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Evaluation of cytotoxicity of oils used in coenzyme Q_{10} Self-Emulsifying Drug Delivery Systems (SEDDS)

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Abstract

The objective of the present study was to develop a suitable method for evaluation of cytotoxicity of the oils used in Self-Emulsified Drug Delivery Systems (SEDDS) using Coenzyme Q10 (Co Q_{10}) as a model compound. For this purpose, three methods of sample preparation were tested, namely (i) suspensions, (ii) homogenization, and (iii) nanoemulsions of oils in Dulbecco's Modified Eagle's Media (DMEM). Studies were carried out by incubating the sample or control with Caco-2 cells grown on transwell insert systems as well as in flat bottom 96-well plates. The cell viability was assessed by using WST-1 and propidium iodide reagents while the monolayer integrity was assessed by mannitol permeability and Trans Epithelial Electrical Resistance (TEER). The cytotoxicity of oils was found to be dependent on the method of sample preparation; nanoemulsions being the least cytotoxic. In conclusion, nanoemulsification is a useful tool for cytotoxicity evaluation of substances, which exhibit poor aqueous/dimethyl sulfoxide (DMSO) solubility.

Keywords: Caco-2; Cytotoxicity; Coenzyme Q_{10}; Oils; Poorly water soluble drug

1. Introduction

Coenzyme Q_{10} (Co Q_{10}, ubiquinone/ubiquinol) is a well known fat-soluble micronutrient, found in the mitochondria of all living cells. Co Q_{10} deficiency is often implicated in several diseases (Sarter, 2002; Folkers and Simonsen, 1995; Folkers et al., 1991, 1993). Due to its high lipophilicity, the oral delivery of Co Q_{10} is challenging. The poor bioavailability in case of Co Q_{10} has been attributed to its slow absorption from gastrointestinal tract and poor aqueous or DMSO solubility. Several approaches have been used in order to enhance its solubility and bioavailability. One such approach is Self-Emulsifying Drug Delivery Systems (SEDDS) for Co Q_{10}, which includes a medium chain triglyceride and a low HLB surfactant. These oil-based formulations were prepared previously by solubilizing Co Q_{10} in triglyceride oils such as peanut oil, corn oil and soybean oil with the help of polyglycolyzed glycerides (PGG) as emulsifiers (Kommuru et al., 2001). In addition, the solubility of Co Q_{10} in these oils and surfactants was also reported in the study. However, no cytotoxicity study had been reported. Excipients in the formulation are usually selected from Generally Recognized As Safe (GRAS) list of ingredients as published by the FDA. If not listed in GRAS compounds, their toxicity concern is of utmost importance (Huggestt and Verschuren, 1996).

A convenient and reliable model for the evaluation of cytotoxicity is the use of human epithelial Caco-2

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cell line (Sakai et al., 1998). Caco-2 cells, originally derived from a human colon adenocarcinoma, are used extensively as an in vitro model for rapid screening of intestinal absorption (Sakai et al., 1998) and cytotoxicity studies (Liu et al., 1999). When cultured on semi-permeable membranes, Caco-2 cells differentiate into a highly functionalized epithelial barrier with morphological and biochemical similarity to that of small intestinal columnar epithelium (Hilgers et al., 1990). These cells develop effective tight junctions and also express numerous brush border enzymes such as lactase, peptidase, etc.

The integrity of the cell monolayer was confirmed by measuring the Trans Epithelial Electrical Resistance (TEER) (El-Sayed et al., 2002) as well as monitoring the transport of paracellular markers (Artursson et al., 1993). The measurement of cell proliferation and cell viability was carried out in order to predict the effect of various substances on human intestine. WST-1 reagent assay was used for evaluating the plasma membrane damage while the DNA-propidium iodide staining assay was used to evaluate the extent of damage to the cell nuclear membrane.

One of the major difficulties encountered in cell culture studies with hydrophobic test compounds is the technique by which an oily component can be brought into contact with the cells growing in an aqueous environment. Cosolvents such as DMSO are used to improve the miscibility of numerous hydrophobic compounds in aqueous media. However, for compounds having poor DMSO solubility such as Co Q_{10} this problem needs to be addressed. The lack of miscibility of the aqueous media such as Dulbecco's Modified Eagel's Media (DMEM) with oily or lipophilic substances poses difficulty in estimation of the permeability characteristics and other pharmacokinetic parameters using cell culture technique. The low density oils tend to float in the well or in the insert donor chamber, which prevents their contact with cells, leading to erroneous and erratic results. The present study explores the possibility of utilizing different sample preparation techniques such as suspensions or dispersions, homogenized microemulsions and nanoemulsions using low concentrations of surfactants to facilitate the contact of the oily components with cells. The present experiments were carried out in order to find the most suitable and reliable method for evaluation of the cytotoxic effect of oils commonly used in SEDDS.

2. Materials and methods

2.1. Materials

Myvacet 9-45 was obtained from Quest International (Hoffman Estates, IL) and Neobee M-20 was obtained as a gift from Stepan Company (Maywood, NJ). Captex-200 and Captex-355 were supplied by Abitech Corp. (Columbus, OH). Cremophor EL was obtained from BASF Corp. (Mount Olive, NJ). Triglyceride oils (peanut oil, corn oil and soybean oil) were purchased from Spectrum Chemicals (Gardena, CA). Cell proliferation reagent WST-1 and propidium iodide reagent were purchased from Roche Diagnostic Corp. (Indianapolis, IN). A clone of Caco-2 cells (C2BBe1) were obtained from American Type Culture Collection (ATCC) (Rockville, MD). DMEM, phenol red free, phosphate buffered saline (PBS), fetal bovine serum (FBS), human transferrin, penicillin and streptomycin solution and trypsin EDTA solution were obtained from Life Technologies, Inc. (Rockville, MD). Transwell® cell culture assembly with polycarbonate inserts, pre-coated with collagen (0.4 μm pore size, 6.5 mm diameter) were purchased from Corning Costar Corporation (Cambridge, MA). Glucose, sodium bicarbonate and D-[1-^{14}C]Mannitol (Mannitol specific activity, 55 mCi/mmol) were purchased from Sigma Chemicals Company (St. Louis, MO). Sodium dodecyl sulfate (SDS) was purchased from Bio-Rad Laboratories (Hercules, CA).

2.2. Cell culture

A clone of Caco-2 cells (C2BBe1) were grown as per incubation protocol provided by ATCC. Briefly, cells were grown in phenol red free DMEM (supplemented with 10% FBS, 4.5 g/l glucose, 4 mM glutamine, 1.5 g/l sodium bicarbonate, 50 units/ml penicillin and streptomycin and 0.01 g/l human transferrin) in 75-cm^2 cell culture flasks until they reached 85–90% confluence after 6–7 days. Cells with passage numbers less than 20 were used for all the experiments. The cells were seeded at a density of 20,000 cells/well in flat bottom 96-well micro-titer tissue culture plates and 200,000 cells/well onto polycarbonate Transwell® inserts (0.4 μm pore size, 6.5 mm diameter) and allowed to grow in a humidified atmosphere at 37 °C (with 5% CO_2).

The culture media was replaced with fresh media every 48 h.

2.3. Preparation of samples

SNEDDS was prepared by taking the oily mixture of Co Q_{10} and lemon oil at a ratio of 50:50, was accurately weighed into screw-capped glass vial and melted in a water bath at 37 °C. Cremophor EL and Capmul MCM-C8 were added to the oily mixture using a positive displacement pipette and then stirred with a magnetic bar. A detailed method of preparation and characterization of SNEDDS for CoQ is published by Nazzal et al. (2002). Required concentrations of SNEDDS for cytotoxicity studies were prepared by diluting with DMEM. Samples were prepared using three different methods: (1) suspensions of oils were prepared just prior to the test. Ten microlitres of oil was measured using a positive displacement pipette and added to10 ml of DMEM (free of supplements). These were taken in a glass vial fitted with screw cap. The mixture was then vortexed for 5 min using Vortex-genie (VWR Scientific), (2) homogenization of oils was obtained by stirring the oil and DMEM mixture in a glass vial for 5 min using a PT 10-35 Polytron homogenizer at #4 setting, (3) nanoemulsions using a surfactant: A 0.1% (v/v) nanoemulsions of each of the oils mentioned were prepared by adding 10 μl of oil and 500 μg of Cremophor EL in 10 ml DMEM in a screw cap glass vial. The mixture was then subjected to homogenization for 5 min by using a PT 10-35 Polytron homogenizer at #4 setting.

2.4. Determination of particle size

The particle size of oily suspensions, homogenized oils and nanoemulsified oils was determined by using Nicomp Particle Sizing System (PSS) ZW380 Application Version 1.61A (Santa Barbara, CA). One milliliter of the sample was taken in a 6 mm × 50 mm disposable culture tube and the intensity was adjusted in order to obtain the particle size distribution. The volume diameter was obtained from the particle sizing system output.

2.5. Effect of oily samples on cell proliferation

The Caco-2 cells were grown in a 96-well plate to 95% confluence (determined by observing under microscope). The cells were washed with PBS, and incubated with 200 μl of test sample (oils such as Myvacet 9-45, Neobee M-5, corn oil, peanut oil, Captex-200, Captex-355 and Soybean oil) or with controls. DMEM alone was used as negative control and 0.1% w/v SDS was the positive control. The cell viability was determined after incubation for 1, 2, 3 and 4 h.

2.6. Analysis by WST-1 assay method

Following the pre-determined incubation time intervals, 20 μl of WST-1 reagent was added directly to the cells in phenol red free DMEM. The plates were further incubated for 15 min so as to allow the reaction between the mitochondrial dehydrogenase released from viable cells and the tetrazolium salt of the WST-1 reagent. The intensity of the colored compound formed (formazan dye) was then quantified using an ELISA reader (Tecan SpectraFlour Plus). The absorbance was measured at 450 nm with a reference at 620 nm. Each assay was performed in triplicate and the cell viability was expressed as a percentage of the absorbance of cells exposed to test samples compared to the controls.

2.7. Propidium iodide staining test

Caco-2 cell monolayers were treated with various oils in phenol red free DMEM in the apical chamber and media free of oils in the basal chamber. Following the treatment for specified time intervals of 1, 2, 3 and 4 h; 0.1 ml of the medium was withdrawn from the apical side and added to 300 μl of propidium iodide solution (50 μg/ml). The fluorescence was measured at 490 nm (excitation) and 630 nm (emission) (Tecan SpectraFlour Plus, CA). The intensity of fluorescence produced upon treatment with various oils is presented as a ratio to that of control.

2.8. Permeability of ^{14}C-radiolabeled mannitol

One potential effect of excipients on Caco-2 cell monolayers would be the disruption of tight junctions. The cell monolayer integrity can be determined by measuring the flux of paracellular marker, mannitol. Therefore, transport experiments were conducted with [^{14}C]-radiolabeled mannitol in the presence and absence of oils. For this purpose the cells were grown

on collagen coated transwell inserts with 0.4 μm pore size as described above in Section 2.2. The apical side of the Caco-2 cells grown on inserts was then treated with phenol red free DMEM containing various oils along with paracellular marker, while the basal chamber was supplemented with DMEM alone. After the incubation in presence of oils for 1, 2, 3, and 4 h time intervals, samples were withdrawn (100 μl from the apical side and 1 ml from the basal side) and the percentage of mannitol permeation was determined.

2.9. Analytical method for permeability evaluation

[^{14}C]Mannitol was analyzed by liquid scintillation counting using Beckman LS 6500 counter. Mannitol concentration was chosen such that initial donor concentration was approximately 100,000 disintegrations per minute (DPMs). Permeability of mannitol was calculated by considering the initial amount in donor chamber as 100%.

2.10. Evaluation of Caco-2 cell monolayer viability by measurement of recovery of TEER

TEER values of cell monolayers treated in presence or absence of test samples for 1, 2, 3 and 4 h were measured using the EVOM^{TM} epithelial voltohmeter (World Precision Instruments, Sarasota, FL). After treatment with test samples, the cells were washed with PBS and fresh DMEM media was added to both apical and basolateral chambers. The cells were then incubated for 24 h (37 °C and 5% CO_2) and recovery of TEER value was monitored at the end of 24 h.

2.11. Statistical analysis

All experiments were performed in triplicates, and the data was compared using ANOVA with a level of significance (α) set at 0.05. When the differences in the means were significant, post hoc comparison was conducted using Tukey–Kramer multiple comparison test.

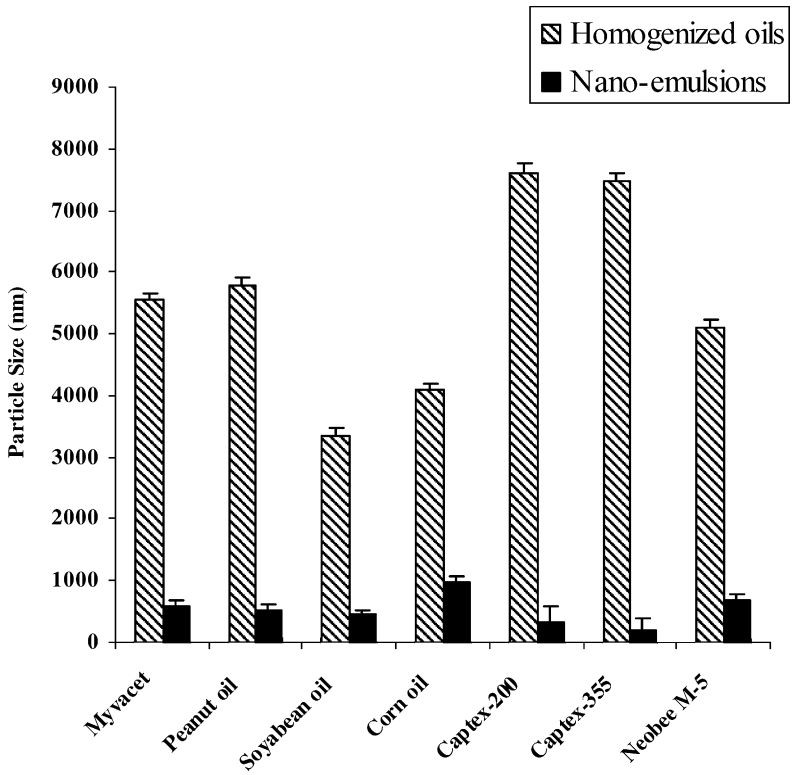


Fig. 1. Particle size of various homogenized and nanoemulsified oils.

3. Results

3.1. Effect of homogenization and nanoemulsification on particle size of oily samples

The particle size of the oil sample depends largely on the method of preparation. The oils when suspended or dispersed in DMEM formed larger globules and the particle size was in the millimeter range (data not shown). When the oils were prepared by homogenization the particle size range was 3000–7500 nm, whereas the particle sizes for the nanoemulsions were less than 1000 nm. Fig. 1 shows the effect of these two sample preparation techniques on particle size.

3.2. Effect of oils on Caco-2 cell viability evaluated by WST-1 method

Fig. 2a shows the cytotoxic effects of oils on Caco-2 cell monolayer after treatment at 1, 2, 3 and 4 h with or without the oily suspensions. The viability of cells treated with DMEM alone, is taken as 100% survival. The cytotoxic effect of SNEDDS was evaluated by quantifying the absorbance of the color produced due to cleavage of WST-1 reagent by viable cell mitochondrial dehydrogenase. Cell viability was not significantly different as presented in Fig. 2 when compared to control. No significant difference was seen between the viability of cell monolayers after treatment with oily suspensions of Myvacet 9-45, peanut oil and soybean oil as compared to negative control. The monolayers treated with other oils namely corn oil and Captex-200, showed a significantly lower percentage of cell survival as compared to that of control. Incubation of up to 4 h had no significant effect on the cytotoxicity pattern of the oils. The effect of oils prepared by homogenization followed a different pattern as compared to that of the suspensions. The microemulsions showed no significant effect as compared to that of control. Fig. 2b shows the percent survival of Caco-2 cell monolayer after treatment with the microemulsions of (homogenized) oils for 1, 2, 3 and 4 h. Fig. 2c shows the percent survival of Caco-2 cells with or without treatment with nanoemulsions with 0.5% surfactant, Cremophor EL. No significant difference was seen in the cell viability of Caco-2 cell monolayers after treatment with nanoemulsions as compared to the control for any of the incubation time intervals. As shown in Fig. 2c, the percent survival of cells after treatment with nanoemulsions for 1hr was more that 80%, whereas SDS 0.1% w/v treated cell monolayers showed only 13% survival.

3.3. Effect of oily samples on Caco-2 cell monolayer determined by propidium iodide staining method

The DNA released from the cells to the apical medium was quantified using propidium iodide stain-

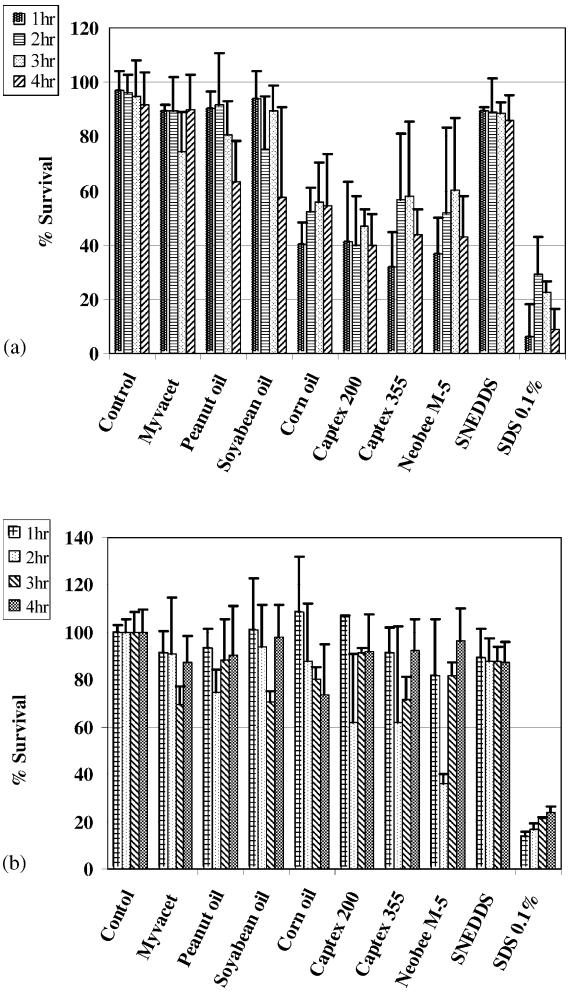
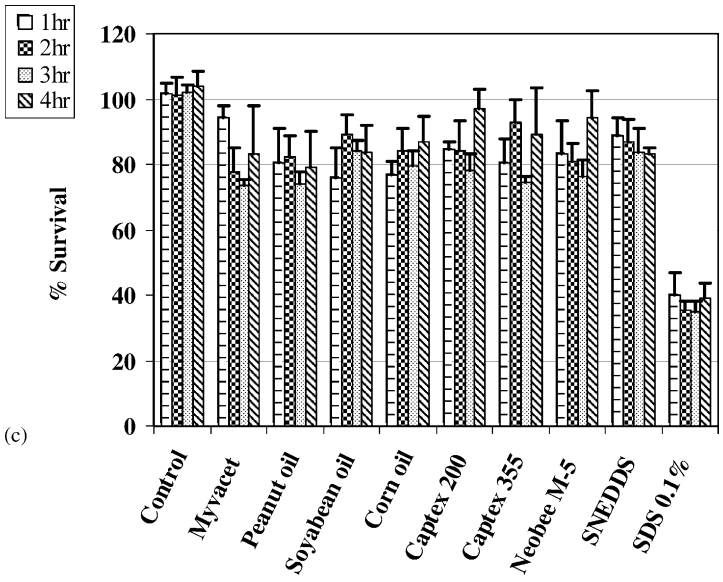


Fig. 2. (a) Cytotoxicity of oils as suspensions on Caco-2 cell monolayer using WST-1 method (% survival compared to control). (b) Cytotoxicity of homogenized oils (microemulsions) using WST-1 reagent. (c) Cytotoxicity of nanoemulsions of oils using WST-1 reagent.



(c)

Fig. 2. (*Continued*).

ing Fig. 3a and b show the ratio of the amount of DNA released by treatment of Caco-2 cell monolayers with homogenized oils and nanoemulsified oils respectively to the amount of DNA released from control monolayers. Although treatment with oils for 1, 2, 3 and 4 h induced the release of DNA from cells, the amount released was not significantly different from that of the cells treated with negative control (DMEM alone). Treatment with 0.1% w/v SDS as a positive control for the same time intervals greatly induced the release of DNA from cells with a ratio of 4:1 compared to the control.

3.4. Permeability of ^{14}C-radiolabeled mannitol

[^{14}C]-radiolabeled mannitol was used as a marker to evaluate the effect of oils on paracellular permeation. Fig. 4 shows the percent of mannitol permeation through Caco-2 cell monolayer after treatment with oils for 1, 2, 3 and 4 h. The mannitol concentration in all the donor wells prior to treatment was taken as 100%. Permeability of mannitol was found to be not more than 10% even after treatment with oils for 4 h, and was not significantly different from the negative control (DMEM alone). Up to 10% permeation of mannitol is reported to be acceptable for permeability studies. In the present study, considering the exposure time of 4 h, the maximum permeation of 10% indicates minimal leakage of the monolayer. In contrast, the permeability of mannitol increased significantly for monolayers treated with positive control, 0.1% w/v SDS and it was found to follow a time-dependent pattern. Percent permeability of 0.1% w/v SDS after 1, 2, 3 and 4 h treatment was $21.8 \pm 1.2\%$, $28.6 \pm 4\%$, $32.65 \pm 1.1\%$ and 37.28%, respectively.

3.5. Evaluation of Caco-2 cell monolayer integrity by measurement of TEER

The effect of oils on the monolayer integrity was examined by measuring the TEER value before and after treatment of Caco-2 cell monolayers with the above mentioned oils for predetermined time intervals of 1, 2, 3 and 4 h. All the oils showed TEER values identical to those of the negative control after treatment with oils for 1 h. Although there was a slight decrease in TEER after 2, 3 and 4 h, it was not significantly different from that of negative control monolayers. The TEER of monolayers treated with 0.1% w/v of SDS for 1, 2, 3 and 4 h reduced drastically and was significantly different as compared to the nega-

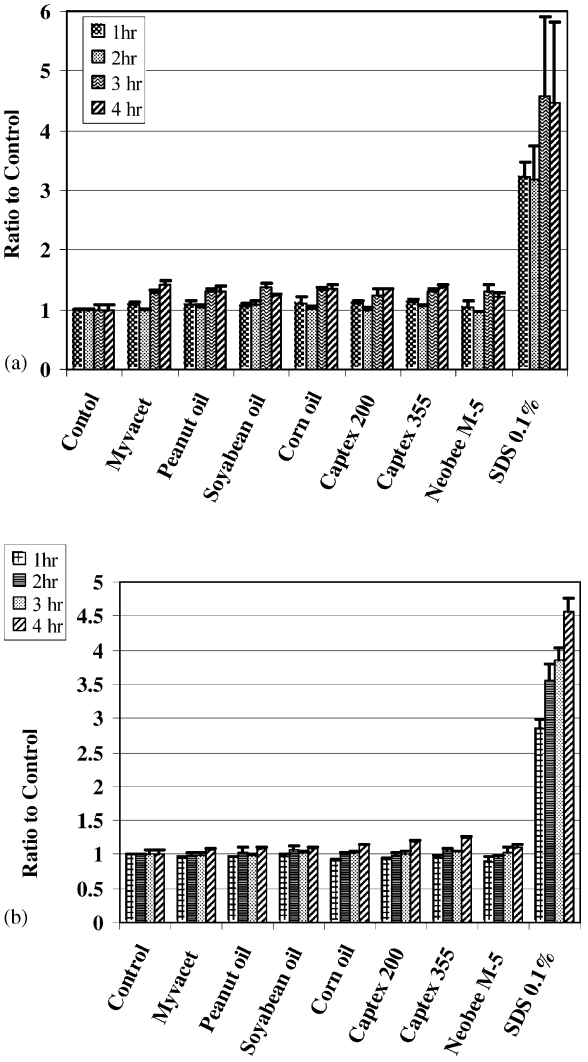


Fig. 3. (a) Cytotoxicity of oils as homogenized samples using propidium iodide staining method (ratio to control). (b) Cytotoxicity of nanoemulsions of oils using propidium iodide staining (ratio to control).

tive control (Table 1). The effect of oils on the recovery of TEER value was examined by measuring the TEER of treated cell monolayers after a 24 h incubation of the cells in DMEM. The TEER values for the monolayers treated with all of the tested oils showed complete recovery and were found to be similar to the values obtained before the experiments were performed.

4. Discussion

Co Q_{10} is reported to possess poor bioavailability and poor delivery properties owing to its solubility (Chopra et al., 1998). We have previously reported some unconventional eutectic based drug delivery systems (Nazzal et al., 2002) to enhance the solubility and bioavailability. Conventional SEDDS utilize oils, surfactants and co-surfactants to obtain an isotropic mixture. While these preparations enhance bioavailability, the toxicity due to oils, surfactants and co-surfactants often pose problems. Due to lack of documentation on the cytotoxicity and dosage limits for these oils, we were interested in testing the cytotoxicity of various excipients used in the formulation. Essential oils have been used extensively in pharmaceutical formulations as solubilizing agents and flavoring agents. These oils are included in the self-emulsifying systems due to their solubilizing properties and their P-glycoprotein inhibiting action. During the last few decades there has been growing concern regarding the use of these oils as excipients for oral consumption. A number of essential oils used as flavoring agents are recognized by the Code of Federal Regulations (CFR) as GRAS compounds, and so are waived from regulatory agency approval for their incorporation in ingested materials. However, reports show that these oils could prove to be cytotoxic if not used in limited concentrations (Burkey et al., 2000). Hence, a number of oils used for solubilizing Co Q_{10} were evaluated by the above mentioned methods, for their cytotoxicity on Caco-2 cell monolayers. In the current study, various oils used in SEDDS were evaluated for their cytotoxicity.

The method of sample preparation is of prime importance in the proper screening of compounds using cell culture models. Poor aqueous solubility and poor DMSO solubility of many highly lipophilic substances proves to be the major hurdle as there is non-uniform exposure of the cell monolayer to such compounds. Improved delivery systems such as microemulsions, nanoemulsions and self-emulsified systems are being extensively used to improve the bioavailability of poorly soluble drugs (Nazzal et al., 2002). By adopting a similar approach for in vitro presentation of such compounds to the cell monolayers, it may be possible to create a more reliable method for assessing cytotoxicity. Therefore in the present study, sample preparation was carried out using three different methods,

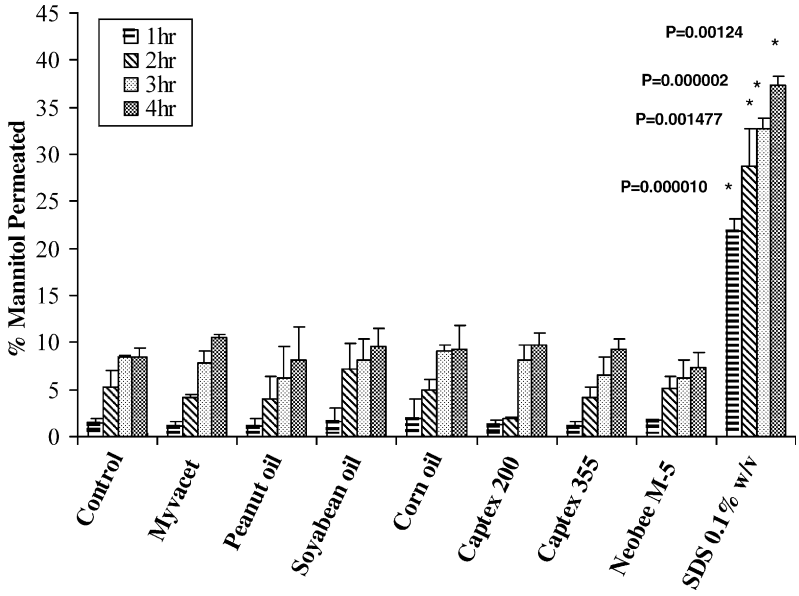


Fig. 4. Cytotoxicity of nanoemulsions of oils assessed by paracellular marker permeation.

namely (1) suspensions or dispersions of samples in aqueous media, (2) homogenization of samples, and (3) nanoemulsions of samples using a surfactant.

The cytotoxicity was evaluated by WST-1 assay method, DNA-propidium iodide staining assay, Mannitol permeability and TEER measurement. Various tetrazolium salts such as MTT (Dimitrijevic et al., 2000), XTT and MTS have been widely used for measuring cell proliferation (Bromberg and Alakhov, 2003). Tetrazolium salts are cleaved by mitochondrial dehydrogenase in living but not dead cells forming a colored compound, formazan the measure of which indicates cell viability. WST-1 is a newer tetrazolium salt that can be used for the non-radioactive, spectrophotometric quantification of cell growth and viability in proliferation and chemosensitivity assays (Ishiyama et al., 1996). WST-1 reagent was selected over other tetrazolium salts, because it is cleaved into a water-soluble product unlike MTT. It has a wider linear range and accelerated color development, which is advantageous for fast and reliable simultaneous mass scale evaluation of different compounds. Another measure of cell viability, DNA-propidium iodide assay is based on the fluorescence developed due to interca-

Table 1
Cytotoxicity of oils assessed by membrane integrity (TEER values expressed in $\Omega\,cm^2$)

	Exposure time				
	0 min	1 h	2 h	3 h	4 h
Control	599 ± 1.15	597 ± 1.53	578 ± 4.73	543 ± 6.43	511 ± 10.15
Myvacet	590 ± 10.00	579 ± 9.02	557 ± 2.52	538 ± 7.21	492 ± 4.93
Peanut oil	596 ± 16.01	588 ± 2.89	572 ± 4.93	544 ± 4.51	506 ± 6.03
Soyabean oil	587 ± 15.28	578 ± 7.64	554 ± 3.21	539 ± 17.04	497 ± 22.50
Corn oil	590 ± 10.02	563 ± 5.77	538 ± 7.64	528 ± 6.81	506 ± 5.03
Captex-200	596 ± 7.00	560 ± 10.00	534 ± 5.13	516 ± 5.51	479 ± 19.01
Captex-355	593 ± 6.08	563 ± 10.41	550 ± 5.51	506 ± 5.03	476 ± 10.69
Neobee M-5	598 ± 3.21	540 ± 10.00	525 ± 4.36	500 ± 10.00	443 ± 29.57
SDS 0.1%	593 ± 6.08	408 ± 7.64	356 ± 11.00	302 ± 2.52	259 ± 17.93

lation of propidium iodide with DNA when the nuclear membrane is damaged (Wrobel et al., 1996). The TEER value is a measure of electrical resistance, which increases with time as the cells form tight junctions, which can be used to measure the integrity of cell monolayer. However, it is less sensitive than other methods such as paracellular marker permeability. Hence more sensitive method, mannitol flux was calculated to assess the damage to Caco-2 cells in presence or absence or test compounds. Sodium dodecyl sulfate (SDS), a potent surfactant, was used as positive control for all the methods at a concentration of 0.1% w/v at which it has been reported to be significantly cytotoxic (Sakai et al., 1998).

The suspensions of oils such as corn oil, Captex-200, Captex-355 and Neobee M-5 and SNEDDS released low amounts of dehydrogenase from viable cell mitochondria as compared to the control, while the homogenized oils and nanoemulsions showed no significant decrease in cell viability as compared to the control (Fig. 2a–c). This membrane damage caused by suspensions of same oils could be explained by the fact that when the oils are presented as suspensions, there could be minimal stability of the suspension due to absence of surfactant, leading to migration of the lower density oil droplets to the top and the retention of higher density oils at the bottom. Captex-200 and Captex-355 (diesters of caprylic acids on propylene glycol) and Neobee M-5 (caprylic/capric triglycerides) may form a layer at the bottom, thus increasing the contact area and time, reducing the intake of nutrients from the aqueous media. The same oils when presented as homogenized oils and nanoemulsions failed to be cytotoxic to Caco-2 cells. These results indicate that homogenized samples may be more reliable indicators of the cell viability. To further confirm the usefulness of homogenized oils or nanoemulsions of oils for cytotoxicity/permeability studies in cell culture methods, the effect of these oils as microemulsions and nanoemulsions was evaluated using propidium iodide staining method, mannitol permeability and by measurement of the TEER recovery. The results were in accordance with the WST-1 method (Fig. 2a–c). None of the oils tested were found to be cytotoxic when presented as homogenized oils or nanoemulsions (Fig. 3a and b). This indicates that the above two methods of sample preparation namely, homogenization and nanoemulsification are suitable for carrying out cell culture experiments with poorly water soluble and poor DMSO soluble substances.

In the present study, Myvacet 9-45, a diacetylated monoglyceride of C-18 fatty acids, was found to be non-cytotoxic by all the methods used. Medium chain oils such as Myvacet 9-45 provide higher solubility than long chain oils. Myvacet 9-45 has been used in an optimized self-emulsifying formulation of Co Q_{10} (Kommuru et al., 2001) and Myvacet 9-08 has been used as a solvent for evaluating the oral bioavailability of phenobarbital (Yska et al., 2000).

Peanut oil is a triglyceride of long chain fatty acid, which is commonly used as a solvent for topical preparations, acts as an absorption enhancer. A review on the safety assessment of peanut oil as reported in International Journal of Toxicology (2001), supports the use of peanut oil, hydrogenated peanut oil, peanut acid, and peanut glycerides in cosmetic formulations, but there are no studies reported on the cytotoxic effects of this oil when ingested orally. When evaluated at a concentration of 0.1% (w/v), peanut oil was found to be non-cytotoxic by all the methods of cytotoxicity evaluation as well as by all three methods of sample preparation (Figs. 2–4).

Soyabean oil is a triglyceride of long chain fatty acid and is frequently employed as a solvent for topical as well as oral formulations. There was no significant cytotoxicity observed at the concentration used (0.1% w/v). The method of sample preparation had no significant difference on the cell viability, which is in accordance with an earlier study reported. Olson and Visek (1990) on the kinetics of cell-mediated cytotoxicity of soyabean oil using the animal model, which reported that 5% soyabean oil is less cytotoxic as compared to 20% soyabean oil. Others have reported that the high molecular volume oils such as soyabean oil had no significant toxicity unless it was used in significantly greater concentration than their critical aggregation concentration (Warisnoicharoen et al., 2003).

In the present study, corn oil showed some cytotoxicity when presented as suspension or dispersion (Fig. 2a). However, it failed to show any cytotoxicity when presented as homogenized or nanoemulsified oil, indicating the importance of sample presentation in such studies (Fig. 2b and c).

Captex-200 and Captex-355 are the medium chain-triglycerides, used as a vehicle, solubilizer, as energy source and viscosity modifier. These have

been previously utilized in SEDDS formulation of Co Q_{10} (Kommuru et al., 2001). Captex-355 is a fully refined and deodorized medium-chain triglyceride inter-esterified with coconut oil. It is soluble in numerous organic solvents and miscible with mineral oil and vegetable oils. Its oral LD_{50} is reported to be >36 ml/kg in rats and >25 ml/kg in mice. In the current study we found that it can be used safely up to a concentration of 0.1% w/v.

Neobee M-5 is a medium chain caprylic or capric acid triglyceride, which is a source of fat as a nutrient in a variety of specialized nutritional products. This is a specialized fat that is similar to conventional oils but is used as a carrier for flavors, vitamins, essential oils and colors. In the present study, though Neobee M-5 reduced the cell viability, it was not found to be significantly different from the control (Fig. 2a).

Therefore, amongst the oils tested, Myvacet 9-45, peanut oil and soyabean oil were found to be non-cytotoxic by all the methods of evaluation. Sample preparation had no effect on the assays used to assess the cytotoxicity levels. This indicates that all the three methods of sample preparation provided uniform sample exposure for these test compounds as well as SNEDDS. However, corn oil, Captex and Neobee M-5 showed variable results, depending on the methods of sample preparation. When these oils were used as suspension or dispersion, significant cytotoxicity was observed indicating the damage to cell membrane. However, the same oils were used in the form of homogenized samples or nanoemulsions they proved to be non-toxic. This difference in the cytotoxicity could be attributed to the contact time of the oils with the Caco-2 cells. When oils are presented to the cells as suspensions or dispersions, which are not stabilized by any additional surfactant, the possibility of formation of large oil globules or aggregates leads to an increased concentration of oils in certain areas of the cell monolayer, and subsequent disruption of the cells. It is proposed that when the oils are used in high concentrations above their critical aggregation concentrations, aggregated forms of oils such as soyabean oil mediated the toxicity in presence of surfactant, which would solubilize the components of the cell membrane thus leading to disruption of membrane integrity. Homogenization or nanoemulsification of these oils would give fine droplet sizes (Fig. 1), which would provide uniform distribution and contact of the oils with the Caco-2 cells and thus reduce the dumping effect of these oils.

5. Conclusion

Caco-2 cell culture technique can be utilized for compounds with poor aqueous and DMSO solubility provided that the appropriate method of sample preparation is used. Myvacet 9-45, peanut oil and soybean oil were found to be non-cytotoxic in emulsion form by all the methods of evaluation. Therefore, these oils are more suitable. Among the three methods of sample preparations tested, homogenization and nanoemulsification provided a suitable tool for cytotoxicity and permeability evaluation of different compounds. Corn oil, Captex-200 and Captex-355 were found to be cytotoxic when used as suspensions or dispersions. However, all these oils were found to be non-cytotoxic when presented as homogenized or nanoemulsified samples.

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