Natural sesame oil is superior to pre-digested lipid formulations and purified triglycerides in promoting the intestinal lymphatic transport and systemic bioavailability of cannabidiol

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Abstract

Lipid-based formulations play a significant role in oral delivery of lipophilic drugs. Previous studies have shown that natural sesame oil promotes the intestinal lymphatic transport and oral bioavailability of the highly lipophilic drug cannabidiol (CBD). However, both lymphatic transport and systemic bioavailability were also associated with considerable variability. The aim of this study was to test the hypothesis that pre-digested lipid formulations (oleic acid, linoleic acid, oleic acid with 2-oleoylglycerol, oleic acid with 2-oleoylglycerol and oleic acid with glycerol) could reduce variability and increase the extent of the intestinal lymphatic transport and oral bioavailability of CBD. The *in vivo* studies in rats showed that pre-digested or purified triglyceride did not improve the lymphatic transport and bioavailability of CBD in comparison to sesame oil. Moreover, the results suggest that both the absorption of lipids and the absorption of co-administered CBD were more efficient following administration of natural sesame oil vehicle compared with pre-digested lipids or purified trioleate. Although multiple small molecule constituents and unique fatty acid compositions could potentially contribute to a better performance of sesame oil in oral absorption of lipids or CBD, further investigation will be needed to identify the mechanisms involved.

Keywords:

Cannabidiol, lipid-based formulation, intestinal lymphatic transport, natural sesame oil

1. Introduction

The oral route is the preferred method of administration of drugs due to convenience and patient compliance. Many natural compounds and synthetic drugs or drug candidates can possess high lipophilicity and poor water solubility. Despite pharmacological activity *in vitro*, oral bioavailability of such lipophilic molecules is usually low, which limits further development of these compounds. Lipid-based formulations represent a common approach to increase bioavailability of lipophilic drugs [1–6].

Due to the poor water-solubility of lipophilic compounds, bile surfactants can promote the micellar solubilisation in the intestinal lumen and absorption of lipophilic drugs [7]. The main organic solutes secreted in bile are bile acids, phospholipids, and cholesterol. These molecules improve the solubility of lipophilic drugs by forming mixed micelles that diffuse to the membrane of the enterocyte. However, the capacity of this mechanism is limited without the presence of dietary lipids or pharmaceutical lipid excipients [8,9].

Most dietary lipids and pharmaceutical lipid excipients are not absorbed intact following oral administration. Instead, they undergo a complicated digestion and intraluminal processing in the upper gastrointestinal (GI) tract. The most common dietary lipids and pharmaceutical lipid excipients are triglycerides of different chain lengths. Following oral administration, triglycerides are partially digested in the stomach by gastric lipases, which preferentially hydrolyse the ester bonds of triacylglycerol, producing free fatty acids and diglycerides [7,10]. When remaining triglycerides and diglycerides are released into the duodenum, pancreatic secretions in the small intestine continue the triglyceride digestion process. The sn1- and sn3- ester bonds of triglycerides are hydrolysed by pancreatic lipase to yield fatty acids and 2-monoacylglycerol [11].

The amphiphilic products of the lipid digestion process, together with bile salts and phospholipids, form mixed micelles. Lipophilic drugs co-administered with dietary lipids or with lipid excipients frequently associate with the lipophilic core of mixed micelles. These micelles diffuse through the unstirred aqueous layer

to the membrane of the enterocytes. It is believed that due to the lower pH near the membrane the mixed micelles disassemble and monomers cross the membrane of the cell [7,10,12,13]. The long-chain fatty acids and monoglycerides are re-esterified to triglycerides that form a hydrophobic core of large lipoproteins (chylomicrons), with which lipophilic drugs can associate and therefore can be transported to the intestinal lymphatic system rather than the portal vein [14,15].

Previous experiments have shown that oral administration of cannabidiol (CBD) together with sesame oil improves the bioavailability and lymphatic transport of CBD [16,17]. CBD is one of the main components of cannabis and is metabolized extensively in animals and humans, and has no psychiatric effects, as opposed to other phytocannabinoids [18]. CBD potentially has high medicinal value and has been reported to be of therapeutic benefit in many types of disease, such as cancer, anxiety, schizophrenia, and immune system disorders [19–23]. However, the oral bioavailability of CBD is limited by its poor water solubility and substantial hepatic first pass metabolism, whereby it is metabolised by oxidation predominantly by CYP3A4 and CYP2C19 [24,25].

Lipid-based drug delivery is a promising approach to improve the oral bioavailability of CBD. Zgair *et al.* have previously reported that when CBD is co-administered orally with sesame oil, the bioavailability of the drug is around 3-fold higher compared to administration without lipids [16]. A subsequent study demonstrated that the levels of CBD in lymph fluid were 250-fold higher than in plasma and substantially above the immunomodulation activity threshold [17]. Furthermore, both *in vitro* and *in vivo* studies have shown that CBD can alleviate the symptoms of inflammation caused by auto-immune diseases through its effects on T lymphocytes and its ability to decrease the release of cytokines in the lymphatic system [17,26]. It has been shown that CBD can reduce the levels of pro-inflammatory cytokines TNF-α and lL-1b of cells from immune cells at high concentrations of 20 µg/mL and microglial cells at a concentration above 1 µM [17,23]. Thus, targeting CBD to the immune cells within the lymphatic system can have a great significance for the

treatment of autoimmune diseases.

Even though the co-administration of CBD with digestible vegetable (sesame) oil leads to high concentrations within the intestinal lymphatic system and higher bioavailability compared to lipid-free formulation, this is associated with substantial variability [16,17]. Due to the complexity of the lipid digestion process in the intestinal lumen, we have hypothesized that the intraluminal digestion could be the rate-limiting step in the processing of the formulation and the absorption of CBD through the lymphatic system, and thus, a source of variability. Therefore, to reduce the variability and increase the efficiency of the lymphatic transport of CBD we have designed a library of pre-digested formulations of CBD consisting of excipients which are a product of intestinal digestion of triglyceride. These formulations do not have to go through digestion process in the intestine to facilitate the absorption and lymphatic transport of CBD, and if hypothesis is correct, should lead to lower variability and potentially to enhanced lymphatic transport and bioavailability of CBD.

Sesame oil mostly contains linoleic and oleic acids, and these two fatty acids are the primary fats obtained from the natural human diet [27,28]. Many studies have reported that these long-chain fatty acids can facilitate the delivery of the lipophilic drug through the intestinal lymphatic system [9,29–32]. However, there is a limited number of reports that directly compare the natural vegetable oil and the corresponding free fatty acids and monoglycerides, or purified triglyceride for the enhancement of the intestinal lymphatic transport and oral bioavailability of lipophilic drugs [33].

Therefore, the aim of this study was to test the hypothesis that pre-digested lipid formulations could reduce variability and increase the extent of the intestinal lymphatic transport and oral bioavailability of CBD compared to digestible natural vegetable oil or purified triglyceride.

2. Materials and method

2.1 Materials

Sesame oil, oleic acid (~ 97%), linoleic acid (≥ 99%), glycerol (≥ 98%), glycerol trioleate (≥ 97%) and 4,4-dichlorodiphenyltrichloroethane (DDT) were purchased from Sigma-Aldrich (Dorset, UK). Cannabidiol (CBD, ≥ 98%) was obtained from THC Pharm (Germany). The 2-oleoylglycerol (≥ 94%) was custom-synthesised by BiBerChem Research Limited (Newcastle upon Tyne, UK). Rat plasma was purchased from Sera Laboratories International (west Sussex, UK). All organic solvents and water were of high-performance liquid chromatography (HPLC) grade or higher and purchased from Fisher Scientific (Leicester, UK).

2.2 Lipid-based formulations

The five tested formulations can be classified into two groups: digestible or pre-digested lipid-based vehicles. The digestible lipid-based vehicles included sesame oil and glycerol trioleate (GT). The pre-digested lipid-based vehicles included oleic acid (C18:1, OA), linoleic acid (C18:2, LA), oleic acid with 2-oleoylglycerol (2:1, molar ratio, 2OA) and oleic acid with glycerol (3:1, molar ratio, OG). All six lipid-based formulations contained fully solubilised CBD at a concentration of 12 mg/mL.

2.3 *In vivo* pharmacokinetic and biodistribution studies

All animal work protocols in this study were reviewed and approved by the University of Nottingham Ethical Review Committee in accordance with the Animals [Scientific Procedures] Act 1986. Male Sprague Dawley rats (340-380 g, Charles River Laboratories, UK) were used for the *in vivo* pharmacokinetics and biodistribution experiments. The animals were housed in a controlled temperature and humidity environment with a 12-hour light-dark cycle in University of Nottingham Bio Support Unit (BSU), with free access to water and food.

A jugular vein cannulation surgery was performed in rats under general gaseous anaesthesia for pharmacokinetics study. All rats were allowed to recover for two nights post-surgery and fasted overnight before oral administration of the tested formulations. All formulations (12 mg/mL CBD) were prepared on the day of the experiment and were administered by oral gavage at a CBD dose of 12 mg/kg. Blood samples (0.2 mL) were collected from cannula at 1, 2, 3, 4, 6, 8, 10, and 12 hours' time points. The plasma samples were obtained by centrifugation at 3000 *g* for 10 minutes and stored at –80 °C until analysis by HPLC. The pharmacokinetic parameters were calculated by a non-compartmental approach using Phoenix WinNonlin 6.3 software (Pharsight, Mountain View, CA, USA, Version 6.3).

The bio-distribution study was performed after obtaining the plasma t_{max} data from the pharmacokinetic studies. Rats were fasted overnight with free access to water before the experiment. All formulations were prepared and administered in the same manner as for pharmacokinetic studies. Animals were humanely sacrificed at t_{max} or one hour prior to t_{max} (t_{max}-1 h) following oral administration. Approximately 50 µL of lymph fluid was withdrawn from superior mesenteric lymph duct using a 1 mL syringe with 25G needle. Mesenteric lymph nodes (MLNs) were also collected and carefully isolated from the surrounding tissue. Blood samples were also collected from the posterior vena cava and serum samples were isolated by centrifugation (3000 *g*, 10 minutes). All obtained samples were stored at –80 °C until analysis by HPLC.

2.4 Bioanalytical procedures

The analysis of CBD in serum, plasma and tissue samples was performed using a previously reported method [16,34]. Briefly, for the determination of CBD in serum or plasma, 10 µL of DDT solution (internal standard, IS) in acetonitrile was spiked into 100 µL of the sample. Cold acetonitrile (450 µL) was added for protein precipitation and the sample was vortex-mixed, followed by addition of 450 µL water. The drug and internal standard were extracted by

vortex-mixing with 3 mL of *n*-hexane for 5 min. Following the centrifugation at 1160 *g* for 10 min, the upper organic phase was transferred to a clean test tube and evaporated to dryness under nitrogen at 37 °C. The residual was reconstituted in 100 µL acetonitrile and 40 µL sample was injected into the HPLC system.

Due to very high concentrations of CBD in lymph fluid, a 10 µL sample was diluted into 90 µL of blank rat plasma. The isolated MLNs were homogenized (POLYTRON® PT 10-35 GT, Kinematica AG, Luzern, Switzerland) with HPLC-grade water in the ratio of 1:2 (w/v). Then 100 µL of diluted lymph fluid or MLN homogenate samples were used for HPLC sample preparation in the same manner as serum or plasma samples above.

A Waters Alliance 2695 separations module HPLC system with a Waters 2996 photodiode array detector was used for all biological sample analysis in this work. The separation was achieved by ACE C18-PFP column (3 µm, 150 mm × 4.6 mm) protected by ACE C18-PFP 3 µm guard column at 55°C. The mobile phase consisted of acetonitrile: water 62:38 (v/v) in isocratic conditions at 1 mL/min flow rate. Retention times of CBD and internal standard (DDT) were 8.7 and 22 minutes, respectively. The lower limit of quantification of CBD was 10 ng/mL for plasma, serum and lymph fluid samples, and 20 ng/mL for MLNs.

2.5 Data analysis

All data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's multiple comparisons tests was used to assess significance of differences among the experimental groups. A *p* value of less than 0.05 was considered statistically significantly different. Statistical analysis was performed using GraphPad Prism version 7.0d (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 *In vivo* pharmacokinetics of CBD administered in different lipid-based formulations

Plasma concentration-time profiles of CBD following oral administration of six tested lipid-based formulations are shown in Figure 1. The pharmacokinetic parameters derived from plasma concentration-time profiles are summarised in Table 1. There was no statistically significant difference in the area under the plasma concentration-time curve (AUC) for pre-digested lipid-based formulations or purified triglyceride in comparison to the sesame oil group. However, the C_{max} was significantly lower for linoleic acid (LA) and 2-oleoylglycerol with oleic acid (2OA) vehicles, and t_{max} was substantially prolonged and variable in 2OA group.

3.2 Bio-distribution of CBD into mesenteric lymph, MLNs and serum following oral administration in different lipid-based formulations

The bio-distribution of CBD into serum, mesenteric lymph and MLNs was assessed following oral administration of sesame oil, oleic acid, linoleic acid, oleic acid with glycerol and glycerol trioleate formulations. Oleic acid with 2-oleoylglycerol (2OA) vehicle did not proceed to the biodistribution assessment stage due to the extremely unfavourable plasma pharmacokinetic profile of CBD administered in this formulation, especially prolonged and variable t_{max} (Figure 1 and Table 1). The animals were sacrificed and all samples were collected at plasma t_{max} and one hour prior to t_{max} (t_{max} – 1h) following oral administration. The triglyceride levels and CBD concentrations were measured in the rat serum and lymph fluid and shown in Figure 2 and 3, respectively. Administration of sesame oil formulation resulted in higher triglyceride serum concentrations compared to the oleic acid group, the glycerol with oleic acid group and the glycerol trioleate group at t_{max} – 1 hour (Figure 2(a)). The triglyceride concentrations in lymph were also the highest following the administration of sesame oil compared to all other groups at t_{max} (Figure 3(b)). There were no statistically significant differences in CBD concentrations in serum or lymph fluid samples at both time points for the

sesame oil group in comparison to the other formulation groups.

The levels of CBD in MLN are shown in Figure 4. There were no significant differences in CBD concentrations in MLN following administration of all pre-digested lipid-based formulations or purified lipid-based formulation compared to sesame oil vehicle at both time points.

4. Discussion

It has been reported previously that lipid-based formulations can increase the intraluminal solubility of lipophilic drugs and enhance the drug absorption. Moreover, oral administration of highly lipophilic drugs in lipid-based formulations containing long-chain triglycerides or long-chain fatty acids results in intestinal lymphatic transport and therefore avoids hepatic first-pass metabolism [9,35]. CBD is a highly lipophilic drug which has high affinity to both rat and human chylomicrons and extensive intestinal lymphatic transport when administered orally with a long-chain triglyceride vehicle (sesame oil) [16,17]. This leads to extremely high concentrations of the drug within the mesenteric lymph fluid and mesenteric lymph nodes and enhanced immunomodulation [17]. However, despite the efficiency of the sesame oil vehicle in promoting the intestinal lymphatic transport of CBD, it is also associated with considerable variability in intestinal lymphatic transport and bioavailability [17].

The digestion of triglycerides in the intestinal lumen is a complicated multi-step process. Therefore, we have hypothesized that the digestion of lipids in the intestinal lumen is a primary source of variability observed in the oral bioavailability and intestinal lymphatic transport of the highly lipophilic drug CBD [16,17]. Therefore, in this work, pre-digested lipid-based formulations were investigated and compared to the natural sesame oil-based formulation, as well as purified triglyceride vehicle.

It was found that the C_{max} of CBD following the administration of the digestible sesame oil-based formulation was higher than for pre-digested lipid-based formulations (Table 1). However, there is no statistically significant difference in AUC between pre-digested lipid-based formulations or purified triglyceride versus sesame oil-based formulation (Table 1). Moreover, variability similar to that of the sesame oil vehicle has been found in the linoleic acid and glycerol trioleate groups. Thus, pre-digested lipids or purified triglyceride have not reduced the variability and have not improved the oral bioavailability of CBD compared to natural sesame oil. In fact, quite consistently, a tendency for

higher AUC (although not statistically significant) was observed in the natural sesame oil group compared to all other pre-digested or purified vehicles. A lack of statistical significance in this observed tendency could be largely attributed to high variability, especially in the sesame oil, linoleic acid and purified triglyceride groups.

Unlike other pre-digested lipid-based formulation groups, in case of 2-oleoylglycerol with oleic acid-based formulation the CBD absorption has been substantially delayed. It is unclear at this stage why the 2-oleoylglycerol excipient led to delayed and variable absorption of CBD. However, due to the unfavourable effect of this specific vehicle on plasma pharmacokinetics of CBD, this formulation did not proceed for further biodistribution studies.

The biodistribution studies were carried out at plasma t_{max} and t_{max} -1 hour time points. It has been previously reported that the maximum concentration in lymph for drugs with substantial intestinal lymphatic transport appears at earlier time point compared to the peak concentrations in plasma [17]. In addition to determination of CBD, triglyceride concentration has been also measured in lymph fluid and serum samples to assess the correlation between the absorption of CBD and the co-administered lipids.

In the current study, administration of natural sesame oil-based formulation resulted in the highest CBD concentrations and highest levels of TG in serum at one hour prior to t_{max} (Figure 2(a)) and lymph fluid at t_{max} (Figure 3(b)) compared to all other formulations. There are no significant differences in the CBD levels in the mesenteric lymph node tissues for purified triglyceride or pre-digested formulations compared to the sesame oil-based formulation (Figure 4). However, there is still a substantial variability that could be seen for all lipid-based formulations, and neither pre-digested lipid-based formulations nor trioleate vehicle improve the CBD levels in intestinal lymphatic system compared to sesame oil-based formulation.

In theory, the pre-digested lipids do not have the need for hydrolysis by

intestinal lipases and instead can directly form mixed micelles together with endogenous bile salts and phospholipids. This could lead to faster and more efficient absorption of co-administered lipophilic drug with lower variability. However, the t_{max} for the linoleic acid group was the same as the sesame oil group, and t_{max} for oleic acid group and glycerol with oleic acid group was the same as for trioleate glycerol (Table 1). Therefore, the intraluminal digestion step does not seem to affect substantially the rate of absorption of lipids and of co-administered CBD.

In this study, pre-digested lipids or purified triglycerides have not improved the CBD bioavailability and lymphatic transport compared to the natural vegetable (sesame) oil. Moreover, taken together, the results suggest that both the absorption of lipids and the absorption of co-administered CBD was more efficient following administration of natural sesame oil vehicle than of pre-digested lipids or purified trioleate.

Although the exact reasons for better performance of natural sesame oil vehicle compared to pre-digested lipids or purified trioleate are unclear, there could be several potential explanations. Sesame oil is a natural oil containing various lipids, mainly triglycerides and phospholipids. Triglycerides present in sesame oil have different chain lengths and degrees of saturation [28,36–38]. It is possible that the various triglyceride composition in sesame oil might provide a more favourable composition for mixed micelles in the intestinal lumen compared to a single type of triglyceride. Even though the levels of phospholipids in sesame oil are relatively low, they might facilitate emulsification of the triglycerides in the intestinal lumen and formation of mixed micelles.

Moreover, there are several minor constituents in the natural plants and seed oils that may have bioactive properties that can affect the absorption of lipids and co-administered drugs. These minor, mostly small molecule constituents can have protective antioxidant effect on the triglyceride, but also can potentially serve as co-factors in multiple key stages of absorption of lipids,

such as lipid digestion or chylomicron assembly. Among such well-known minor components of natural lipids are tocopherols. Notably, α-tocopherol and γ-tocopherol are the most abundant tocopherols present in natural oil [39,40]. The levels of tocopherols in sesame oil vary from 0.21 to 0.8 g/kg oil [41]. The absorption of α-tocopherol and γ-tocopherol is similar to that of lipids, in association with the mixed micelles in the intestinal lumen [42]. Tocopherols are regarded as fat soluble antioxidants, which can prevent the oxidation of unsaturated fatty acids [40].

Another potential minor constituent identified in the plant and vegetable oils are phytoestrogens. The lignan family are the main phytoestrogens and can reach substantial amounts in natural vegetable oils [43]. The metabolism of lignans in the GI tract is associated with gut bacteria leading to formation of enterolignans [44]. The content of lignans in sesame oil varies from 6.5 to 17.3 g/kg oil [39]. The main lignans present in sesame oil are sesamin, sesamolin and sesaminol. Sesamin is present in the sesame oil at highest levels (0.07% to 0.61%) compared to other lignans [45,46]. It has been reported that the plant lignans have antioxidant properties, which provide the thermal and storage stability to the lipids [47,48]. Some lignans in sesame oil can prevent lipid peroxidation in cells [49,50]. Therefore, it might be hypothesised that lignans in sesame oil prevent the triglyceride oxidation or peroxidation before or after digestion in the GI tract.

5. Conclusion

In this study, we have compared pre-digested lipids and purified trioleate to the sesame oil as the lipid vehicle for oral delivery of CBD *via* the intestinal lymphatic system. The *in vivo* studies have shown that the pre-digested or purified lipids do not improve the extent or decrease the variability of CBD bioavailability and lymphatic transport in comparison to the natural sesame oil. Therefore, the intraluminal digestion step does not seem to affect substantially the rate and extent of absorption of lipids and of co-administered CBD. Moreover, taken together, the results suggest that both the absorption of lipids and the absorption of co-administered CBD was more efficient following administration of natural sesame oil vehicle than of pre-digested lipids or purified trioleate.

The various small molecule constituents or the diverse forms of fatty acids in the natural oil could contribute, in a synergistic effect, to the absorption of lipids and co-administered lipophilic drugs. The mechanisms, and constituents responsible for more efficient absorption of lipids and co-administered highly lipophilic drug (CBD) following administration of natural sesame oil compared to pre-digested or purified lipids should be further investigated. Moreover, future studies should assess if these beneficial effects on absorption of lipids and co-administered lipophilic drug are limited to sesame oil or could be also achieved using other natural vegetable oils.

Declaration of interests

The authors declare that they have no conflicts of interest.

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Legends to Figures

Figure 1. Plasma concentration-time profile of CBD (mean ± SD) following oral gavage administration of 12 mg/mL CBD solution in sesame oil, glycerol trioleate (GT), oleic acid (OA), linoleic acid (LA), oleic acid with 2-oleoylglycerol (2:1, molar ratio, 2OA), and oleic acid with glycerol (3:1, molar ratio, OG). The CBD dose was 12 mg/kg for all formulations.

Figure 2. Triglyceride (TG) and CBD concentrations in serum. SO, sesame oil; OA, oleic acid; GT, glycerol trioleate; LA, linoleic acid; OG, oleic acid with glycerol (3:1, molar ratio). CBD was orally administered in lipid-based formulations at a dose of 12 mg/kg to rats. (a) The concentration of CBD and triglyceride level in rat serum at one-hour prior to t_{max} (t_{max} – 1 h). (b) The concentration of triglyceride and CBD in rat serum at t_{max}. All data are presented as mean ± SD, *n=4*. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons test. All experimental groups were compared to the sesame oil group. * P <0.05.

Figure 3. Triglyceride (TG) and CBD concentrations in lymph fluid. SO, sesame oil; OA, oleic acid; GT, glycerol trioleate; LA, linoleic acid; OG, oleic acid with glycerol (3:1, molar ratio). CBD was orally administered in lipid-based formulations at a dose of 12 mg/kg to rats. (a) The concentration of triglyceride and CBD in lymph fluid at one-hour prior to t_{max} (t_{max} – 1 h). (b) The concentration of triglyceride and CBD in lymph fluid at t_{max}. All data are presented as mean ± SD, *n=4*. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons test. All experimental groups were compared to the sesame oil group. * P < 0.05, ** P < 0.01.

Figure 4. Distribution of cannabidiol (CBD) into mesenteric lymph nodes (MLN). SO, sesame oil; OA, oleic acid; GT, glycerol trioleate; LA, linoleic acid; OG, oleic acid with glycerol (3:1, molar ratio). CBD was orally administered in lipid-based formulations at a dose of 12 mg/kg to rats. (a) Concentrations of CBD in MLN at one-hour prior to t_{max} (t_{max} – 1 h). (b) Concentrations of CBD in

MLN at t_{max}. All data are resented as mean ± SD, *n=4*. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons test. All experimental groups were compared to the sesame oil group.