centrifuged at 4000 rpm at 4 °C for 15 min to obtain plasma samples, which were immediately processed. The sample preparation comprised the addition of 50 μL internal standard (minoxidil 200 ng/mL) and 100 μL methanol to 50 μL plasma. This solution was vortexed for 30 s and centrifuged for 5 min at 14 rpm, and the supernatant was diluted five times in methanol prior to analysis.

The samples were analyzed using UPLC-MS/MS system (Waters, Pittsburg, USA). Compound separation was achieved using a C18 column (Kinetex, 50 mm × 2.1 mm, 2.6 μm; Phenomenex, Torrance, USA). The mobile phases were water (A) and acetonitrile (B), both acidified with 0.1% formic acid at the following gradient: 0 min, 95% A; 0.17 min, 95% A; 1.17 min, 40% A; 2.17 min, 30% A; 3.17 min, 0% A, 5.33 min, 0% A; 5.50 min, 95% A; 7.00 min, 95% A. The flow rate was maintained at 0.4 mL/min. Samples and column temperatures were maintained at 10 °C and 30 °C, respectively. The injection volume was 1.0 μL, and data

acquisition was performed using a triple-quadrupole mass spectrometer (Xevo TQS, Waters, Pittsburg, USA) under the following conditions: nebulizer (N_2) and drying gas (N_2) flow rates, 150 L/h and 1000 L/h, respectively; capillary voltage, 4 kV; ionization source temperature, 350 °C; cone voltage, 50 V, collision gas (Ar) flow rate, 0.15 mL/min; collision energy, 24 eV. Linearity for CBD was between 5 and 255 ng/mL with $r > 0.995$.

2.6. In vivo study in humans

2.6.1. Volunteers

A total of 28 volunteers were pre-screened for eligibility and 12 volunteers of both genders (6 males and 6 non-pregnant females) were screened for study participation (Fig. 1), after obtaining informed consent from subjects. According to the inclusion criteria, volunteers were 18–50 years old, had a body mass index (BMI) of 18.50 – 29.99 kg/m^2, and were non-smokers. In addition, the volunteers were in good physical and mental health as established by their medical history, physical examination, electrocardiogram, vital signs, biochemistry, and hematology results.

The main exclusion criteria included a relevant presence or history of surgery or medical disorder (e.g., cardiac, gastrointestinal, respiratory, hepatic, renal, endocrinal, neurologic, psychiatric, or hematologic events within the last 3 months) potentially interfering in study results, history or chronic abuse of alcohol and/or drugs, known hypersensitivity to the medicament or its correlates, as well as to formulation excipients, use of medicaments that potentially interfere with kinetics/dynamics of the investigated medicament or any other medicament considered clinically significant, regular consumption of grapefruit, donation or loss of > 450 mL of blood within the last 3 months, a positive test result for drugs during all periods of study, and use of hemp/cannabis products at least 1 week prior to study initiation.

Data regarding the current clinical condition of the research participant, relevant and concomitant diseases, personal and family history, evaluation of contraceptive methods, treatments, surgical procedures, and/or previous/concurrent medications were recorded. In addition, vital signs, including blood pressure, heart rate, temperature, and a record of weight, height, and BMI were documented. The physical evaluation included a general inspection of the skin and appendages, oropharynx, musculoskeletal, respiratory, cardiovascular, digestive, genitourinary, neurological, extremities, and limbs. Cardiac evaluation and laboratory tests were considered fundamental for the screening and selection process. During the screening/selection stage, no participant was positive in serology tests or a pregnancy test (β-hCG for women). However, some parameters in hematology, biochemistry, and urine type I exams presented with abnormalities, which were classified as not clinically significant or presented within the normal range after evaluation and/or retesting to confirm the alterations (repeat).

This study was conducted in compliance with the ICH E6 (R2) – Good clinical practice, as well as local regulations (Resolution n° 466 of December 12, 2012, and Resolution n° 251 of August 07, 1997). The research protocol and documents were approved by the Ethical Committee of the Medicine Faculty of Jundiaí (Approval number 3898310).

2.6.2. Experimental protocol

The clinical study was performed as a randomized, double-blind, monocentric, and controlled crossover design at Azidus Brazil Contract Research Organization (Valinhos, Brazil). Each visit to the research center lasted 24 h, 12 h before, and 12 h post-dosing. A washout period of at least 7 days was performed between each pharmacokinetic day. Twelve healthy volunteers were randomized to receive a single dose of cannabis formulations (standardized to 40 mg CBD) with 200 mL of still water under fasted conditions on separated kinetic days. On each dosing occasion, volunteers were fasted for at least 8 h overnight before and 4 h after dosing. The water intake was limited to 1 h before and 1 h after dosing, except for 200 mL of water during formulation administration. Blood samples (6 mL each) were collected at pre-dosing and 0.25, 0.50, 0.75, 1, 1.33. 1.66, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12 and 24 h after

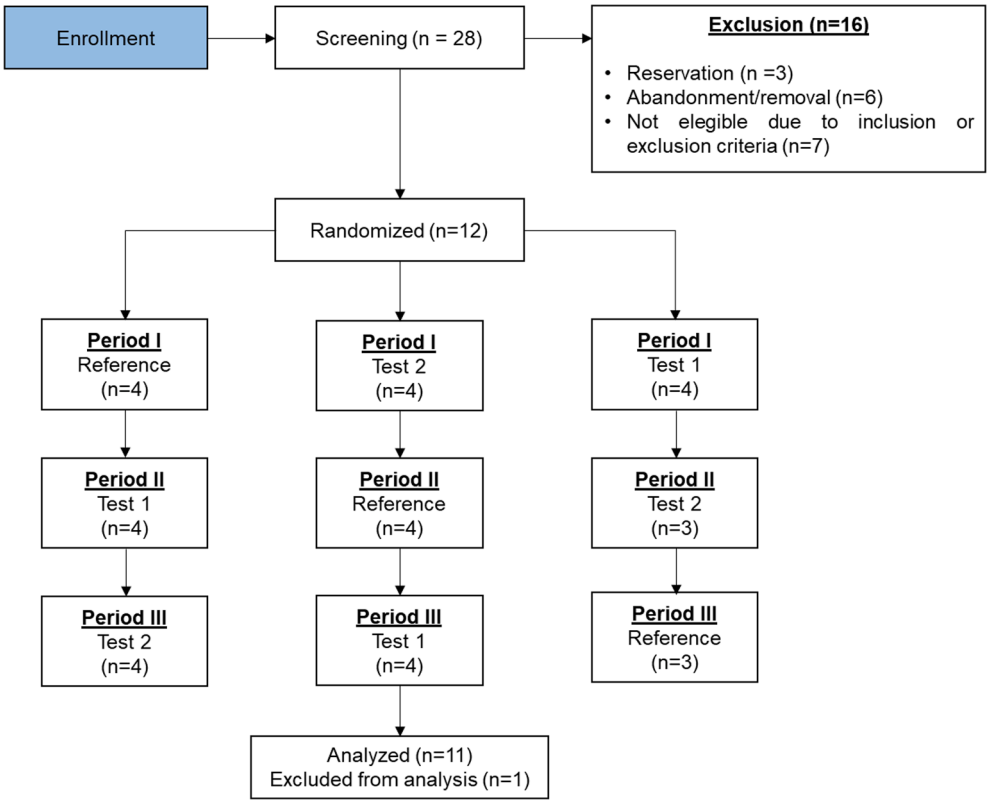

Fig. 1. Study population.

product administration.

Dinner before pharmacokinetic testing days, as well as meal and fluid intake, were all standardized as normocaloric and normoproteic. Meals were served 4 and 8 h after administering respective formulations. In addition, participants were asked to avoid alcoholic beverages or xanthine- containing foods, as well as coffee, tea, and soft drinks 48 h before pharmacokinetic testing days.

2.6.3. Assay and CBD and 11-OH-THC quantification

Blood samples were centrifuged at 3500 rpm at 4 °C for 10 min to obtain plasma; then samples were stored at −80 °C until analysis. The plasma samples underwent liquid–liquid extraction to isolate the analytes, CBD and 11-OH-THC. Accordingly, plasma samples were prepared by transferring 400 μL of plasma to a tube, followed by the addition of 1.6 mL of cold acetonitrile. This mixture was vortexed for 1 min, and 1.6 mL of water was added. Next, the mixture was vortexed for 1 min, followed by the addition of hexane (3 mL). The mixture was vortexed again for 1 min and then centrifuged at 4000 rpm for 2 min at 4 °C. The samples were frozen, and the supernatant was separated from the congealed aqueous phase. The solvent was then evaporated at 40 °C and the sample was resuspended in 150 μL acetonitrile + 1% formic acid and 50 μL water before analysis.

The samples were analyzed using UPLC-MS/MS system (1260 Infinity, Agilent, Santa Clara, CA, USA). For CBD quantification, the compound separation was achieved using a C18 column (Symmetry, 50 mm × 2.1 mm, 3.5 μm; Waters, Pittsburg, USA). The mobile phases were (A) water and (B) acetonitrile, both acidified with 0.1% formic acid. The flow rate was maintained at 0.4 mL/min for 8 min in gradient elution. The samples and column temperatures were maintained at 25 °C and 40 °C, respectively. The injection volume was 40.0 μL and data acquisition was performed in a mass spectrometer (Qtrap 5500, AB Sciex, Framingham, USA). For CBD, linearity was achieved between 0.05 and 20 ng/mL, with $r > 0.995$.

For 11-OH-THC, compound separation was achieved using a C18 column (Xbridge, 50 mm × 3.0 mm, 3.5 μm; Waters, Pittsburg, USA). The mobile phases were water acidified with 0.1% formic acid and methanol. The flow rate was maintained at 0.4 mL/min for 10 min. The samples and column temperatures were maintained at 10 °C and 40 °C, respectively. The injection volume was 40.0 μL and data acquisition was performed using a mass spectrometer (Qtrap 5500, AB Sciex, Framingham, USA). For 11-OH-THC, linearity was achieved between 0.05 and 4 ng/mL, with $r > 0.995$.

2.7. Statistical analysis

For the *in vivo* study in mice, pharmacokinetic parameters were determined by non-compartmental analysis using Phoenix WinNonLin ® software (version 7.0). The pharmacokinetic parameters evaluated were maximum plasma concentration (C_{max}), time to reach the maximum concentration (T_{max}), and area under the concentration–time curve (AUC). AUC was determined using trapezoidal log/linear method. The absolute bioavailability was determined by the administering CBD (10 mg/kg diluted in MCT) through the lateral caudal vein, calculated using the following equation: F (%) = [(Intravenous Dose × Oral AUC) / (Oral Dose × Intravenous AUC)] × 100. One-way analysis of variance (ANOVA), followed by Tukey's test, was used to determine statistically significant differences among the experimental groups. Statistical significance was set at $p < 0.05$.

For the *in vivo* study in humans, the pharmacokinetic parameters and statistical analysis were calculated using the software Phoenix WinNonLin® version 8.3. The formulation equivalence assessment was performed using 90% confidence intervals (based on the Schuirmann test) for pharmacokinetic parameters transformed into natural logarithms (ln). The construction of these intervals is based on the residual mean square of the ANOVA, which was constructed according to the 3 × 3 crossover design and contained the sequence effects (fixed effect), subject within the sequence (random effect), formulation (fixed effect), and period (fixed effect). For the formulations to be considered equivalent, the confidence interval of the ratios of the means must range between 80 and 120%; however, for data with ln transformation, the confidence interval ranges between 80 and 125%. For the pharmacokinetic parameter T_{max}, the non-parametric Wilcoxon confidence interval (90%) was used for the individual difference T1 and T2 minus R. Results are expressed as geometric means obtained by the method of least squares. Graphs and correlation analyses were performed using GraphPad Prim 5® (GraphPad Software, Inc., San Diego, CA, USA)

3. Results

3.1. In vitro study

For the three formulations, the distribution of physical phases and the distribution pattern of CBD across the digestion phases after 30 min of digestion are shown in Table 2 and Fig. 2, respectively. The SEDDS formulation presented no oily phase, whereas MCT and GML formulations exhibited a similar behavior with, 3.8 ± 0.5% and 5 ± 1% of samples, respectively, remaining in the oily phase.

Fig. 2 presents the CBD distribution into the phases after 30 min of digestion and demonstrates that CBD distribution is formulation dependent. Most CBD (86 ± 2%) in the SEDDS formulation was recovered in the aqueous phase. Conversely, only 5.6 ± 0.8% and 13 ± 2% CBD was recovered in the aqueous phase of MCT- and GML-based formulations, respectively. For these samples, most of the CBD was recovered in the oily phase. In formulations presenting oily phase, the total CBD recovery across three phases (oil, aqueous, and pellet) was less than the theoretical CBD content. According to Williams et al. (Williams et al., 2012), the lower recovery observed for these formulations can be attributed to the difficulty isolating of the oily phase using bench-top centrifugation. In some cases, the residual oil adheres to the inside of the sample tube, preventing the complete removal of the oily phase for quantification.

3.2. In vivo study in mice

Fig. 3 represents the plasma concentration of CBD *vs.* time profiles of formulations following oral administration to mice at 100 mg/kg. The corresponding C_{max}, T_{max} and $AUC_{0\text{-}6h}$ parameters obtained in these *in vivo* experiments are listed in Table 3. SEDDS afforded the highest systemic exposure, with a C_{max} of 1248.77 ng/mL for CBD and $AUC_{0\text{-}6h}$ four times that of the MCT formulation. The bioavailability of the SEDDS formulation was approximately 1.5 times higher than the value obtained for the GML formulation and 3.25 times higher than that of the MCT formulation. A statistically significant difference was observed between the SEDDS and MCT formulations ($p < 0.05$).

3.3. In vivo study in humans

Figs. 4 and 5 present the pharmacokinetic results of CBD and 11-OH-THC for SEDDS, GML, and TCM formulations following oral administration at 40 mg of CBD and 2 mg of THC. For CBD, the SEDDS demonstrated the highest C_{max} and $AUC_{0\text{-}12h}$, with values 1.98 and 1.48 times higher than MCT formulation, respectively; GML resulted in C_{max} and $AUC_{0\text{-}12h}$ values 1.85 and 1.31 times higher than MCT formulation, respectively. In addition, the GML formulation exhibited the highest

Table 2
Distribution of physical phases of tested lipid-based formulations.

Formulation	Oily Phase (%)	Aqueous phase (%)	Pellet (%)
MCT	5 ± 1	95 ± 4	2.2 ± 0.8
GML	3.8 ± 0.5	93 ± 4	3.2 ± 0.3
SEDDS	0.00	98 ± 3	3.4 ± 0.5

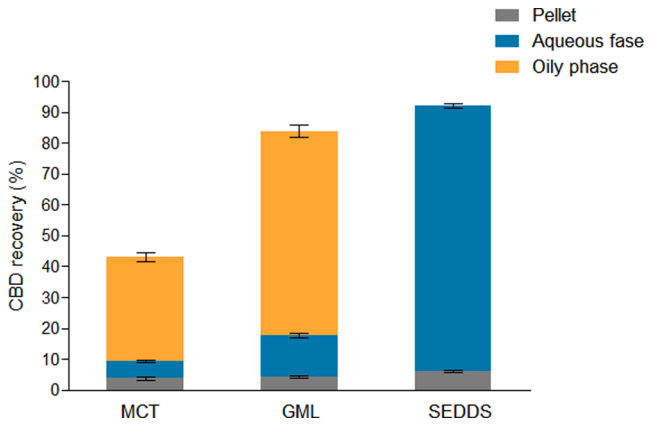

Fig. 2. Distribution pattern of CBD across the physical phases formed following 30 min of digestion of MCT, GML and SEDDS formulations.

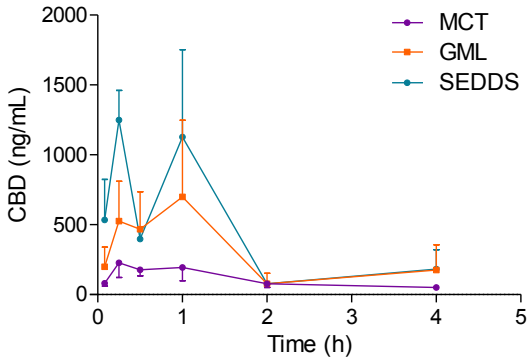

Fig. 3. Mouse CBD plasma concentration *vs.* time (mean ± SEM) after an oral single dose administration of MCT, GML and SEEDS formulations containing 100 mg of CBD.

Table 3
Pharmacokinetic parameters obtained following single dose oral administration in mice of 100 mg/kg of CBD in different formulations.

Formulation	C_{max} (ng/mL)	T_{max} (min)	$AUC_{0\text{-}6h}$ (min*ng/mL)	F (%)
MCT	266 ± 104,2	30	44.184 ± 16.196	2.4
GML	699 ± 547,18	60	118.649 ± 96.626	5.3
SEDDS	1248 ± 212,59*	30	175.629 ± 86.185*	7.8

* Statistically significantly different from MCT

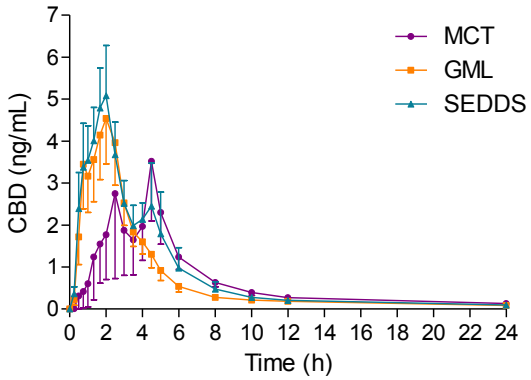

Fig. 4. Human CBD plasma concentration *vs.* time (mean ± SEM) after an oral single dose administration of MCT, GML, and SEDDS formulations containing 40 mg of CBD and 2 mg of THC.

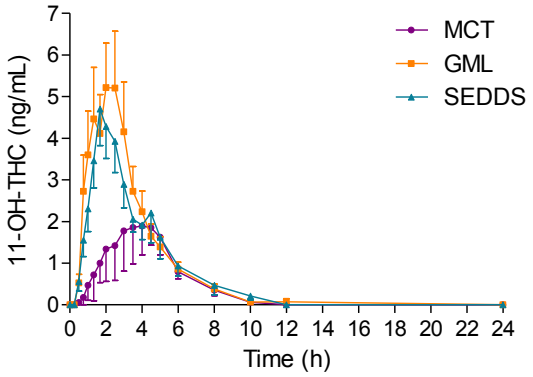

Fig. 5. Human 11-OH-THC plasma concentration *vs.* time (mean ± SEM) after an oral single dose administration of MCT, GML, and SEDDS formulations containing 40 mg of CBD and 2 mg of THC.

C_{max} and $AUC_{0\text{-}12h}$ for the metabolite 11-OH-THC, with values 2.97 and 2.5 times higher than the MCT formulation, respectively. Moreover, the SEDDS and GML formulations presented a CBD T_{max} of approximately 1.6 h, while the MCT formulation presented a T_{max} value of 4.2 h. Both the SEDDS and GML formulations significantly differed from the MCT-based formulation ($p < 0.05$); however, no statistical difference was observed between the SEDDS and GML. The results are listed in Table 4.

The side effects observed throughout the study are shown in Table 5. Most adverse events were classified as mild in intensity (85%) and expected predictability (75%). Among the 20 adverse events, 15 had a causal relationship with drugs, classified as possible (75%), and 5 as unlikely (25%). Throughout the study, no adverse events were classified as serious.

3.4. Correlations

Correlation data of the *in vitro* digestion test and *in vivo* AUC for mice or humans, and the AUC correlation between mice and humans are presented in Fig. 6. Percentual recovery of CBD in the aqueous phase after digestion of MCT (5.6 ± 0.8%), GML (13 ± 2%), and SEDDS formulations (86 ± 2%) showed no correlation with AUC after oral administration to mice ($r^2 = 0.75$) and humans ($r^2 = 0.66$). Interestingly, results from the AUC between mice and humans showed correlation ($r^2 = 0.98$).

4. Discussion

The oral bioavailability of CBD is considerably low owing to its systemic metabolism. CBD bioavailability is approximately 6% under fasted conditions, with up to 75% metabolized before entering circulation (Millar et al., 2018). CBD undergoes extensive hepatic and intestinal metabolism by cytochrome P450 (CYP) enzymes (CYP2C19 and CYP3A4), and by the uridine 5′-diphospho-glucuronosyltransferase (UGT) enzymes (UGT1A7, UGT1A9, and UGT2B7). CYP3A4 appears to

Table 4
Pharmacokinetic parameters obtained following single dose oral administration in humans of 40 mg of CBD and 2 mg of THC in different formulations. Results are expressed as Geometric Means obtained by the method of least squares.

Formulation	CBD			11-OH-THC		
	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}12h}$ (min*ng/mL)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}12h}$ (min*ng/mL)
MCT	3.495*	4.257*	11.960*	2.449*	3.575*	6.391*
GML	6.465	1.591	15.758	7.249	1.999	16.082
SEDDS	6.943	1.683	17.740	6.546	1.953	14.823

* Statistically significantly different

Table 5
Summary of adverse events total and segmented by treatment.

Side Effects	MCT (%)	GML (%)	SEDDS (%)	TOTAL (%)
Headache	–	2 (22)	–	2 (10)
Somnolence	5 (83)	5 (56)	3 (60)	13 (65)
Dizziness	1 (17)	–	1 (20)	2 (10)
Nausea	–	1 (11)	1 (20)	2 (10)
Vomiting	–	1 (11)	–	1 (5)

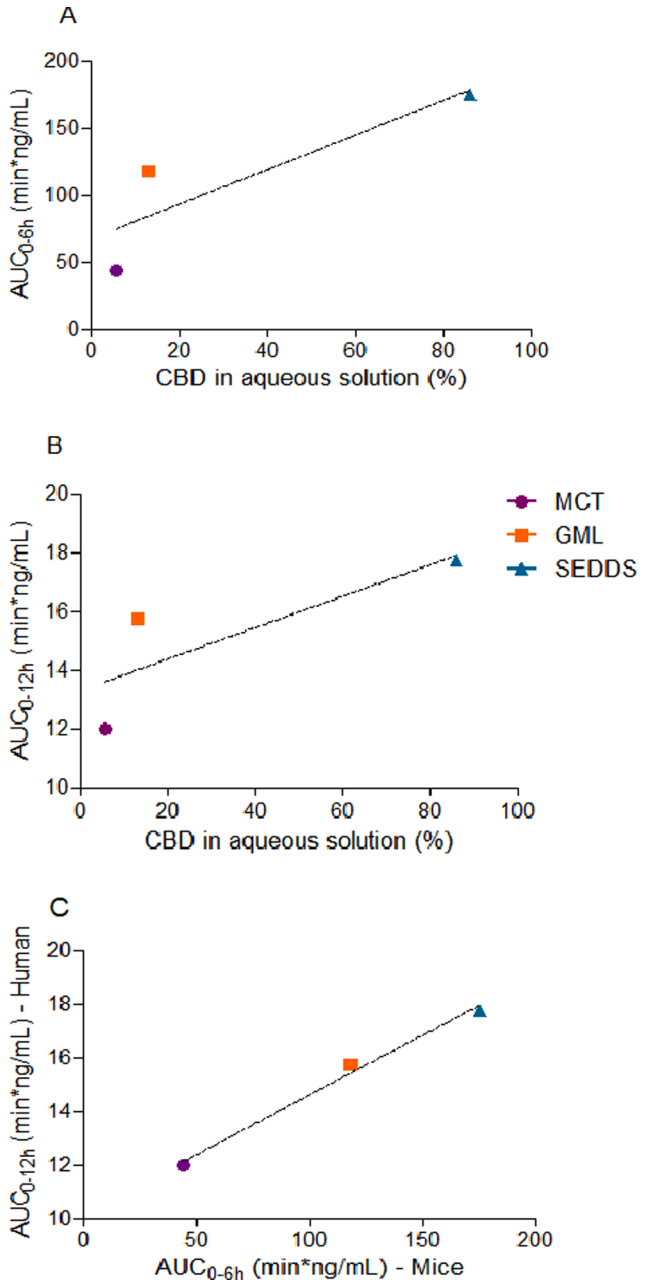

Fig. 6. Correlation data of % CBD in aqueous phase after *in vitro* digestion test and AUC of plasma *vs.* time after oral administration of MCT, GML or SEDDS formulation to (A) mice, (B) humans, and (C) AUC correlation between mice and humans.

be the main enzyme involved in CBD metabolism, and CYP2C19 may be related to the conversion of CBD into 7-OH-CBD, which is metabolized to 7-carboxy-CBD by CYP3A4 (Perucca and Bialer, 2020). THC is mainly metabolized by isozymes CYP2C9, CYP2C19 and CYP3A4 to 11-OH-THC, which known to possess psychoactive activity (Lemberger et al., 1972; Schwope et al., 2011).

Co-administration of high-fat meals can improve CBD bioavailability by 4-fold compared with the fasted state (Taylor et al., 2018). This effect may be related, at least in part, to lipid digestion and the action of bile and enzymes for micelle formation which can enhance the dissolution rate and solubility of lipophilic drugs, as well as lymphatic absorption, as discussed in previous studies (Birnbaum et al., 2019; Charman et al., 1997). However, the administration of medicines with food has some disadvantages. For example, inconsistency in meal times, food composition, lipid type, and proportion can produce variable bioavailability, impacting clinical outcomes (Perucca and Bialer, 2020). In contrast, Charman et al. (Charman et al., 1993) have shown that a long-chain monoglycerides formulation can mitigate the food effect of danazol, a BCS Class II drug, and suggested that this effect can be attributed to monoglycerides undergoing less lipid hydrolysis when compared with to non-hydrolyzed long-chain triglycerides. Considering the discussed studies and the poor water solubility of CBD, the formulation composed of partially hydrolyzed long-chain triglycerides (GML) could promote CBD bioavailability through lymphatic absorption; this effect could be further improved following the addition of a surfactant by enhancing dissolution rate.

Accordingly, three LBFs were prepared to evaluate the effect of MCT and long- chain triglycerides (GML), as well as the potential of the SEDDS, on the oral absorption of CBD. We selected MCT oil in the present study, as most commercially available CBD formulations employ this agent as a vehicle, allowing a valuable comparison with GML and SEDDS formulations, which reportedly promote superior bioavailability of lipophilic molecules, such as CBD. The formulations were evaluated by performing *in vitro* digestion, *in vivo* pre-clinical pharmacokinetic study in mice and a human pharmacokinetic assessment. For the study in mice, the CBD content in the formulation was 100 mg/mL; in humans, the amount of extract in the formulation was reduced to achieve the maximum THC content (<0.2%) established by the Brazilian Agency (ANVISA) for Cannabis products, resulting in a CBD content of 40 mg/mL. In addition, 11-OH-THC was monitored during the pharmacokinetic study in humans.

4.1. *In vitro study*

The fate of an incorporated drug with respect to phases formed during the dispersion and digestion of LBF provides an indicator of its potential utility in aiding oral absorption of poorly water-soluble drugs, such as CBD (Williams et al., 2012). CBD recovered in the aqueous phase represents the fraction available for rapid absorption, whereas the oily phase and pellet are considered to represent the drug not readily available for absorption following oral administration (Zgair et al., 2016). The MCT- and GML-based formulations presented a similar digestion pattern, separating into an oily phase, an aqueous phase, and a pellet; the SEDDS formulation separated only into the aqueous phase and pellet (Table 2). The absence of the oily phase in the SEDDS formulation indicates the complete hydrolysis of the sample (Sek et al., 2002).

Sek et al. (2002) have evaluated the *in vitro* digestion profiles of long-chain triglycerides and MCTs, as well as the phase behavior of their lipolytic products. The authors observed that after 30 min of digestion, the MCT sample was completely hydrolyzed, resulting only in an aqueous phase and pellet, whereas the GML sample formed an oily phase, an aqueous phase, and pellet; these findings were in contrast to the observations in the present study, where both MCT and GML formulations separated into three phases: oily, aqueous and pellet. These differences in the distribution pattern can be attributed to the presence of the Cannabis extract in formulations employed in the present study, which also contain triglycerides in its composition. In terms of the SEDDS formulation, complete digestion could be attributed to the lower amount of lipids compared with the oral MCT and GML solutions, as the

lipids content of the SEDDS formulation is partially substituted by the surfactant (polyoxyl castor oil) and cosolvent (polyethylene glycol 400); in addition to the properties of the surfactant and the cosolvent.

As mentioned, CBD recovered in the aqueous phase represents the fraction readily available for absorption, thus potentially predicting the effect of different LBFs (Type I and Type III) on CBD bioavailability. On comparing Type I formulations, the MCT-based formulation presented a CBD recovery of approximately 6%, whereas the GML-based formulation exhibited a CBD recovery two-fold that of the MCT formulation (13 ± 2%). The presence of GML in LBFs enhances oral bioavailability owing to its high monoglyceride content, improving the solubilization of lipophilic drugs, and its ability to maintain/increase drug solubility in post-digestive phases via mixed micellar formation (Maisine® CC product information, Gattefossé, Saint-Priest, France).

It has been shown that the solubility of drug substances with log P of approximately 4–8 is favored by the presence of long-chain triglycerides (Nielsen et al., 2001), corroborating the findings of the present study, where the CBD with a log P = 6.5 afforded better solubility in the GML formulation. In previous study, the addition of 15% GML to a formulation containing cinnarizine, a BCS Class II drug with log P = 5.8, reportedly improved the drug solubilization (up to 85% of the dose) after *in vitro* digestion, as cinnarizine was solubilized in mixed micelles formed by glycerides the digestion of GML, as well as by the complexation of cinnarizine and fatty acids (Maisine® CC product information, Gattefossé, Saint-Priest, France).

We compared Type I (GML) and Type III (SEDDS) formulations, which used glyceryl monolinoleate as an oily vehicle. In the present study it was detected that CBD recovery in the aqueous phase of the SEDDS formulation was 6.6 times greater, in line with reports developing SEDDS formulations as prominent solutions for enhancing the oral delivery of cannabinoids (Izgelov et al., 2020; Knaub et al., 2019; Nakano et al., 2019). The increased solubilization of SEDDS can be spontaneous formation of a thermodynamically stable o/w nanoemulsion or dispersion upon contact with the aqueous environment of the gastrointestinal tract. Nanodroplets entrap the lipophilic drug within their lipid core, thus stabilizing it in water with surfactants (Knaub et al., 2019).

4.2. In vivo study in mice

In the present study, *in vivo* mice experiments revealed that GML formulation presented a 2.6-times higher C_{max}, and 2.7-times higher $AUC_{0\text{-}6h}$ for CBD that the MCT formulation, corroborating the *in vitro* study findings. These improvements could be potentially attributed to a faster rate of digestion and, consequently, to increased absorption of the GML formulation. Long-chain glycerides from GML are absorbed by the intestinal lymphatic system, further increasing the bioavailability of CBD.

Conversely, Izgelov et al. (Izgelov et al., 2020) have reported that differences in MCTs and long-chain glycerides were not substantial for Type I formulations considering the oral absorption of cannabinoids in rats; however, these differences were more prominent for Type II formulations. Another study evaluated bioavailability after oral administration of seocalcitol, a BCS Class II drug with log a P of 4.8, dissolved in MCTs and long-chain triglycerides, and revealed no difference in the *in vivo* bioavailability of seocalcitol (Grove et al., 2005). Feng et al. (Feng et al., 2021) have investigated pharmacokinetic profile of CBD following administration of six LBFs composed of digestible oil (sesame or glycerol trioleate) and pre-digested oils (oleic acid, linoleic acid, 2-oleoylglycerol with oleic acid and oleic acid with glycerol) at a dose of 12 mg/kg. No statistical difference was observed in the AUC; however, the sesame oil formulation improved CBD exposure. The sesame oil formulation presented a higher C_{max} than the linoleic acid and 2-oleoylglycerol with oleic acid formulations.

It is worth mentioning that cannabinoids used in the study by Izgelov et al. (Izgelov et al., 2020) and seocalcitol used by Grove et al. (Grove et al., 2005) were isolated substances; however, the present study evaluated the bioavailability of a CBD-rich extract, which could explain the different behaviors observed. Nevertheless, these results demonstrate the difficulty in establishing a clear trend regarding the type of lipid that could promote the best bioavailability performance for each substance, reinforcing the importance of investigating each vehicle preclinically, after the *in vitro* development.

The SEDDS formulation exhibited 4.7-times higher C_{max}, and 4-times greater $AUC_{0\text{-}6h}$ than the MCT formulation. Furthermore, the SEDDS revealed a C_{max} and $AUC_{0\text{-}6h}$ values 1.8-times and 1.5-higher than the GML formulation, respectively. These results are consistent with those observed in *in vitro* tests and can be related to the capacity of the polyoxyl 40 castor oil and polyethylene glycol to promote oil-in-water emulsification. In addition, polyoxyl 40 castor oil can inhibit CYP3A isoenzymes (Goole et al., 2010). Thus given that CBD and THC are primarily metabolized by liver isozymes CYP2C19 and CYP3A4 (Lucas et al., 2018), synergistic actions of the active ingredient and emulsifier might increase the bioavailability of cannabinoids.

4.3. In vivo study in humans

In the present pharmacokinetic study undertaken in humans, the GML formulation exhibited C_{max} and $AUC_{0\text{-}12h}$ values that were 1.8- and 1.3-times greater than those of the MCT formulation, whereas the SEDDS formulation presented C_{max} and $AUC_{0\text{-}12h}$ values 2- and 1.5-times higher than those of MCT formulation, respectively (Table 4). For 11-OH-THC, the GML formulation presented C_{max} and $AUC_{0\text{-}12h}$ values 3- and 2.5-times greater than those of the MCT formulation; SEDDS presented C_{max} and $AUC_{0\text{-}12h}$ values that were 2.7- and 2.4-times higher than those of MCT formulation. Accordingly, GML and SEDDS afford a significant reduction in the coefficient of variation for most pharmacokinetic parameters, which can lower interindividual variabilities. These results suggest that the bioavailability of cannabinoids is significantly influenced by the physicochemical characteristics of lipids, the length of the fatty acid chain and, its susceptibility to digestion.

GML is composed of a mixture of mono-, di-, and triglycerides. Monoglycerides can improve the solubility of cannabinoids after the digestion process via micellar formation. This process occurs more rapidly in GML than in MCT, possibly explaining the reduced time required to reach maximum concentration (T_{max}). Surfactants and cosolvents also promote the faster micellar formation and cannabinoids dispersion in gastrointestinal fluids and unstirred water layer. In addition, the influence of surfactants and cosolvents can involve other mechanisms to improve bioavailability, as previously discussed.

The SEDDS has been extensively investigated in attempts to improve the bioavailability of CBD. For example, Atsmon at al. (Atsmon et al., 2018) have developed self-nanoemulsifying drug delivery systems (SNEDDS) with a mean particle size of approximately 30 nm to deliver CBD and THC in capsules. The pharmacokinetic profile was compared with the oromucosal spray Sativex, the market reference indicated as palliative treatment for spasticity related to multiple sclerosis, in an open-label, randomized, crossover single-dose study performed in 14 healthy male volunteers. The capsules containing CBD and THC in SNEDDS reportedly improved C_{max} by 1.6-times in both compounds while yielding bioavailability values that were 31% (CBD) and 16% (THC) higher than those of Sativex. The pharmacokinetic profile of 11-OH-THC was similar to that observed for THC (Atsmon et al., 2018).

Furthermore, SEDDS capsules based on Vesisorb® technology were developed to deliver CBD from a hemp extract. A randomized, double-blind, crossover study was performed in 16 healthy volunteers under fasted conditions to compare the SEDDS formulation (mean particle size, 40–50 nm) with a MCT solution containing the same concentration of CBD. Compared with the MCT formulation, the SEDDS formulation improved C_{max} by 4.4-times and $AUC_{0\text{–}8h}$/ $AUC_{0\text{–}24h}$ by 2.85-/1.70-times. In addition, the formulations presented a reduction in the coefficient of variation, thus indicating a decrease in interindividual

variabilities (Knaub et al., 2019).

Izgelov et al. (Izgelov et al., 2020) have reported similar CBD exposure between sesame oil and a SNEDDS with mean a particle size of 39 ± 8 and polydispersity index value of 0.3, in a study examining 12 volunteers using synthetic CBD. No statistically significant differences were observed between the two formulations. However, the SNEDDS formulation elicited a faster T_{max} (2 h) than the sesame oil formulation (4 h). Individual examination revealed a bimodal AUC for sesame oil, with two profiles between individuals, with early and delayed absorption (until the sixth hour). This phenomenon is termed flip-flop pharmacokinetics and can be observed for poorly water-soluble drugs when the rate of absorption is slower than the rate of elimination (Yáñez et al., 2011). Conversely, SNEDDS resulted in a more uniform and predictable CBD absorption curve.

In the present study, no statistically significant difference was observed between GML and SEDDS; however, a similar pharmacokinetic profile with more uniform absorption was noted for both GML and SEDDS when compared with MCT. These results suggest that the absorption rate of GML is similar to that of SEDDS, which supports the assumption that this partially hydrolyzed oil could be beneficial for improved cannabinoid pharmacokinetics.

Considering the high interindividual variability of cannabinoids, the single dose administered and the limited number of participants in the present study and discussed reports could be considered limitations. Nevertheless, GML and SEDDS considerably improved the pharmacokinetics of cannabinoids and potentially reduced interindividual variabilities, consistent with the results of previous studies. Furthermore, the potential of GML for cannabinoids delivery is evident, which can be considered a stable and safe vehicle compared with formulations containing multiple excipients and considerable quantities of emulsifiers, such as SEDDS.

Adverse events were assessed based on intensity, predictability, causality, and outcome. Based on the results presented in Table 5, no adverse events were classified as serious, thus indicating that the examined formulations were safe when administered under fasting conditions within the recommended dose.

4.4. Correlations

Predictive models that precisely describe the relationship between *in vitro* properties and *in vivo* response can reduce human tests, optimize, and improve formulation quality, compare pharmacokinetic profiles between different formulations, predict drug bioequivalence, and reduce costs of pharmaceutical development. A comprehensive correlation between *in vitro* dissolution and *in vivo* concentration–time can have regulatory significance, as it can determine the biopharmaceutical rate with a good level of confidence (Birnbaum et al., 2019). This correlation study is qualitative with no regulatory purpose; however, it can be useful during early stages of LBF development.

LBF development aims to provide drug solubility and stability with suitable excipients, maintaining solvent properties after dilution and digestion *in vivo*. The *in vitro* digestion test simulates the digestion process under physiological conditions and is considered an interesting technique to evaluate the rate of digestion of lipid formulations and the fate of co-administered drugs by analyzing phase behavior. After the digestion process, the quantification of drugs in the different phases (oily, aqueous and pellet) can determine drug dispersibility in the aqueous phase and potentially predict dissolution rate and absorption *in vivo*. The *in vitro–in vivo* correlation (IVIVC) has been evaluated in several studies and appears dependent on different factors that remain poorly clarified.

For example, droplet size had a poor influence on the bioavailability of drugs such as probucol (Nielsen et al., 2008) and danazol (Porter et al., 2004); however, decreasing the droplet size reduced the inter-and intra-individual variability of oral cyclosporine absorption (Kovarik et al., 1994). Considering other examples, no correlation was observed between the *in vitro* digestion test results and bioavailability for four different fenofibrate LBFs administered to Wistar rats (Do et al., 2011), as well as no difference in bioavailability when administered to landrace pigs, where fenofibrate was precipitated or promoted dispersibility (Griffin et al., 2014). However, some degree of correlation was observed when four different cinnarizine LBFs were administered to dogs (Larsen et al., 2013). Feeney et al. (2016) have evaluated the IVIVC for different LBFs by comparing eight drugs. A linear correlation was detected only for two drugs (danazol and griseofulvin).

Physicochemical properties could not be directly related to the evaluated results, however, a better correlation was observed for poorly soluble drugs with high melting points (Feeney et al., 2016). The findings in these reports indicate that the IVIVC is complex and may depend on factors such as the intrinsic characteristics of the drug, concentration and animal model. To the best of our knowledge, no published studies have evaluated the IVIVC of cannabinoids.

In the present study, we observed no correlation between the *in vitro* digestion test and *in vivo* tests, for both mice ($r^2 = 0.75$) (Fig. 6A) and humans ($r^2 = 0.66$) (Fig. 6B). In contrast, a correlation was observed between mice and humans ($r^2 = 0.98$) (Fig. 6C). Interspecies differences in gastrointestinal anatomy and physiology may hinder the use of these models for predicting human outcomes (Kararli, 1995). However, the pharmacokinetic profile depend on the intrinsic characteristics of compounds, such as solubility, permeability, lipophilicity, and metabolism (Li et al., 2013), which are factors that seem to determine the correlation between *in vitro* tests and *in vivo* outcomes, as discussed previously. Despite different cannabinoid doses administered to mice and humans, the findings of the present study suggest a correlation between mice and humans for cannabinoids, at least in terms of qualitative analyses.

5. Conclusions

Cannabinoids have great potential for treating a wide range of pathologies; however, their low bioavailability is challenge for develop formulations with an optimal delivery. The main objective of the present work was to increase the bioavailability of CBD, which could improve efficacy, reduce side effects, and reduce the cost of treatment. Lymphatic absorption has been proven to benefit the bioavailability of drugs undergoing marked first-pass metabolism. In addition, the absorption rate of poorly water-soluble drugs, such as CBD, influences pharmacokinetics and bioavailability. Thus, we proposed a formulation containing a partially hydrolyzed long-chain triglycerides and SEDDS for improving pharmacokinetic profile. The obtained results suggest that the length of lipid chains and their susceptibility to digestion can influence CBD and THC delivery. GML and SEDDS significantly improved the pharmacokinetic properties, exhibiting higher bioavailability, C_{max} and T_{max}, than the MCT formulation. Therefore, the GML and SEDDS formulations can benefit cannabinoid therapy. Furthermore, the correlation studies presented in this report can contribute to future developments.

CRediT authorship contribution statement

Manuel A.A. De Prá: Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing. **Renata Vardanega:** Methodology, Investigation, Writing – review & editing. **Carla G. Loss:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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