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Piperine-pro-nanolipospheres as a novel oral delivery system of cannabinoids: Pharmacokinetic evaluation in healthy volunteers in comparison to buccal spray administration

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ABSTRACT

Nowadays, therapeutic indications for cannabinoids, specifically Δ^9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) are widening. However, the oral consumption of the molecules is very limited due to their highly lipophilic nature that leads to poor solubility at the aqueous environment. Additionally, THC and CBD are prone to extensive first pass mechanisms. These absorption obstacles render the molecules with low and variable oral bioavailability. To overcome these limitations we designed and developed the advanced pro-nanolipospheres (PNL) formulation. The PNL delivery system is comprised of a medium chain triglyceride, surfactants, a co-solvent and the unique addition of a natural absorption enhancer: piperine. Piperine was selected due to its distinctive inhibitory properties affecting both Phase I and Phase II metabolism. This constellation self emulsifies into nano particles that entrap the cannabinoids and the piperine in their core and thus improve their solubility while piperine and the other PNL excipients inhibit their intestinal metabolism. Another clear advantage of the formulation is that its composition of materials is approved for human consumption. The safe nature of the excipients enabled their direct evaluation in humans. In order to evaluate the pharmacokinetic profile of the THC-CBD-piperine-PNL formulation, a two-way crossover, single administration clinical study was conducted. The trial comprised of 9 healthy volunteers under fasted conditions. Each subject received a THC-CBD (10.8 mg, 10 mg respectively) piperine (20 mg)-PNL filled capsule and an equivalent dose of the oromucosal spray Sativex® with a washout period in between treatments.

Single oral administration of the piperine-PNL formulation resulted in a 3-fold increase in Cmax and a 1.5-fold increase in AUC for THC when compared to Sativex®. For CBD, a 4-fold increase in Cmax and a 2.2-fold increase in AUC was observed. These findings demonstrate the potential this formulation has in serving as a standardized oral cannabinoid formulation. Moreover, the concept of improving oral bioavailability described here, can pave the way for other potential lipophilic active compounds requiring enhancement of their oral bioavailability.

1. Introduction

The growing body of evidence regarding the therapeutic advantages of cannabis has placed the plant, specifically the cannabinoids Δ^9-tetrahydrocannabinol (THC) and Cannabidiol (CBD), at the front of modern medicine [1,2].

Although the oral route is the most convenient for patients, the reported oral bioavailability of these compounds is low and variable (approximately 9–13%). Poor oral absorption of the cannabinoids is the result of their lipophilic nature, poor aqueous solubility and mostly, susceptibility to significant first pass metabolism [3,4]. While both THC and CBD are prone to oxidation processes starting at the intestine, only CBD undergoes direct glucuronidation. In addition, there have been reports regarding THC inclination to P-gp (P-glycoprotein) efflux, further limiting its absorption [5–9].

Our group has devised a lipid-based formulation that overcomes the absorption challenges of BCS (biopharmaceutical classification system) class II molecules such as THC and CBD. This lipid carrier is termed pro-nanolipospheres (PNL) and is a self-nano emulsifying drug delivery system (SNEDDS). The formulation consists of a medium chain triglyceride, surfactants, phospholipids and a co-solvent that dissolves all the excipients and the required drug. The term we use for this constellation

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is “pre-concentrate”. Upon contact with water, the pre-concentrate self-emulsifies into an o/w nano-emulsion. The resulting nano particles entrap the lipophilic drug in their core, which consists of the triglyceride, thus achieving a solubilized state in an aqueous medium [10,11]. The nanometric size of the particles is of great importance, since it enables penetration to the inter-villous spaces at the intestinal brush border, thus increasing the available surface area for absorption [12].

The PNL formulation serves as the basis for the development of a more sophisticated formulation, an Advanced PNL, with the added value of containing the natural absorption enhancer piperine [13]. We have found that the PNL excipients not only aid in increasing solubility in the GI tract, but they also inhibit first pass metabolism mechanisms such as CYP family enzymes and the P-gp efflux pump [10].

There are reports regarding the ability of several natural alkaloids and phenolic compounds to inhibit certain Phase I and Phase II metabolic processes. Among them is the alkaloid piperine, naturally found in black pepper [14–16].

We have succeeded in incorporating piperine into the PNL formulation following a screening of other potential absorption enhancers such as curcumin, resveratrol. The piperine is solubilized in the pre-concentrate with THC or CBD. When it is dispersed in water, a homogenous, visibly clear emulsion is obtained with nano particles of around 30 nm size. Moreover, the obtained zeta potential (ζ) for the formulation is high enough to maintain stability throughout their passage in the GI tract. The formulation remains clear without precipitation of any of the ingredients for up to two months in its dispersed state. Thus, the Piperine-PNL (P-PNL) formulation is physically and thermodynamically stable.

In the freely moving rat model, incorporating THC and CBD into the piperine-PNL vehicle results in a 9 and 6-fold increase in oral bioavailability, respectively, when compared to THC and CBD solution and an additional 1.47 and 2-fold increase when compared to THC/CBD-PNL administration [13]. We maintain that this enhanced absorption is result of P-PNL's ability to increase solubility of the cannabinoids, inhibit Phase I and Phase II metabolism as well as efflux mechanisms.

The PNL serves as a platform for synchronized delivery of both piperine and the model drug to the enterocyte site. This achieves not only increased solubility of the lipophilic drug and absorption enhancer, but also successful inhibition of intestinal first pass metabolism mechanisms.

The potential in our preclinical results and the need for an oral cannabinoid formulation in the market led us to investigate the behavior of the THC/CBD-piperine-PNL formulation in a clinical setting. Moreover, the GRAS (generally recognized as safe) status of PNL excipients facilitated the transition into the clinical sphere.

So far, the FDA has approved THC formulations for the treatment of nausea and vomiting associated with cancer chemotherapy as well as an appetite booster for AIDS patients [17,18]. The non-psychoactive cannabinoid CBD, although yet to be marketed, has an extremely safe profile in human beings, and it has been clinically evaluated for the treatment of anxiety, movement disorders, inflammation, pain, and epilepsy [19–22]. Interestingly, there is growing evidence that combined administration of CBD and THC has a superior effect. Researchers claim that CBD can not only potentiate the therapeutic effects of THC but also diminish its undesirable effects such as anxiety, panic, sedation, dysphonia and tachycardia [21,23]. Due to an established therapeutic rational for the combination of THC and CBD, we aimed to evaluate pharmacokinetic profiles of both compounds in a combined administration. Therefore, we developed a THC-CBD-piperine-PNL formulation for which we maintained particle size of 50 nm and less.

We conducted a two-way crossover, single administration clinical study with 9 healthy fasting volunteers. Each subject received a THC-CBD piperine-PNL capsule and the oromucosal spray Sativex® with a washout period in between formulations. Sativex® contains both cannabinoids and is approved in Israel and in some states in Europe for treatment of patients with multiple sclerosis. The primary goal of this study was to compare the oral THC-CBD-piperine-PNL formulation to the Sativex® spray in terms of plasma exposure and bioavailability. This work will pave the way for a safe, standardized oral cannabinoid formulation.

2. Materials and methods

2.1. Chemicals

All chemicals, unless otherwise specified, were purchased from Sigma-Aldrich (Rehovot, Israel). THC was purchased from THC Pharm GmbH – The health concept, Germany. CBD was purchased from STI Pharmaceuticals Ltd., UK. BioPirine® a standardized extract from the fruit of *Piper nigrum* L (black pepper) or *Piper longum* L (long pepper) containing 95% piperine was kindly donated by Sabinsa Corporation, USA. Prof. Raphael Mechoulam, The Hebrew University of Jerusalem, Israel, kindly provided Cannabigerol (CBG). For the preparation of PNLs, Tween 20 was purchased from Merk KGaA, Germany, Span 80 from Ofer chemicals lab suppliers, Israel, lecithin (EPIKURON® 200) from Cargill, USA, tricaprine (CremerCOOR®; MCT C10-95) from CREMER Oleo Division, France, hydrogenated castor oil (HCO 40) from BASF The Chemical Company, Germany and ethyl lactate (PURASOLV® EL) was purchased from Corbion purac, Spain. Sativex® was purchased from Armon HaNatziv Pharmacy, Jerusalem.

2.2. THC-CBD-piperine-PNL preparation

THC-CBD-piperine-PNL was prepared by the pre-concentrate method. This preparation process was based on the formulation design of CBD-piperine-PNL and THC-piperine-PNL described before [13]. Briefly, an amphiphilic co-solvent (ethyl lactate) and soy phospholipid (lecithin) were initially placed in a clean scintillation tube at a ratio of 4:1 respectively, and heated to 40 °C until completely dissolved. Then, triglyceride tricaprin, polyoxyl 40-hydroxy castor oil, Tween 20, and Span 80 were added at the ratio of 1:1:1:1; the mixture was gently stirred and heated to 40 °C until a homogenous solution was formed. Further, THC, CBD, and piperine were added to the pre-concentrate (in this order) and gently stirred and heated to 40 °C until a homogenous solution was formed. The THC:CBD ratio in our formulation was approximately 1:1 as dictated by Sativex® composition. Each capsule contained 10.8 mg of THC and 10 mg CBD. BioPerine®, a patented extract containing 95% piperine by Sabina Corp, was found to be safe and has earned self-affirmed GRAS status. According to the GRAS monograph, a maximum dose of BioPirine® should not exceed 15 mg/kg/day. In our clinical studies, we have followed this data regarding the safety of piperine. The dose of piperine we decided to use was 20 mg, according to the dose broadly administered in various clinical studies. The composition is presented in Table 1.

Table 1
Composition of the THC-CBD-piperine-PNL pre-concentrate formulation.

Component type	Ingredient	Relative composition % (w/w)
Active ingredients	THC	1.08
	CBD	1
Absorption enhancer	Piperine	2
Surfactants	Tween 20	13.5
	Span 80	13.5
	HCO 40	13.5
Soy phospholipid	Lecithin	8
Triglyceride	Tricaprin	13.5
Organic co-solvent	Ethyl lactate	34

2.3. Particle size, poly dispersity index (PdI) and ζ potential determination

Particle size and ζ potential were determined using Zetasizer Nano ZS ZEN 3600 (Malvern Instruments Ltd., Malvern, Worcestershire, UK). Prior to particle size and ζ, potential determination 200 μL of the pre-concentrate were vortex-mixed in 1800 μL distilled water at 37 °C for 30 s, forming a dilution in a ratio of 1:10 (v/v). The measurements were taken using Folded Capillary Cells (Malvern Instruments Ltd., Malvern, UK). Before the measurements were taken, the cells were flushed through with ethanol followed by de-ionized water to facilitate wetting and cleaning of the cell.

2.4. THC-CBD-piperine-PNL soft gelatin capsules preparation

Alsepa® omega 3 capsules were emptied using a syringe equipped with a 23G needle. The emptied capsule was washed with ethanol using a syringe with a needle of the same diameter and left for drying. This enabled us to obtain empty soft gelatin capsules. 900 mg of the formulation containing the THC-CBD-piperine-PNL were injected into the capsule shell. The hole created by the needle was sealed using a minute amount of melted gelatin. The capsules were prepared < 24 h prior to the study and stored at 4 °C. The capsules were filled in lab conditions. 20% of the capsules were randomly selected and their active content amount was verified.

2.5. Study design

The study was performed on 9 healthy male volunteers, age 20–30. It was an open label, cross-over single-arm two-way study (Cycle 1 and Cycle 2). Each volunteer received a THC-CBD-piperine-PNL capsule and Sativex®, each administrated in a separate setting. Written informed consent to participate in this study was signed by all volunteers, which was approved by the institutional review board of the Hadassah Medical Center, Jerusalem, Israel and by the Israeli Ministry of Health. Study participants were not to use THC, CBD or cannabis for 4 weeks prior to the beginning of the study. Individuals were screened for the presence of any clinically significant illness, as detected by history, physical examination, and/or clinical laboratory tests which might put the individual at increased risk of adverse events or that might interfere with absorption, distribution, metabolism, or excretion of study medications. Criteria for exclusion from study participation included history of psychosis, other than caffeine or nicotine dependence or simple phobia, consumption of grapefruit, grapefruit juice, Seville oranges, pomelo-containing products, within 14 days prior to Day 1. Prior to the study a clinically significant history of drug allergies, abnormal heart function as well as any history of adverse events associated with cannabis intoxication or dependence was examined. Additionally, those who had donated 0.5 L blood within 30 days of drug administration were excluded from the study. Blood pressure, heart rate and body temperature were measured while the study participant was sitting after resting for 10 min. Participants were required to have ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic blood pressure, ≤ 100 bpm heart rate and body temperature ≤ 37.5 °C. Lastly, subjects with mouth ulcerations, damaged oral mucosa and/or oral cavity (route of Sativex® administration) could not participate in the study.

2.6. Study protocol

Volunteers were admitted to the pain unit at Hadassah Medical Center in Jerusalem for the study. The total duration of each Cycle was approximately 24 h. Subjects remained confined to the pain unit of Hadassah medical center until completion of Day-1 (blood withdrawal 12 h). After that, the volunteers were released overnight. The volunteers returned to the pain unit the following morning for final blood sampling (Day-2; 24 h post-dose). Volunteers fasted for at least 8 h prior to the administration of the study drug (overnight). Unsweetened tap or mineral water was allowed at all times before and during the study unless otherwise specified. A meal was served approximately 4 and 8 h following study drug administration. Vital signs were measured (while sitting and after resting 10 min.) in the morning of each Cycle prior to drug administration, 30 min, 1 h, 2 h, 12 h and 24 h post-dose for monitoring and safety assessment. In Cycle 1, 9 volunteers received a single dose of 4 actuations of Sativex® which were administered within 1–2 min by the study physician. The dose of THC and CBD administered was as follows: THC 10.8 mg and CBD 10 mg. Sativex® actuations were directed sublingually and at the buccal mucosa. In Cycle 2 the same 9 volunteers received a single oral dose of THC-CBD-piperine-PNL capsule with 200 mL of water. The dose of THC and CBD was the same as in Cycle 1. 15 blood samples of 8 mL were drawn throughout each Cycle at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12 and 24 h post dose. All subjects completed the study. There was a 21 day washout period between Cycle 1 and Cycle 2.

2.7. Study end points

2.7.1. Primary outcome measures

The following pharmacokinetic parameters were calculated for THC and CBD using non-compartmental analyses: maximum observed plasma concentration (C_{max}), time to maximum observed plasma concentration (T_{max}), area under the curve to the time of last measurable concentration ($AUC_{0–24\ h}$).

2.7.2. Safety variables and endpoints

The safety variables included the following: vital signs (Systolic blood pressure, Diastolic blood pressure, pulse rate and body temperature) results and changes from baseline. Occurrence of adverse events throughout the study (from the moment the subject signs the informed consent form).

2.8. Recording of adverse events

At each contact with the subject, the investigator searched for adverse events by specific questioning and by physical examination. Possible adverse effects are mainly dizziness, somnolence and disorientation. These effects are the most frequently reported by clinical studies conducted with both tested cannabinoids. All adverse events occurring during the study period were recorded. The clinical course of each event was followed until resolution, stabilization, or until it has been determined that the study treatment or participation was not the cause.

2.9. Sample handling

Blood samples were centrifuged immediately after blood collection at 3220*g* for 15 min. Plasma was separated and divided into two aliquots. Plasma samples were stored in polypropylene vials at − 70 °C in a vertical position pending analysis.

2.10. Plasma analysis and analytical method for CBD and THC

Plasma aliquots of 2 mL were spiked with 20 μL of internal standard cannabigerol (CBG; 1 μg/mL). ACN (5 mL) was added to each test tube (tubes A) and vortex-mixed for 5 min. The extraction of THC, CBD and CBG was performed by *N*-hexane (5 mL) that was added to each test tube A, followed by 5 min vortex-mixing. After centrifugation at 3220*g* for 7 min, the *N*-hexane organic layer was transferred to fresh glass test tubes (tubes B). The organic layer was then evaporated to dryness (Vacuum Evaporation System, Labconco, Kansas City, MO). Thereafter, an additional extraction procedure was performed, i.e., once again *N*-hexane (5 mL) was added to each test tube A, followed by 5 min vortex-mixing. After centrifugation at 3220*g* for 7 min. The organic layer was transferred to tubes B and evaporated to dryness (Vacuum Evaporation

System, Labconco, Kansas City, MO). Then, tubes B were reconstituted in 80 mL of ACN:water (80:20). The resulting solution (80 μl) was injected into the HPLC-MS-MS system.

Chromatography was performed under reverse phase conditions using a Shimadzu (Kyoto, Japan) UHPLC System, series Nexera, consisting of a Shimadzu CBM-20A LITE controller, two Shimadzu LC-30AD pumps, including a Shimadzu Prominence DGU-20A5R degasser, a Shimadzu SIL-30AC autosampler and a Shimadzu CTO-20AC column oven. The chromatographic separations were performed on a Kinetex™ (Phenomenex, Torrance, CA, USA) column (C18, 2.6 μm particle size, 100 Å pore size, 50 × 2.1 mm), protected by a SecurityGuard™ (Phenomenex, Torrance, CA, USA) ULTRA cartridges (C18, 2 × 2.1 mm).

The compounds were detected by an AB Sciex (Framingham, MA, USA) Triple Quad™ 5500 mass spectrometer using electrospray ionization (ESI) and a multiple reaction monitoring (MRM) mode of acquisition. Air was produced (SF 2 FF compressor, Atlas Copco, Belgium) and purified using an NM20Z nitrogen generator (Peak Scientific, Inchinnan, Scotland). Purified air was used as the source, and exhaust gases and purified nitrogen were used as curtain and collision gases. A receiver was placed between the compressor and the nitrogen generator for a large and stable supply of air. Optimal detection conditions were determined by constant infusion of 100 ng/mL solutions of CBD, THC and CBG in 9:1 ACN:water using the integrated syringe pump (5 μL/min). Transitions were selected and their settings were determined using Analyst Software in compound optimization mode.

The injection volume was 10 μL. Oven temperature was maintained at 40 °C, and the autosampler tray temperature was maintained at 5 °C. Chromatographic separation was achieved using a linear gradient program at a constant flow rate of 0.3 mL/min over a total run time of 7 min.

THC, CBD and their internal standard, CBG, were detected in positive ion mode. Their transitions are shown in Table 2. The molecular ion of the compounds $[M + H]^+$ was selected in the first mass analyzer and fragmented in the collision cell followed by detection of the products of fragmentation in the second mass analyzer. The TurboIonspray® probe temperature was set at 500 °C with the ion spray voltage at 5000 V. The curtain gas was set at 25.0 psi. The nebulizer gas (Gas 1) was set to 15 psi, the turbo heater gas (Gas 2) was set to 10 psi, and the collision gas (CAD) was set to 8 psi. The entrance potential (EP) was set at 10 V. The collision energy potentials (CE), collision cell exit potentials (CXP) and declustering potentials (DP) for the monitored transitions are given in Table 2. The dwell time was 70 ms. Data acquisition and analysis were performed on a Dell Optiplex 960 computer with Analyst 1.6.2 software distributed by AB Sciex.

Quantitative calibration (0–250 ng/mL) of THC and CBD was performed before every batch of samples using peak-area ratios (compound versus internal standard). The calibration curve ($y = a + bx$) was obtained by weighted ($1/y$) linear least-squares regression of the measured peak-area ratio (y) versus the concentration added to the plasma (x).

Table 2
Multiple reaction monitoring (MRM) transitions and parameters for CBD, THC and CBG (IS) in positive ion mode. *m/z*: mass to charge ratio; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential; V: volts; eV: electronvolts; Rt: retention time.

Name	Precursor (*m/z*)	Product (*m/z*)	DP (V)	CE (eV)	CXP (V)	Rt (min)
CBD	315.1	123.1	1	43	18	2.7
		193.0	40	34	20	
THC	315.1	193.0	40	34	20	3.4
		123.1	1	43	18	
CBG	317.1	193.1	80	23	24	2.7
		123.1	80	43	14	

2.11. PK analysis

The concentration vs. time data were analyzed by a non-compartmental PK analysis using WinNonlin® (version 5.2, Pharsight, Mountain View, CA). Following this analysis PK parameters were obtained.

2.12. Statistical analysis

All values are expressed as mean ± standard error of the mean (SEM) if not stated otherwise. To determine statistically significant differences among the experimental groups, the parametric *t*-test was used. Any p values < 0.05 were termed significant.

3. Results

3.1. Characterization of THC-CBD-piperine-PNL

Particles of 20 nm in diameter were formed upon introduction of the THC-CBD-piperine-PNL pre-concentrate into the water phase. The PdI value of the nanoparticles formed was 0.23, indicating a narrow and favorable particle size distribution (PdI < 0.5).

3.2. PK profiles of THC and CBD given in piperine-PNL

Concentration vs. time profiles of THC and CBD following single oral administration of the optimized P-PNL formulation at a dose of 10.8 mg THC and 10 mg CBD vs. buccal administration of Sativex® at the same dose, are presented in Figs. 1 and 2, respectively.

Single oral administration of the P-PNL formulation resulted in a 3-fold increase in Cmax and a 1.5-fold increase in AUC for THC compared to Sativex®. Additionally, a 4-fold increase in Cmax and a 2.2-fold increase in AUC for CBD compared to Sativex® were observed (Table 3).

Figs. 3 and 4 represent the terminal slopes obtained following oral administration of THC-CBD-piperine-PNL and Sativex® buccal spray, plotted on a semi-logarithmic graph. Evidently, the slopes of the terminal phases in both study groups are identical. These data indicate that there is no difference in the elimination phases between these two formulations.

3.3. Drug-related adverse events recorded during the study

The adverse events observed during our clinical study are summarized in Table 4. These include anxiety, somnolence, thirst, dizziness, auditory hallucinations, disorientation, abdominal pain, balance disorder, reflux, hand numbness, emesis, nausea and tingling tongue. All adverse events were ranked as mild to moderate and were resolved without medical intervention within 10 min to 2 h post dose. The most common adverse event in both groups was somnolence. All subjects completed the study.

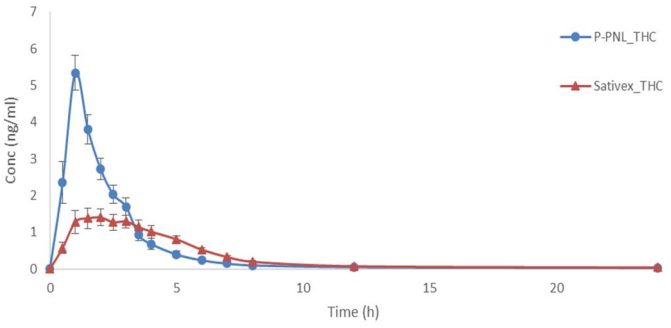

Fig. 1. Plasma THC concentration vs. time plot (mean ± SEM) following oral administration of THC-CBD-piperine-PNL and buccal administration of Sativex® at a dose of 10.8 mg (n = 9 for each group).

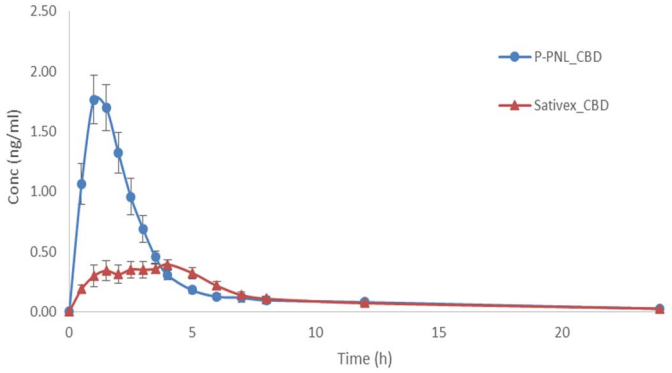

Fig. 2. Plasma CBD concentration vs. time plot (mean ± SEM) following oral administration of THC-CBD-piperine-PNL and buccal administration of Sativex® at a dose of 10 mg (n = 9 for each group).

Table 3
AUC and Cmax values (mean ± SEM), Tmax (median, range) obtained following single oral administration of CBD-THC-Piperine-PNL and Sativex® buccal spray in healthy human volunteers, at a dose of 10.8 mg THC, 10 mg CBD and 20 mg piperine in case of the of THC-CBD-piperine-PNL (n = 9 for each group).

	AUC (h∗ng/mL)	C_{max} (ng/mL)	T_{max} (h)	K_{el} (h^{-1})
Sativex®-CBD	3.1 ± 0.4	0.5 ± 0.1	3 (1–5)	0.1 ± 0.02
P-PNL-CBD	6.9 ± 1.3*	2.1 ± 0.4*	1 (0.5–1.5)	0.1 ± 0.03
Sativex®-THC	8.0 ± 1.0	1.8 ± 0.2	2 (1–4)	0.1 ± 0.03
P-PNL-THC	11.6 ± 0.9⸗	5.4 ± 0.01⸗	1 (1–1.5)	0.1 ± 0.02

* A significant difference ($p < 0.05$) from Sativex®-CBD corresponding values was found.
⸗ A significant difference ($p < 0.05$) from Sativex®-THC corresponding values was found.

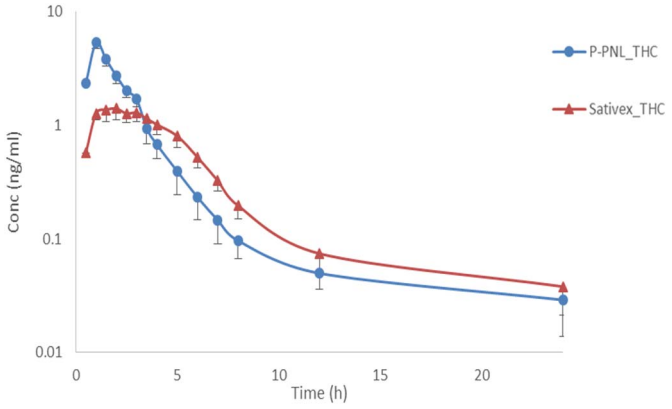

Fig. 3. A semi-logarithmic plot of plasma concentration time profiles in human volunteers for THC obtained following single oral administration of THC-CBD-piperine-PNL and Sativex® buccal administration at a dose of 10.8 mg THC and 20 mg piperine in the case of THC-CBD-piperine-PNL (n = 9 for each group).

4. Discussion

The transition of cannabis from a recreational drug to a standardized medicine has yet to be completed. This is partially the result of the biased opinion held by society, but mainly an outcome of development challenges ranging from regulatory aspects to pharmaceutical difficulties.

In the few countries in which the use of cannabis is legalized as a medicinal drug, patients are presented with limited options for consumption. Mostly, they turn to smoking, which provides a relatively high bioavailability of up to 56% for THC and 18–44% for CBD and quick onset of action [6,24]. However, the smoking route holds health hazards and leads to high inter patient variability, ultimately failing to meet the needs of a reliable, safe cannabinoid medication.

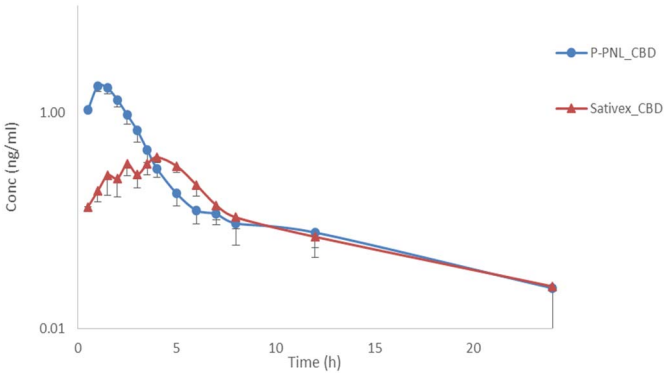

Fig. 4. A semi-logarithmic plot of plasma concentration time profiles in human volunteers for CBD obtained following single oral administration of THC-CBD-piperine-PNL and Sativex® buccal administration at a dose of 10 mg CBD and 20 mg piperine in the case of THC-CBD-piperine-PNL (n = 9 for each group).

Table 4
Drug related adverse events following single, randomized administration of oral modified low dose THC-CBD-piperine-PNL formulation and buccal Sativex® spray (10.8 mg THC, 10 mg CBD and 20 mg piperine in the case of THC-CBD-piperine-PNL).

Adverse events	# of cases		Severity	Duration (h)	Results
	Sativex®	P-PNL			
Anxiety	0/9	1/9	Moderate	0.5	Resolved w/o medical intervention
Somnolence	4/9	3/9	Moderate	1–2	
Thirst	2/9	0/9	Mild-Moderate	0.5	
Dizziness	1/9	2/9	Moderate	1–1.5	
Auditory hallucinations	1/9	0/9	Mild	1	
Disorientation	1/9	0/9	Moderate	1	
Abdominal pain	1/9	0/9	Mild	0.5	
Balance disorder	0/9	1/9	Moderate	0.5	
Reflux	0/9	1/9	Moderate	0.5	
Hand numbness	0/9	2/9	Mild	0.5	
Emesis	0/9	1/9	Moderate	Received food and relieved	
Nausea	0/9	1/9	Mild-Moderate	0.2–1.5	
Tingling tongue	0/9	1/9	Moderate	0.5	
Withdrawal	0/9	0/9			

A substantial percentage of cannabis using patients consumes cannabinoids via the oromucosal pathway, specifically the spray Sativex®. The advantage of this route is assumingly bypassing the hurdles of first pass metabolism associated with intestinal absorption since it is not drained via the portal vein. Sativex® is a solution indicated for the relief of spasticity and neuropathic pain in multiple sclerosis patients, approved in various countries in Europe as well as in Israel. Nevertheless, Sativex® possesses some drawbacks in terms of side effects, compliance and inter-patient variability. The Sativex® solution contains ethanol and propylene glycol as the vehicle of administration, which over a long-period treatment may be harmful to the oral environment. These excipients often lead to lesions, mouth ulcerations, pain and soreness. In such cases, the treatment has to be interrupted until complete healing of the oral mucosa [25].

The oral route of administration is optimal for its advantages of patient compliance and adherence, simplicity in manufacturing and safety. However, oral absorption of cannabinoids is very limited. Although THC and CBD readily penetrate the enterocyte layer when taken orally, their absorption is slow and erratic. This is because of their lipophilic properties and thus low solubility in the unstirred layer of the

intestine [6]. Moreover, both molecules are prone to extensive intestinal as well as hepatic first pass metabolism. THC elimination via metabolism is mediated by CYP family enzymes, mainly, CYP 2C9 and 3A4. There are many resultant metabolites, however the most prominent metabolic pathway is hydroxylation at C11 primary by CYP 2C9, which leads to an active metabolite 11-OH-THC [6]. It was also demonstrated that p-glycoprotein (P-gp) contributes to THC disposition pattern [26]. The extent of CBD absorption resembles that of THC. CBD undergoes Phase I as well as direct Phase II metabolism processes. These pathways are buffered through CYP2C19 and CYP3A4, and glucuronidation via UGT 1A9 [7].

In our pre-clinical study, we incorporated THC or CBD into a self-nano emulsifying drug delivery system, the pro-nanolipospheres (PNL).

The PNL formulation serves as a vehicle for increasing solubility of the molecules at the aqueous milieu of the intestine and as a means to inhibit first pass metabolism mechanisms. We further developed an advanced PNL formulation that contains the absorption enhancer piperine and the model molecules THC/CBD. The piperine itself is of lipophilic nature, thus although with great inhibitory potential, poor solubility usually mars its performance as an absorption enhancer. The PNL is a platform for simultaneous delivery of both the drug and piperine in their solubilized state to the enterocyte monolayer. Thus, we have succeeded in substantially increasing the oral bioavailability of THC and CBD with the aid of piperine-PNL. Based on the promising results in-vivo in the freely moving rat model, we have evaluated the performance of piperine-PNL formulation in clinical conditions presented in this paper. The matrix of PNL is composed of excipients of GRAS status, which reduces the risk of acute/chronic toxicity. As such, PNLs are suggested to be safe for oral consumption [12]. The safety of the alkaloid piperine that we have incorporated into our PNLs, was evaluated in several studies conducted by in-vitro and in-vivo models [27,28].

Although pre-clinical experiments were conducted for each cannabinoid separately, i.e., THC-piperine-PNL and CBD-piperine PNL, for the clinical study we developed a combined PNL formulation. The rationale behind this alteration is the reported “entourage effect” of the cannabis plant. This phenomenon relates to a synergism created when administrating THC together with other phytocannabinoids from the plant, specifically, CBD [23,24]. The reference formulation in our trial was Sativex®. We chose this formulation, since it is the only known formulation containing both THC and CBD. In addition to its suitable composition, the Sativex® formulation is approved in Israel.

The development and optimization of the combined PNL formulation relied upon the preclinical composition with adjustments in excipient concentration. The ratio of THC and CBD was approximately 1:1 as in the Sativex® formulation. Dispersion in water of the final PNL, resulted in a homogenous visibly clear dispersion with a Polydispersity index of 0.23, nano particles of 40 nm and zeta potential of 12.5 mV. Thus, the nano emulsion formed, was stable enough for passage in the GI tract. Bkerman et al. [12], have previously demonstrated the importance of smaller particle size and its inverse relationship with intestinal absorption. Thus, in our development we aimed for a particle size of 50 nm or less. The theory behind this prerequisite condition is that particles of nano metric range can penetrate inter-villous space at the brush border of the gut wall. Thus, nano particles gain additional surface area available for absorption [10,12].

The clinical trial presented in this paper, a two-way cross over, single administration design was chosen, in which all subjects receive both compared treatments. Patients received capsules of the liquid oral formulation, containing 10 mg of each cannabinoid and 20 mg of piperine. The dose of piperine was chosen according to the dose broadly administrated in various clinical studies. The doses for THC and CBD were adjusted following a previous preliminary investigation. The format of the clinical trial was as described above, since our primary goal was to evaluate the behavior of the oral formulation in clinical terms and to prove its non-inferiority to other available formulations.

In this trial, as seen in pre-clinical studies, AUC and Cmax were significantly higher for each cannabinoid following P-PNL delivery system administration compared to Sativex® (Figs. 1, 2). Interestingly, the magnitude of Cmax elevation for CBD following P-PNL delivery system administration was higher than for THC. The C_{max} of THC was increased approximately by 3-fold whereas the C_{max} of CBD was increased by 4-fold. These results reinforce our previously suggested hypothesis that the observed difference can most probably be attributed to the different metabolic pathways of THC and CBD. As opposed to THC, CBD is subjected not only to Phase I metabolism but also to Phase II metabolism. The substantial elevation we observed in CBD may be related to inhibition of Phase II glucuronidation process induced by the piperine component of our P-PNL formulation. This hypothesis has so far remained theoretical, since CBD is a substrate of Phase I as well as of Phase II metabolism. Thus, the effect of piperine on Phase II metabolism cannot be conclusively evaluated using a compound that is subjected to both mechanisms. Further investigation with proper model molecules should be conducted.

Evidently, the PK profile of THC and CBD obtained following THC-CBD-piperine-PNL oral administration is significantly different from the PK profile of these cannabinoids following Sativex® administration. This difference stems to the different administration routes oral and buccal, correspondingly.

Buccal drug delivery can offer several advantages over oral delivery. The oral mucosa is highly vascularized, and therefore any drug diffusing into the oral mucosa membranes has direct access to the systemic circulation via capillaries and venous drainage. Thus, drugs that are absorbed through the oral mucosa can directly enter the systemic circulation, bypassing the GI tract and first-pass metabolism in the liver. However, leakage of the drugs to the GI tract following this route of administration cannot be ruled out.

The absorption of both cannabinoids was significantly faster from the P-PNL formulation compared to Sativex®, with T_{max} values of 1 h for both THC and CBD versus 3 h for THC and 2 h for CBD, respectively (Table 3).

It should be noted that this study was conducted under fasting conditions. Upon administration of THC-CBD-piperine-PNL following fed conditions, especially after high fat meal consumption, the T_{max} may be significantly increased. This is because lipids in the GI tract provoke delay in gastric emptying, i.e., gastric transit time is increased.

The low bioavailability and long T_{max} values following Sativex® administration indicate that the absorption of THC and CBD was not solely through the oral mucosa, but rather that the absorption of the cannabinoids was also through the GI tract.

Furthermore, our study results indicate that the main difference, in terms of PK profile between the developed P-PNL and Sativex® is attributed to the absorption phase. This can be seen from the semi-logarithmic representation of the terminal slopes obtained following the administration of P-PNL and Sativex® (Figs. 3 and 4). Terminal slopes starting from about 4 h (post administration) are parallel, indicating no difference in the elimination phase. These results are in line with our pre-clinical studies and show that the higher AUC and C_{max} of THC and CBD were obtained due to an augmented fraction of absorbed cannabinoids.

During our clinical trial no severe cardiovascular or intoxication effects or serious adverse events were observed. It is important to note that the type, the incidence and the severity of the reported adverse events were similar in both groups, indicating that our P-PNL formulation is as safe for use as Sativex®. Additionally, the adverse events observed in the two study groups are similar to those reported in other clinical studies with healthy volunteers using similar or significantly higher doses of THC and CBD. The most common adverse effects listed are somnolence, dizziness and disorientation. Two subjects in the Sativex® group reported being thirsty. This might be the result of its vehicle, which is composed of ethanol and propylene glycol (50%:50% v/v). All the adverse events were resolved without medical intervention

within 0.5 to 2.5 h and all subjects completed the study.

Our clinical studies corroborate the results of our pre-clinical studies and further confirm the potential of P-PNL formulation as a drug delivery system for enhanced oral bioavailability of lipophilic compounds subjected to intestinal Phase I and/or Phase II metabolism. These positive results pave the way to continue the utilization of this new formulation in the clinical-therapeutic setting.

5. Conclusion

The piperine-PNL serves as delivery system for BCS class II molecules such as THC and CBD, characterized with limited oral bioavailability. This lipid based vehicle successfully dissolves the model drug and the absorption enhancer and increases their poor aqueous solubility. This is achieved via creation of stable nano particles that contain the compounds in their core. The formulation delivers the cannabinoids together with piperine to the enterocyte monolayer, enabling the compounds to pass the unstirred layer of the intestine and reach that smallest areas available for absorption. Moreover, the carrier inhibits the intestinal first pass metabolism these molecules are subjected to, resulting, in a substantial increase in oral bioavailability in healthy human beings. Ultimately, we have reached our goal in demonstrating the advantage piperine-PNL has on the marketed product Sativex® and in proving its potential application in a clinical setting. Comparing both formulations in a semi-logarithmic plot, proved that the addition in drug exposure, allowed by piperine-PNL administration, is result of the effect it has on the absorption phase of the cannabinoids and not the elimination phase as was seen in-vivo. In line with our pre-clinical studies, the effect piperine-PNL had on CBD drug exposure was more significant in comparison to THC. We explain this phenomenon by relating to direct glucuronidation CBD undergoes, not visible for THC. This theory ought to be tested with the suitable model drug, isolating each metabolism process CBD undergoes separately. The GRAS status of the excipients enabled the execution of reported clinical study and is a benefit in terms of regulation and further clinical trials in disease models.

Finally, the PNL encompasses the advantages of an oral formulation and is tailored for the specific lipophilicity and metabolism characteristics of the cannabinoids. These properties render it as the ultimate solution for the need in a standardized oral cannabinoid formulation.

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