International Journal of Pharmaceutics 624 (2022) 121947

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Vegetable oils composition affects the intestinal lymphatic transport and systemic bioavailability of co-administered lipophilic drug cannabidiol

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ARTICLE INFO

Keywords:
Natural vegetable oil
Lipid-based formulation
Fatty acid
Lymphatic transport
Cannabidiol

ABSTRACT

Although natural sesame oil has been shown to facilitate the lymphatic delivery and oral bioavailability of the highly lipophilic drug cannabidiol (CBD), considerable variability remains an unresolved challenge. Vegetable oils differ substantially in composition, which could lead to differences in promotion of intestinal lymphatic transport of lipophilic drugs. Therefore, the differences in composition of sesame, sunflower, peanut, soybean, olive and coconut oils and their corresponding role as vehicles in promoting CBD lymphatic targeting and bioavailability were investigated in this study. The comparative analysis suggests that the fatty acids profile of vegetable oils is overall similar to the fatty acids profile in the corresponding chylomicrons in rat lymph. However, arachidonic acid (C20:4), was introduced to chylomicrons from endogenous nondietary sources. Overall, fatty acid composition of natural vegetable oils vehicles affected the intestinal lymphatic transport and bioavailability of CBD following oral administration in this work. Olive oil led to the highest concentration of CBD in the lymphatic system and in the systemic circulation in comparison to the other natural vegetable oils following oral administration in rats.

1. Introduction

Lipid-based formulations represent an emerging oral drug delivery strategy to improve the absorption and bioavailability of lipophilic compounds following oral administration (Kalepu et al., 2013; Porter et al., 2013; Pouton and Porter, 2008). The physicochemical properties of drugs, such as aqueous solubility and biological membrane permeability, are the main variables affecting their oral bioavailability (Chan and Stewart, 1996). Lipid-based vehicles can lead to the increased solubility of lipophilic drugs in the intestinal lumen and eventually enhance their oral bioavailability (Carrière, 2016; Odeberg et al., 2003; Porter et al., 2007).

When long-chain triglycerides (LCT) or long-chain fatty acids are present in an oral lipid vehicle, the co-administered highly lipophilic compounds have the potential for intestinal lymphatic transport through the mechanism of incorporation into chylomicrons in the enterocytes (Kalepu et al., 2013; O'Driscoll, 2002; Porter et al., 2007). Chylomicrons are large lipoproteins that act as means of transport of dietary lipids from enterocytes to lymph lacteals, and eventually to the systemic circulation through intestinal lymphatic system (Cifarelli and Eichmann, 2019; Dixon, 2010; Martins et al., 1996). Chylomicrons are mainly composed of LCT, as well as phospholipids, cholesterol, cholesterol esters and apoproteins (Bragdon and Karmen, 1960; Buttet et al., 2014; Karmen et al., 1963; Nestel et al., 1962). Diet is the primary source of these triglycerides (TG), and it has been reported that LCT-rich meal or formulations facilitate the production of chylomicrons (Black, 2007; Carreiro and Buhman, 2019; Dash et al., 2015; Xiao et al., 2019). Once consumed, dietary TG undergo series of transformations in the intestinal lumen and enterocytes before they are incorporated into chylomicrons. The first step is a hydrolysis of TG in the gastrointestinal (GI) tract on *sn*-1 and *sn*-3 positions to produce fatty acids and 2-monoglyceride. The LCT are then re-synthesised from these lipid digestion products in the

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https://doi.org/10.1016/j.ijpharm.2022.121947
Received 27 March 2022; Received in revised form 4 June 2022; Accepted 19 June 2022
Available online 23 June 2022

enterocytes and packed into chylomicrons. Because of their large particle size (75–1200 nm), chylomicrons cannot penetrate into blood capillaries, and therefore are directly transported into the intestinal lymphatic system, bypassing the portal vein and liver. Avoidance of liver at the first pass makes lymphatic transport of drugs a promising route for drug delivery, especially for compounds with substantial hepatic first pass metabolic loss (Bayly, 2014; Mansbach, 2004; Martins et al., 1996). It has been reported that drugs with lipophilicity of $\log D_{7.4} \geq 5$ and TG solubility of >50 mg/g are good candidates for high association with chylomicrons and the intestinal lymphatic transport (Charman and Stella, 1986; Gershkovich et al., 2008; Trevaskis et al., 2010).

LCT are abundant in most natural vegetable oils (Zambelli et al., 2015; Zhang et al., 2021). The use of natural vegetable oils as a main component of lipid-based formulations for highly lipophilic compounds has been reported in several previous studies. A partial list of examples include soybean oil-induced enhancement of the oral absorption of leukotriene B4 (LTB4) inhibitor ontazolast through lymphatic transport (Hauss et al., 1998), peanut oil-induced improvement in the lymphatic transport and oral bioavailability of some highly lipophilic synthetic cannabinoids (Gershkovich et al., 2007), and olive oil-induced enhancement in absorption of two highly lipophilic immunomodulators following oral administration, in comparison to lipid-free vehicle (Caliph et al., 2009).

Previous studies in our laboratory have reported that sesame oil vehicle leads to efficient transport of a highly lipophilic phytocannabinoid cannabidiol (CBD) through the intestinal lymphatic system following oral administration in rats. CBD concentrations in the lymph fluid were about 250-fold higher than in plasma and the overall systemic exposure was 2.8-fold higher compared to a lipid-free formulation (Zgair et al., 2017, 2016). However, a considerable variability in the concentrations of CBD in lymph fluid, lymph nodes and plasma has been found following the oral administration of sesame oil-based formulation (Feng et al., 2021a, 2021b; Zgair et al., 2017, 2016). The high lipophilicity of the drug (Millar et al., 2020), as well as incomplete digestion of sesame oil or variable efficiency of emulsification in the GI tract could potentially contribute to the variability in plasma and lymph concentrations of CBD (Feng et al., 2021b; Zgair et al., 2016).

Possible approaches to reduce the variability associated with lipid-based drug delivery of CBD have been recently investigated, including the use of pre-digested lipids for the formulation rather than undigested oil (Feng et al., 2021a). However, simple elimination of the lipid hydrolysis step in the GI tract using purified digested excipients did not reduce the variability and led to a reduction in the extent of the lymphatic transport and bioavailability in comparison to a natural sesame oil vehicle. This suggests that the hydrolysis of TG in the GI tract by lipases is not the main factor associated with variability in CBD concentrations in plasma and in the lymphatic system. Therefore, more recently a different approach has been attempted utilising medium-chain triglyceride (MCT) and/or surfactants combined with sesame oil lipid-based formulations to reduce variability (Feng et al., 2021b). This approach enhanced the CBD solubility in the mixed micelles *in vitro* and reduced the systemic blood concentrations variability *in vivo*. However, these mixed lipid-based formulations led to reduced lymphatic transport and bioavailability in comparison with a pure sesame oil vehicle (Feng et al., 2021b).

Vegetable oils differ substantially in their composition and it is likely that these differences could lead to variable performance as a vehicle for delivering lipophilic drugs to the lymphatic system and systemic circulation following oral administration (Gervajio, 2005; Kamal-Eldin and Appelqvist, 1994; Mounts et al., 1988; Ollivier et al., 2006; Sanders, 1980). Evaluating these differences might offer an insight into the factors and ingredients in vegetable oils that are crucial for the enhancement of the lymphatic transport and bioavailability of lipophilic drugs. Therefore, the aim of this study was to compare the composition and drug lymphatic transport and bioavailability enhancement properties of various vegetable oils using CBD as a model compound.

2. Materials and methods

2.1. Materials

Sesame oil, sunflower oil, peanut oil, soybean oil, olive oil, coconut oil, methyl pentadecanoate, potassium bromide (KBr), sodium hydroxide solution (NaOH, 1 M), L-α-phosphatidylcholine (L-a-lecithin, ~60%, from egg yolk), Trizma® maleate, sodium taurocholate hydrate, pancreatin from porcine pancreas (8 × USP specifications), probucol, 4,4-dichlorodiphenyltrichloroethane (DDT) and serum TG determination kit were purchased from Sigma-Aldrich (Dorset, UK). Trimethylsulfonium hydroxide (0.25 M solution in methanol), sodium chloride (NaCl) and calcium chloride ($CaCl_2$) anhydrous were purchased from Fisher Scientific (Leicester, UK). Cannabidiol (CBD, ≥98%) was obtained from THC Pharm (Germany). Rat plasma was purchased from Sera Laboratories International (West Sussex, UK). All other solvents and reagents were of analytical or high-performance liquid chromatography (HPLC) grade, and obtained from Fisher Scientific (Leicester, UK).

2.2. Lipid-based and lipid-free formulations

CBD (12 mg/mL) was solubilised in six different natural vegetable oil vehicles: sesame sunflower, peanut, soybean, olive and coconut oils. A mixture of 80:10:10 propylene glycol:ethanol:water containing 12 mg/mL of solubilised CBD was used as a control lipid-free formulation.

2.3. In vitro lipolysis

The efficiency of digestion of lipid-based formulations and efficiency of micellar solubilisation of CBD following digestion was assessed by means of an *in vitro* lipolysis system as previously described (Benito-Gallo et al., 2015; Feng et al., 2021b; Zgair et al., 2016). Briefly, two distinct buffers were utilised, a complete digestion buffer (fasted state) consisting of 50 mM Trizma® maleate, 150 mM NaCl, 5 mM $CaCl_2$, 5 mM sodium taurocholate hydrate and 1.25 mM L-a-lecithin, and an incomplete buffer consisting of 50 mM Trizma® maleate, 5 mL 150 mM NaCl and 5 mM $CaCl_2$. All ingredients were dissolved in water and the pH was adjusted to 6.8 at 37 °C. Pancreatic lipase (1 g) was added to 5 mL incomplete digestion buffer and vortexed for 15 min. After a 15-min, 1600*g* centrifugation at 5 °C, the lipase extract was obtained by collecting supernatant of the product and kept on ice. Lipid-based formulation (0.1 mL) was pre-mixed with the complete digestion buffer (22.7 mL) for 15 min at 37 °C. To initiate the lipolysis reaction, 2.2 mL lipase extract was then added, and a pH was maintained within a range of 6.8 ± 0.05 using 1 M NaOH solution by means of pH-stat titrator (LabX light v3., T50 Graphix, Mettler Toledo Inc., Leicester, UK). Lipid digestion process was terminated following 1 h reaction and the final lipolysis medium was collected and ultra-centrifuged at 268,350*g*, 37 °C for 1.5 h. As a result, three distinct layers were obtained: the top layer (undigested lipid phase), the middle layer (aqueous micellar phase) and the bottom layer (sediment phase). The three layers were separated and analysed for CBD and TG concentrations.

2.4. In vivo pharmacokinetics and biodistribution

All experimental protocols were carried out and authorised by the United Kingdom Home Office and University of Nottingham Ethical Review Committee in accordance with the Animals [Scientific procedures] Act 1986. *In vivo* pharmacokinetic studies were carried using male Sprague Dawley rats, 340 to 380 g (Charles River Laboratories, UK). The animals were kept in the University of Nottingham Bio Support Unit (BSU) under regulated temperature and humidity, 12 h light-dark cycle, with unrestricted access to water and food.

For pharmacokinetic studies, rats underwent a jugular vein cannulation surgery as previously described (Feng et al., 2021b, 2021a). Lipid-free and lipid-based formulations were administered by an oral gavage

following 48 recovery period and overnight fasting. The formulations were freshly prepared and administered to rats at a dose of 12 mg/kg CBD. Blood samples (0.2 mL) were collected into EDTA-containing tubes at 1, 2, 3, 4, 5, 6, 8, 10, and 12 h after administration. Plasma samples were obtained from the blood by centrifugation (3000 *g*, 10 min) and kept at –80 °C until analysis by means of a validated HPLC method (Zgair et al., 2015). Phoenix WinNonlin Version 6.3 software (Pharsight, Mountain View, USA) was used to calculate the pharmacokinetic parameters by a non-compartmental approach.

For bio-distribution studies, the same formulations were administered to fasting animals. Based on the pharmacokinetic parameters of CBD following oral administration in different formulations, rats were humanely sacrificed at plasma t_{max} and 1 h prior to plasma t_{max} (t_{max} – 1 h) post dosing. Mesenteric lymph fluid, mesenteric lymph nodes (MLN) and blood samples were collected post-mortem, and serum was separated from blood by centrifugation (3000*g*, 10 min). All samples were stored at −80 °C until further analysis.

2.5. Determination of CBD concentrations using HPLC-UV

The lipolysis fractions, rat plasma, serum, lymph fluid and the MLN samples were prepared as previously reported (Feng et al., 2021b, 2021a; Zgair et al., 2017, 2016, 2015). The concentration of CBD in all samples were determined using HPLC system consisting of Waters Alliance separation module and 2996 Photodiode Array Detector, using previously described chromatography conditions (Feng et al., 2021b, 2021a; Zgair et al., 2017, 2016, 2015).

2.6. Separation of chylomicrons from lymph

Chylomicrons were separated from lymph samples by means of density gradient ultracentrifugation, using a methodology modified from a previously published work (Gershkovich and Hoffman, 2005). Due to a limited and variable volume of lymph fluid that could be collected from each rat and high concentration of CBD previously observed in lymph, the collected samples were diluted using various volumes of phosphate-buffered saline (PBS) to make up to 1 mL. One mL diluted lymph fluid samples were then mixed with 0.1425 g KBr to achieve 1.1 g/mL density. A density gradient was then built using PBS buffers with densities of 1.006, 1.019 and 1.063 g/mL. The chylomicrons were separated by density gradient ultracentrifugation (SORVALL® TH-641 Rotor, Thermo Fisher Scientific, 268,350*g*, 35 min, 15 °C), and the top layer of approximately 1 mL was collected. The chylomicron samples were stored at 4 °C for further analysis.

2.7. TG extraction from chylomicrons

TG were extracted from the separated chylomicrons using previously reported lipid extraction methodology with minor modifications (Bahrami et al., 2014). Briefly, separated chylomicrons samples (1 mL) were vortex-mixed for 1 min with 2.4 mL chloroform/methanol mixture (2:1, v/v) and 0.6 mL 0.9% (w/v) sodium chloride (NaCl) solution. Following centrifugation at 1160*g* at 4 °C for 10 min, three distinct layers were obtained: upper layer (clear liquid waste), solid debris (proteins) and lower layer (lipids in solvent). The lowest layer, which contained TG, was transferred to a clean glass test tube. The remaining mixture was extracted two more times and all lower phases were combined and evaporated to dryness under nitrogen at 37 °C. The dry residue was reconstituted in 500 µL chloroform for further analysis.

2.8. Sample preparation for fatty acid analysis by GC-MS/MS

Fatty acid composition was determined for vegetable oils and corresponding chylomicrons separated from lymph. Approximately 0.01 g vegetable oil was dissolved in 2 mL chloroform. One mL of the mixture was then added into 200 µL trimethylsulfonium hydroxide solution (0.25 M in methanol) for the hydrolysis of TG and subsequent methylation of fatty acids. The reconstituted chylomicrons in chloroform samples (300 µL) were also prepared with 200 µL trimethylsulfonium hydroxide solution. After 10 min reaction, the lipid extracts were filtered through a 0.45 µm filter and analysed using GC-MS/MS (Bahrami et al., 2014).

2.9. GC-MS/MS analysis

The composition of the fatty acids was assessed by means of GC-MS/MS (Trace GC Ultra; Thermo Scientific) equipped with an auto-injection system (AS3000) and coupled to a quadrupole mass spectrometer (DSQ II Quadrupole GC-MS/MS; Thermo Scientific). Fatty acids determination conditions in GC-MS/MS have been slightly modified from previously published works (Bahrami et al., 2014; Gedi et al., 2019). The capillary column (Phenomenex Zebron ZB-FFAP, 30 m × 0.22 mm internal diameter, 0.25 µm film thickness) was used, 10 µL of each sample was injected. The oven temperature was maintained at 80 °C for 1 min and then increased to 250 °C at a rate of 5 °C/min for 2 min. The spit flow of the carrier gas (helium) was 25 mL /min. Identification was achieved by comparing the mass spectra with a standard library through the Thermo Scientific Xcalibur software (NIST/EPA/NIH Mass Spectral Library, Version 2.3, NIST 17, Gaithersburg, MD, USA).

2.10. TG levels measurement

TG levels were determined using a commercially available kit (TR0100, Sigma, Gillingham. UK). Manufacturer instructions were followed.

2.11. Data analysis

All results ($n \geq 3$) were reported as mean ± standard deviation (SD). All graphs and statistical analysis were generated using GraphPad Prism v9.2d (GraphPad software, USA). One-way analysis of variance (ANOVA), followed by Dunnett's or Tukey's multiple comparisons test were applied to evaluate the statistical significance of differences between means. In addition, a Student *t-test* was applied for the fatty acids composition analysis. The results were considered statistically significantly different when the *p*-values were below 0.05.

3. Results

3.1. Distribution of CBD into lipolysis fractions following in vitro digestion of vegetable oil formulations

The distribution of CBD into the sediment, aqueous micellar and oil phases following *in vitro* lipolysis of different vegetable oil formulations is presented in Fig. 1. No statistically significant differences were observed in CBD distribution into undigested oil phases between different vegetable oil vehicles (Fig. 1A). The distribution of CBD into the micellar aqueous phase was higher for sesame and sunflower oil-based formulations than for olive, peanut and coconut oil vehicles (Fig. 1B). The distribution of CBD into the sediment phase was higher for peanut and coconut oil vehicles compared to the rest of the vegetable oil formulations (Fig. 1C).

3.2. In vivo pharmacokinetics of CBD following oral administration in different vegetable oils and lipid-free formulation

Pharmacokinetics of CBD was assessed following oral gavage administration of 6 natural vegetable oil-based formulations and the lipid-free formulation in rats. The plasma concentration-time profiles of CBD are presented in Fig. 2, the pharmacokinetic parameters are summarised in Table 1. The maximum concentrations (C_{max}) of CBD in rat plasma following oral administration of sesame oil and olive oil-based

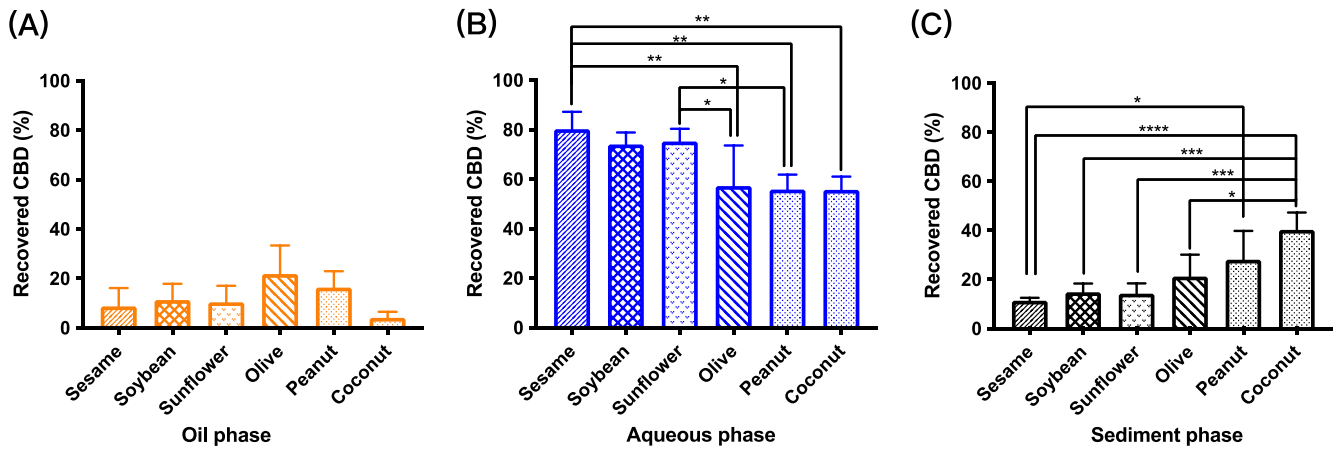

Fig. 1. The distribution of CBD into oil, aqueous micellar and sediment fractions following *in vitro* lipolysis of 12 mg/mL CBD in sesame, soybean, sunflower, olive, peanut and coconut oils. The volume of formulations was 0.1 mL in all cases, lipolysis reaction was set up to run for one hour. (A) Distribution of CBD into oil phase. (B) Distribution of CBD into aqueous micellar phase. (C) Distribution of CBD into sediment phase. All data are presented as mean ± SD (n = 4–6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$.

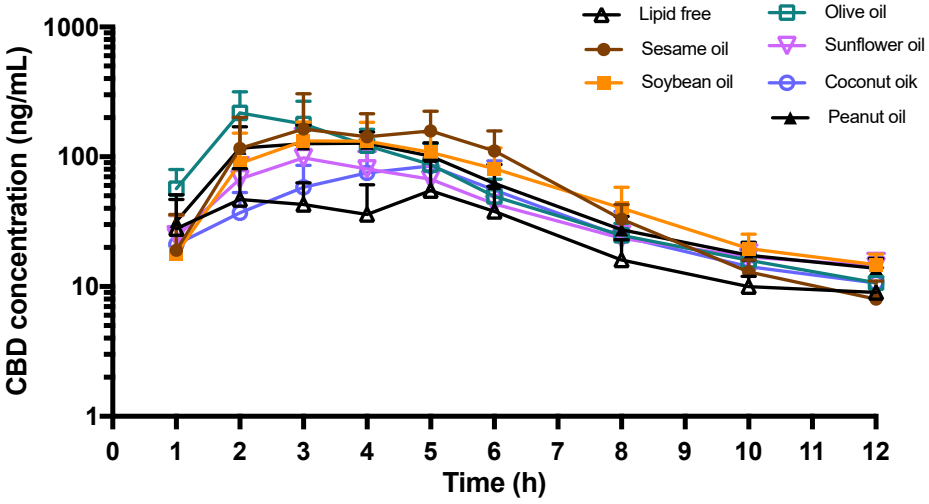

Fig. 2. Individual plasma concentration–time semi-logarithmic plot of CBD (mean ± SD, n = 6) following oral administration at a dose of 12 mg/kg (12 mg/mL) CBD in lipid-free (propylene glycol:ethanol:water, 80:10:10, v/v/v) formulation, sesame oil, soybean oil, peanut oil, olive oil, sunflower oil and coconut oil.

Table 1
Plasma pharmacokinetic (PK) parameters of CBD following oral administration in lipid-free and lipid-based formulations (mean ± SD).

Formulations	$t_{1/2}$ [a] (h)	t_{max} [b] (h)	C_{max} [c] (ng)	$AUC_{0-\infty}$ [d] (h × ng/mL)	n
Lipid-free	2.3 ± 0.6	2	81 ± 30	356 ± 83	6
Sesame	1.5 ± 0.2	3	209 ± 118**	865 ± 342***	6
Soybean	2.2 ± 0.3	3	165 ± 41	775 ± 164**	6
Peanut	2.3 ± 0.4	3	153 ± 36	737 ± 130**	6
Olive	2.0 ± 0.3	2	258 ± 66***	835 ± 130***	6
Sunflower	2.3 ± 0.4	3	112 ± 57	551 ± 136	6
Coconut	1.5 ± 0.4	5	96 ± 36	413 ± 164	6

, * compared to lipid-free formulation (**, $p < 0.01$, ***, $p < 0.001$).
[a] half-life.
[b] time to maximum concentration in plasma.
[c] the maximum concentration in plasma.
[d] AUC from 0 to infinity.

formulations are 2.6-fold ($p < 0.01$) and 3.2-fold ($p < 0.001$) higher than for lipid-free formulation, respectively. The oral administration of sesame oil, soybean oil, peanut oil and olive oil-based formulations resulted in statistically significantly higher ($p < 0.05$) area under the plasma concentration-time curve ($AUC_{0-\infty}$) compared to lipid-free formulation.

3.3. Lymphatic targeting of CBD following oral administration in natural vegetable oil-based vehicles

Rat serum, mesenteric lymph and mesenteric lymph nodes (MLN) were collected at pre-selected time points (plasma t_{max} and 1 h prior to t_{max}), determined based on the pharmacokinetic parameters (Table 1), and assessed for CBD (all samples) and TG levels (lymph fluid and serum). There were no statistical differences in CBD and TG levels in serum among six vegetable oil formulations at both t_{max} and t_{max} – 1 h (Fig. 3).

Figs. 4 and 5 show the concentration of CBD in mesenteric lymph fluid and MLN. Fig. 4 also shows TG levels in lymph fluid samples. There were no statistically significant differences in CBD concentrations in mesenteric lymph fluid at t_{max} – 1 h between all lipid-based formulations. However, oral administration of coconut and soybean oil formulations resulted in higher TG levels in lymph fluid at one hour before plasma t_{max} (t_{max} – 1 h) (Fig. 4A). Olive oil formulation led to 3.7-fold, 2.2-fold, 3.1-fold and 5-fold higher levels of CBD in mesenteric lymph fluid at plasma t_{max} compared to soybean, sesame, sunflower and coconut oil-based formulations, respectively (Fig. 4B). The TG levels in

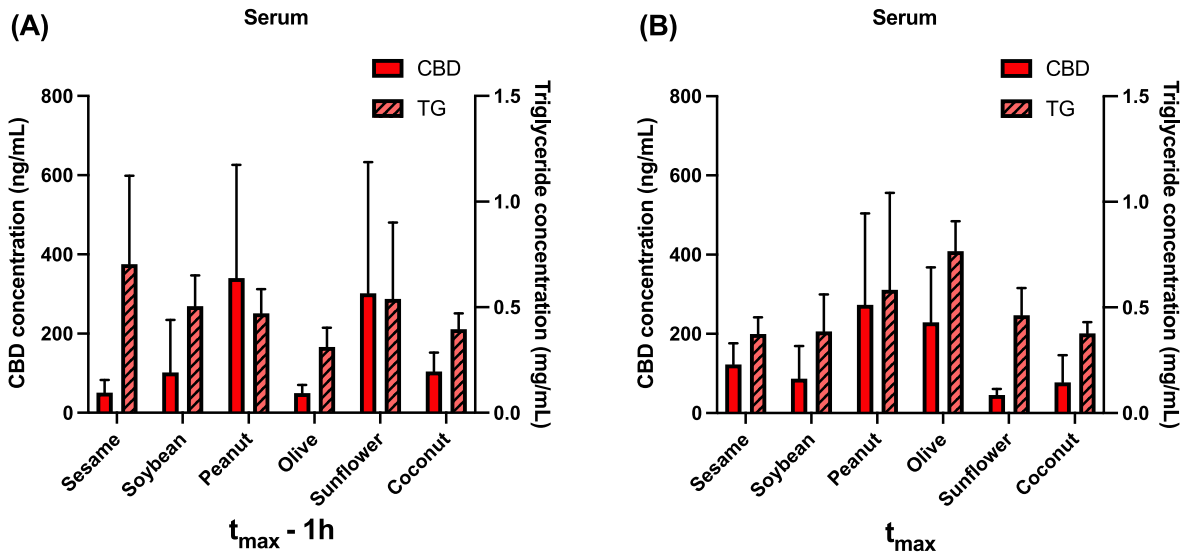

Fig. 3. CBD and TG concentrations in serum. CBD was orally administered in sesame, soybean, peanut, olive, sunflower and coconut oil vehicles at a dose of 12 mg/kg. The concentration of CBD was 12 mg/mL in all formulations. (A) The concentrations of CBD and TG in rat serum at t_{max} – 1 h (one hour prior to plasma t_{max}). (B) The concentration of CBD and TG in rat serum at t_{max}. All data are presented as mean ± SD, n = 4–6. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test.

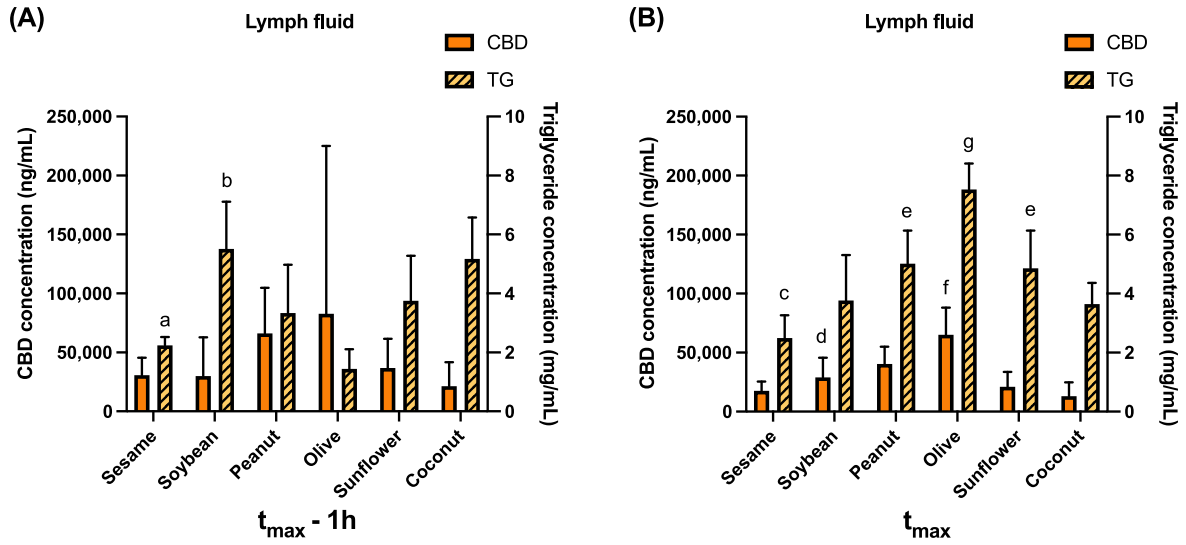

Fig. 4. CBD and TG concentrations in the mesenteric lymph fluid. CBD was orally administered in sesame, soybean, peanut, olive, sunflower and coconut oil-based formulations at a dose of 12 mg/kg. The concentration of CBD was 12 mg/mL in all formulations. (A) The concentrations of CBD and TG in lymph fluid at one hour prior to plasma t_{max} (t_{max} – 1 h). Differences in TG levels: a, $p < 0.05$, *vs.* coconut oil; b, $p < 0.01$, *vs.* sesame and olive oils. (B) The concentrations of CBD and TG in lymph fluid at plasma t_{max}. Differences in TG levels: c, $p < 0.001$, *vs.* olive oil; e, $p < 0.01$, *vs.* olive oil; g, $p < 0.01$, *vs.* soybean and coconut oils. Differences in CBD concentrations: d, $p < 0.05$, *vs.* olive oil; f, $p < 0.01$, *vs.* sesame oil. All data are presented as mean ± SD, n = 4–6. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test.

lymph fluid were highest in the olive group compared to other lipid-based formulation groups at plasma t_{max} (Fig. 4B). No differences in CBD distribution into MLN were observed between various vegetable oils formulations at plasma t_{max} – 1 h and t_{max} (Fig. 5).

3.4. Fatty acids composition of vegetable oils and the corresponding rat chylomicrons

Fatty acid composition of vegetable oil vehicles was assessed and compared to the composition of the corresponding lymph chylomicrons following administration of these vehicles to rats (Table 2). The composition of the fatty acids in the chylomicrons was overall similar to the fatty acids in the administered vehicles in each case. Coconut oil contains a higher proportion of saturated medium-chain fatty acids (C6-C12), as well as C14:0 (myristic acid) in comparison to other vegetable oils. The only medium-chain fatty acid (capric acid, C10:0) found in rat chylomicrons was following oral administration of coconut oil vehicle. In the case of coconut oil administration, no fatty acids longer than 22 carbons were identified in chylomicrons. Intriguingly, a considerable amount of arachidonic acid (C20:4) was found in rat chylomicrons following administration of all vegetable oil vehicles, while it is not present in vegetable oils themselves.

4. Discussion

Although natural vegetable oils have been investigated in the past as

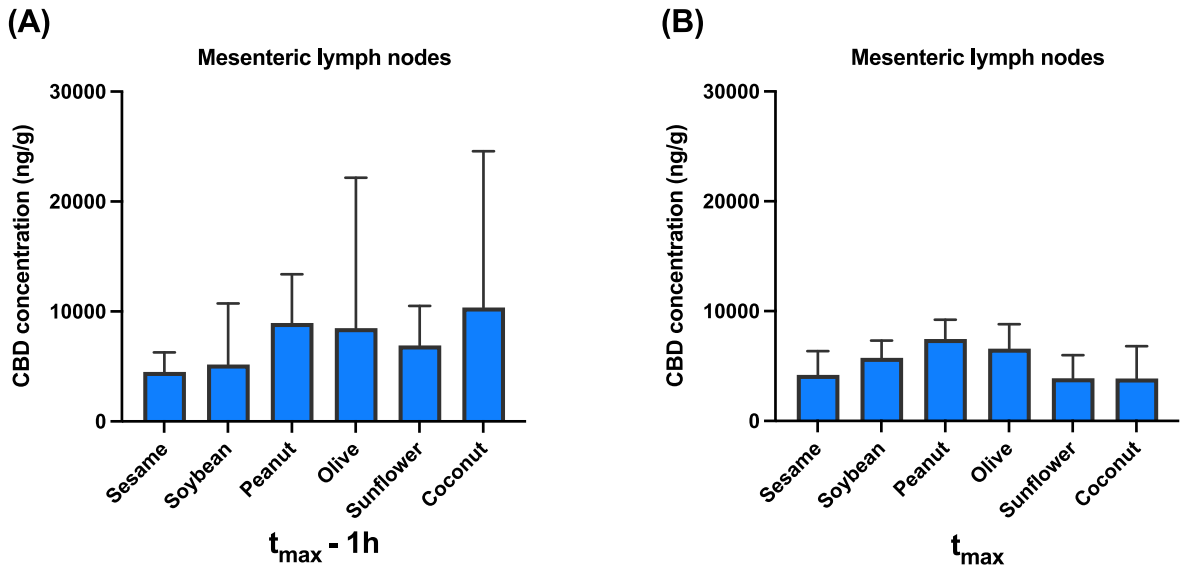

Fig. 5. Distribution of CBD into mesenteric lymph nodes (MLN). CBD was orally administered in sesame, soybean, peanut, olive, sunflower and coconut oil-based formulations at a dose of 12 mg/kg. The concentration of CBD was 12 mg/mL in all formulations. (A) The concentrations of CBD and TG in MLN at one hour prior to plasma t_{max} (t_{max} – 1 h). (B) The concentrations of CBD and TG in MLN at plasma t_{max}. All data are presented as mean ± SD, n = 4–6. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test.

the formulation vehicles to stimulate chylomicrons formation and enhance the intestinal lymphatic transport and oral bioavailability of lipophilic drugs, little research has focused on whether various natural vegetable oil vehicles have similar lymphatic transport and bioavailability enhancement capabilities for lipophilic drugs (Webb and Sanders, 1991). Therefore, in this work we have compared different natural vegetable oils for their ability to promote intestinal lymphatic transport and systemic bioavailability of co-administered CBD. In addition to the list of vegetable oils containing LCT known to facilitate intestinal lymphatic transport, an oil with triglycerides rich in medium-chain fatty acids (coconut oil) has been also included for comparison.

The intraluminal digestion and processing of six vegetable oils has been assessed using the *in vitro* lipolysis system. The extent of the distribution of CBD into the aqueous micellar phase was lower for olive, peanut and coconut oil in comparison to other experimental groups (Fig. 1B). The lipolysis of vegetable oils and micellar solubilisation of CBD following the completion of the lipolysis process is affected by the nature of fatty acids of these oils. It has been shown previously that oral administration of another highly lipophilic drug halofantrine in trilinolein (tri C18:2) vehicle led to higher intestinal lymphatic transport and bioavailability than administration in triolein (tri C18:1) (Holm et al., 2001). This suggests that degree of saturation of fatty acids may impact the lipids digestion and micellar solubility of lipophilic drugs in the GI tract during and following lipid digestion. Therefore, high oleic acid (C18:1) versus linoleic acid (C18:2) ratio in vegetable oils, such as in olive and peanut oils, could be a reason for lower CBD distribution into the aqueous micellar phase in *in vitro* lipolysis experiment compared to other formulations (Fig. 1B and Table 2).

It has been reported that triglycerides containing unsaturated fatty acids are hydrolysed more rapidly than those containing saturated fatty acids (Carroll and Richards, 1958; Gallagher and Playoust, 1969). Moreover, rate and extent of lipid digestion are also affected by fatty acid chain length, with MCT being hydrolysed more efficiently and rapidly in comparison to LCT (Benito-Gallo et al., 2015; Dahan and Hoffman, 2008, 2007; Greenberger et al., 1966). However, despite the fact that coconut oil contains a higher proportion of medium-chain and saturated fatty acids, such as lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), it leads to lower distribution of CBD into the aqueous micellar phase in *in vitro* lipolysis compared to other vegetable oil vehicles (Fig. 1B and Table 2). Furthermore, about 10% of peanut oil contents are very-long-chain fatty acids, such as arachidic (C20:0) and eicosenoic acids (C20:1), which may delay the lipolysis process. As a result, CBD distribution into the aqueous micellar phase following *in vitro* lipolysis of peanut oil-based formulation was lower than for other formulations. Despite the fact that there were no statistically significant differences in the distribution of the CBD into lipid phase, olive and peanut oils showed a trend of higher CBD distribution into the undigested lipid phase when compared to other formulations in *in vitro* lipolysis (Fig. 1A).

Six lipid-based formulations and lipid-free formulation were then assessed *in vivo* in rats. With the exception of sunflower and coconut oil-based formulations, the rest of the formulations significantly enhanced the plasma AUC of CBD in comparison to the lipid-free formulation (Table 1). It is not surprising that the oral bioavailability of CBD following oral administration of the coconut oil-based formulation was comparable to that of the lipid-free formulation. According to the GC-MS/MS assessment, coconut oil contains 30% MCT (Table 2), which have lower participation in chylomicrons assembly process in comparison to LCT. A proportion of CBD dose associated with digested MCT was most probably absorbed through the hepatic portal vein transport pathway with hepatic first-pass metabolism, resulting in a lower overall bioavailability of CBD in co-administered coconut vehicle in comparison to other oil vehicles. In addition, a prolonged t_{max} (5 h) was observed in the coconut oil group. Such prolonged drug absorption might be related to the fact that coconut oil contains more than 80% saturated fatty acids, which are hydrolysed slower than unsaturated fatty acids (Table 1) (Gallagher and Playoust, 1969).

The *in vivo* PK results show that most lipid-based formulations significantly improved the bioavailability of CBD in comparison to the lipid-free formulation, particularly sesame and olive oil-based formulations outperforming other lipid-based vehicles. These *in vivo* findings do not reflect the results of the *in vitro* lipolysis assay. As was previously reported, one of the limitations of *in vitro* lipolysis system is that it only simulates the lipid digestion step in the GI tract rather than the entire absorption and lymphatic transport process (Dahan and Hoffman, 2007, 2006).

In biodistribution studies rat serum, lymph fluid and MLN samples were collected at predetermined time points, and analysed for CBD (serum, lymph fluid and MLN) and TG (serum and lymph fluid) levels. There were no statistically significant differences in the levels of TG or

Table 2
Fatty acid composition of natural vegetable oils and the corresponding rat lymph chylomicrons . Chylomicrons were isolated from lymph fluid following oral administration of corresponding vegetable oil-based formulations.

Fatty acid (% total fatty acid)										
	Saturated fatty acid [b]									
	C8:0	C10:0	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0	C22:0	C24:0
Sesame										
Oil					12.9 ± 0.3	0.2 ± 0.02	8.1 ± 0.6	1.2 ± 0.01	1.0 ± 0.04	0.9 ± 0.2
CM [a]					13.1 ± 0.3		6.3 ± 0.3*			
Soybean										
Oil					15.9 ± 0.2	0.3 ± 0.1	7.9 ± 0.4	0.9 ± 0.02	1.4 ± 0.1	0.7 ± 0.1
CM [a]				0.3 ± 0.01	10.9 ± 0.4*		6.2 ± 0.9*			
Peanut										
Oil					11.9 ± 0.02	0.2 ± 0.02	5.1 ± 0.1	2.7 ± 0.02	7.3 ± 0.3	4.2 ± 0.1
CM [a]				0.6 ± 0.2	13.7 ± 2	0.7 ± 0.2*	5.6 ± 0.5	0.9 ± 0.1***	1.7 ± 0.3****	
Olive										
Oil					12.3 ± 0.2	0.2 ± 0.02	7.8 ± 1.2	1 ± 0.1	0.5 ± 0.1	0.01 ± 0.01
CM [a]				0.3 ± 0.1	12.2 ± 0.6	0.5 ± 0.1	5 ± 0.3*	0.5 ± 0.1*	0.5 ± 0.2	
Sunflower										
Oil					9.5 ± 0.2	0.2 ± 0.03	8 ± 0.1	0.8 ± 0.03	2.3 ± 0.02	1.1 ± 0.3
CM [a]				0.3 ± 0.03	14.4 ± 1.9*		6.8 ± 0.5*			
Coconut										
Oil	3.7 ± 0.1	2.6 ± 0.02	28.5 ± 0.3	21.1 ± 0.4	18.3 ± 0.3	0.2 ± 0.02	8.1 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	
CM [a]		0.6 ± 0.2**	26.5 ± 7.3	14 ± 4.4	18 ± 1		7.7 ± 1.7			
	Unsaturated fatty acid [c]									
	C16:1	C17:1	C18:1	C18:2	C18:3	C20:1	C20:4	C22:1		
Sesame										
Oil	0.2 ± 0.01	0.1 ± 0.01	44.3 ± 0.1	30.4 ± 0.6	0.3 ± 0.1	0.4 ± 0.02		0.2 ± 0.02		
CM [a]			39.5 ± 2.1	38.3 ± 2.3*			2.7 ± 0.7			
Soybean										
Oil	0.1 ± 0.02	0.2 ± 0.01	29.7 ± 0.3	37.9 ± 1	4.1 ± 0.2	0.4 ± 0.1		0.6 ± 0.8		
CM [a]			29.8 ± 0.2	47.3 ± 0.3**	0.4 ± 0.1***	2.6 ± 0.3*	2.6 ± 0.3			
Peanut										
Oil	0.1 ± 0.01	0.1 ± 0.04	50.8 ± 0.5	15.2 ± 0.1	0.2 ± 0	2 ± 0.1		0.2 ± 0.1		
CM [a]	0.7 ± 0.2*		47.6 ± 4.2	23.5 ± 1**	0.5 ± 0.1*	1.4 ± 0.3	3.1 ± 0.9			
Olive										
Oil	0.7 ± 0.04	0.2 ± 0.02	71.6 ± 1	4.8 ± 0.2	0.5 ± 0.01	0.4 ± 0.02		0.1 ± 0.03		
CM [a]	1.2 ± 0.1**	0.1 ± 0.01*	67.8 ± 1.4*	8.6 ± 0.6**	0.6 ± 0****	0.4 ± 0.03	2.3 ± 0.4			
Sunflower										
Oil	0.1 ± 0.01	0.1 ± 0.03	37.6 ± 0.3	39.8 ± 0.1		0.3 ± 0.1		0.3 ± 0.04		
CM [a]			24.4 ± 2.9*	45.4 ± 2.3	3.2 ± 2.6	2.7 ± 1.4	2.9 ± 0.7			
Coconut										
Oil		0.1 ± 0.1	13.8 ± 0.3	2.5 ± 0.2						
CM [a]			11 ± 0.9*	9.4 ± 1.6*		3.5 ± 3.3	10.5 ± 10.5			

*, **, ***, ****; are statistically significantly different from oil group (*, $p < 0.005$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

[a] CM: chylomicrons.

[b] C8:0 = caprylic acid; C10:0 = capric acid; C12:0 = lauric acid; C14:0 = myristic acid; C16:0 = palmitic acid; C17:0 = heptadecanoic acid; C18:0 = stearic acid; C20:0 = arachidic acid; C22:0 = behenic acid; C24:0 = lignoceric acid.

[c] C16:1 = palmitoleic acid; C17:1 = *cis*-10-heptadecenoic acid; C18:1 = oleic acid; C18:2 = linoleic acid; C18:3 = linolenic acid; C20:1 = eicosenoic acid; C20:4 = arachidonic acid; C22:1 = erucic acid.

CBD in serum and MLN samples between all lipid-based formulation groups, which might be attributed to substantial variability (Figs. 3 and 5). Interestingly, lower TG levels in lymph at one hour before plasma t_{max} (t_{max} – 1 h) in olive oil group indicated that the olive oil-based formulation did not produce as many chylomicrons as other lipid-based formulations at that early time point (Fig. 4A). However, at t_{max}, olive oil efficiently promoted the chylomicrons production and showed the highest CBD levels, which suggests that olive oil can lead to higher levels of co-administered CBD in the lymphatic system in comparison to other natural lipids (Fig. 4B). The overall biodistribution results suggest that olive oil was superior in promoting drug transport through the enterocytes and association with chylomicrons in comparison to other vegetable oils.

To understand better the effect of lipid profile of various natural vegetable oils on the enhancement of chylomicron formation and drug association with chylomicrons, fatty acid composition has been assessed for lipid vehicles and the corresponding lymph chylomicrons (Table 2). The data suggest that natural vegetable oils consisting of mainly oleic acid (C18:1) and linoleic acid (C18:2) promote the intestinal lymphatic transport of CBD by stimulating chylomicron formation (Fig. 4 and Table 2). In agreement with these data, it was previously shown in Caco-2 cells that increased oleic acids and linoleic acids concentrations in the medium stimulate the synthesis and secretion of chylomicrons (Field et al., 1988; Williams et al., 2004). Both sesame and olive oils are rich in unsaturated C18 fatty acids, therefore they promote the intestinal lymphatic transport and overall systemic exposure to CBD more efficiently than other vegetable oils.

Interestingly, olive oil has shown lower variability in lymphatic transport and bioavailability of CBD in comparison with sesame oil. The variability in absorption of lipophilic drugs administered in vegetable oil vehicle could be associated with oxidation of the oil. It has been found that photo-oxidation and auto-oxidation are major issues affecting the stability of edible oils (Abbas Ali et al., 2017; Khan and Shahidi, 2002). The formation of fatty acids hydroperoxides is the sign of oxidation, and the main peroxidation product is produced from linoleic acid (Miyazawa et al., 1995; Terao and Matsushita, 1981). Recent oils stability study has shown a decline in linoleic acid levels in nut oils following 14 days of continuous light exposure. However, fewer oxidative changes were

observed in the oleic acid composition (Bai et al., 2019). According to the fatty acid profile of vegetable oils described in Table 2, sesame oil contains higher proportion of C18:2 than olive oil, whereas olive oil is rich in C18:1. Thus, sesame oil could be more prone to oxidation before and after administration, which may explain higher variability in absorption of CBD observed with sesame oil vehicle than with olive oil formulation.

Moreover, the data of fatty acids composition in vegetable oils and in the corresponding lymph chylomicrons (Table 2) suggest that chylomicrons composition is primarily dictated by the composition of the administered oil. Similar conclusions were reached in other studies where size, density and fatty acid profiles of human and animal chylomicrons have changed in accordance to changes in lipid consumption (Cartwright and Higgins, 1999; Fraser et al., 1968; Levy et al., 1991; Sakr et al., 1997; Zilversmit, 1965). Even though dietary lipids affect the fatty acids composition in chylomicrons, it appears that in all cases palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) are major fatty acids that could be found in chylomicrons at different proportions (Kayden et al., 1963; Lai and Ney, 1998). These fatty acids in various proportions may potentially lead to differences in drug loading into chylomicrons. Previously published works reported that MCT appear in human chylomicrons after oral administration of MCT (Swift et al., 1990). Indeed, the current study has also shown that MCT were found in chylomicrons following administration of coconut oil (Table 2).

A trace amounts of odd chain fatty acids such as heptadecanoic acid (C17:0) and *cis*-10-heptadecenoic (C17:1) have been found in both oil and chylomicron samples (Table 2). According to previous research, a proportion of heptadecanoic acid is directly supplied from diet, and the other part of C17:0 is synthesized from stearic acid (C18:0) through endogenous α -oxidation (Jenkins et al., 2015, 2017). It has been suggested that odd chain fatty acids are associated with health benefits such as metabolic regulation. However, there is little research on the lymphatic transport aspect of these fatty acids (Venn-Watson et al., 2020).

Rat lymph chylomicrons contained a certain amount of arachidonic acid (C20:4) following oral administration of all vegetable oil-based formulations. It appears that this fatty acid was added from non-dietary source because it is absent in natural vegetable oils (Table 2). According to previous reports, arachidonic acid is synthesized endogenously from linoleic acid (C18:2) during the absorption/lymphatic transport process, and its metabolites are associated with the regulation of lymphatic contractility (Caselli et al., 1993; Johnston and Gordon, 1981; Pavero et al., 1992). Moreover, arachidonic acid is a major component of phospholipids, which are located on the cell membranes (Martin et al., 2016; Spector, 2009). A class of phospholipids, phosphatidylethanolamine, can be converted to N-acylphosphatidylethanolamine (NAPE), a precursor of two major endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG) (Cadas et al., 1997; Ueda et al., 2013). These endocannabinoids are neurotransmitters that regulate the immune system and other pharmacological effects of cannabis by binding to cannabinoid receptors (Battista et al., 2012; Kano et al., 2009; Lu and Mackie, 2021; Marzo et al., 2004). In addition to their interaction with CB1 and CB2 receptors, anandamide and 2-AG also interact with non-cannabinoid receptors, such as GPR55 and TRPV1 (Corroon and Felice, 2019; Tsuboi et al., 2018). CBD is a phytocannabinoid that also interacts with these receptors of endocannabinoid system (Corroon and Felice, 2019; McPartland et al., 2015). Furthermore, there are studies that demonstrated that intake of unsaturated dietary fats could significantly enhance anandamide and 2-AG concentrations in the brain and intestine (Artmann et al., 2008; Hansen and Artmann, 2008). Therefore, the endogenous addition of arachidonic acid to lymph chylomicrons could suggest potential enhanced activation of the endocannabinoid system following oral administration of CBD with lipids, beyond the interaction of CBD itself with relevant receptors.

It should be noted, that in addition to fatty acids composition investigated in this study, it is also possible that other small molecule ingredients in vegetable oils, such as lignans, vitamin E and phospholipids can contribute to their optimal performance as vehicles facilitating the lymphatic transport of lipophilic drugs (Câmara Grilo et al., 2014; Morris, 2007; Nagy et al., 2013). The involvement of these substances in regulating chylomicron production or lipophilic drug lymphatic transport needs to be further investigated in future studies.

5. Conclusion

In this study, six different natural vegetable oils were evaluated as lipid vehicles to facilitate the intestinal lymphatic transport and oral bioavailability of a lipophilic model drug CBD. The fatty acid composition in oils and corresponding lymph chylomicrons following oral administration of lipids was assessed. According to fatty acid analysis and *in vivo* studies, coconut oil-based formulation has higher MCT content than other oils, which leads to lower lymphatic transport and systemic bioavailability of CBD in comparison to other lipid-based formulations. In contrast, sesame and olive oil vehicles led to higher CBD concentrations in lymphatic tissues and systemic circulation than other natural vegetable oils. This is most likely due to the fact that sesame and olive oils contain higher proportion of oleic (C18:1) and linoleic acids (C18:2), which are known to promote intestinal lymphatic transport. However, sesame oil has higher variability of CBD absorption in comparison to olive oil, most probably because it is more prone to oxidation due to higher linoleic acid content. Therefore, olive oil could be a recommended lipid carrier candidate for CBD and potentially other lipophilic drugs to enhance intestinal lymphatic transport and bioavailability following oral administration.

CRediT authorship contribution statement

Wanshan Feng: Conceptualization, Methodology, Software, Writing – original draft. **Chaolong Qin:** Investigation. **Salah Abdelrazig:** Validation, Formal analysis. **Ziyu Bai:** Investigation. **Mekha Raji:** Investigation. **Randa Darwish:** Validation. **YenJu Chu:** Investigation. **Liuhang Ji:** Investigation. **David A. Gray:** Supervision. **Michael J. Stocks:** Supervision. **Cris S. Constantinescu:** Supervision. **David A. Barrett:** Supervision. **Peter M. Fischer:** Supervision. **Pavel Gershkovich:** Conceptualization, Methodology, Supervision, Resources, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pavel Gershkovich reports financial support was provided by Rosetrees Trust. None.

Acknowledgements

The authors would like to acknowledge Paula Muresan for her help with the density gradient of ultracentrifuge and to thank the Bio-Support Unit (BSU) team in University of Nottingham for excellent technical assistance.

Funding

This work was supported by Rosetrees Trust and Stoneygate Trust [grant number M902].

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2022.121947.

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