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ORIGINAL ARTICLE

Development and Evaluation of Sesamol-Loaded Self Nanoemulsifying Drug Delivery System for Breast Cancer

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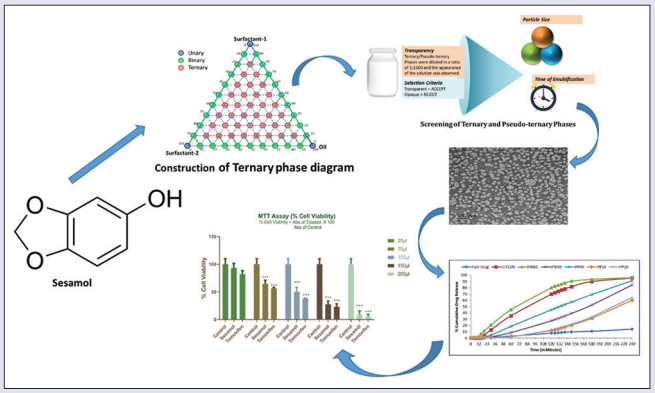
ABSTRACT

Background: Breast cancer is one of the leading causes of cancer-related death among females in the world. Sesamol, which is an herbal phenolic compound, is investigated for its powerful antioxidant and anticancer motility. Sesamol induces growth arrest and apoptosis in malignant cells. However, its pharmaceutical significance is limited due to poor bioavailability. The self nanoemulsifying drug delivery system (SNEDDS) is a type of lipid nanocarrier system that is suitable for the encapsulation of lipophilic drug molecules. **Objectives:** In the present study, Sesamol-loaded SNEDDS were developed, identified, and assessed for the enhancement of its *in vitro* dissolution rate and anticancer efficacy. **Materials and Methods:** Based on the solubilization potential of sesamol, the oil (Isopropyl myristate, Isopropyl palmitate, Caprylic capric triglycerides, Sesame oil) surfactant (Span 80, and Tween 80), and cosurfactant (Poly Ethylene Glycol 400) were chosen for the formulation of Sesamol-loaded SNEDDS. SNEDDS were prepared by an aqueous titration technique. Developed formulations were characterized and assessed for thermodynamic stability, self nanoemulsification efficiency, droplet size, polydispersity index, zeta potential, surface morphology, refractive index, the percent of transmittance, and drug release profile. **Results:** *In vitro* dissolution rate of Sesamol was significantly enhanced from the optimized formulation in comparison with pure drug. The finalized formulation was selected for *in vitro* anticancer effects in human breast cancer cells (MCF-7) by 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide (MTT) assay. MTT assay suggested remarkable anticancer efficacy of finalized Sesamol-loaded SNEDDS against MCF-7 cells compared with standard (Marketed preparation). **Conclusion:** The outcome of this study revealed the incredible potential of SNEDDS in the enhancement of *in vitro* dissolution rate and anticancer efficacy of the poorly soluble drug.

Key words: Droplet size, self nanoemulsification, self nanoemulsifying drug delivery systems, solubility, zeta potential

SUMMARY

- Self nanoemulsifying drug delivery systems (SNEDDS) of Sesamol were developed, identified, and assessed for the enhancement of its *in vitro* dissolution rate and anticancer efficacy
- Developed formulations were characterized and assessed for thermodynamic stability, self nanoemulsification efficiency, drop size, polydispersity index, zeta potential, surface morphology, refractive index, the percent of transmittance, and drug release profile. The best formulation was selected for *in vitro* anticancer efficacy in human breast cancer cells (MCF-7) by 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide assay
- The study outcome revealed the potential of SNEDDS in the enhancement of *in vitro* dissolution rate and anticancer efficacy of the poorly soluble drug.



Abbreviations used: MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide; SNEDDS: Self Nano-Emulsifying Drug Delivery System; RI: Refractive Index; ZP: Zeta Potential; PDI: Polydispersity Index; DPPH: 2,2′-diphenyl-1-picrylhydrazyl; DCFH-DA: 2, 7-Dichlorodihydrofluorescein diacetate; SPAN 80: Sorbitan Monooleate 80; TWEEN 80: Polysorbate 80; IPM: Isopropyl Myristate; IPP: Isopropyl Palmitate, CCTG: Caprylic Capric Triglycerides; HLB: Hydrophilic Lipophilic Balance.

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INTRODUCTION

Cancer, also known as malignancy, is the uncontrolled growth of abnormal cells.[1] It is one of the main causes of mortality worldwide. In 2008, 8 million deaths were recorded as a result of malignant diseases, and this figure is estimated to reach 11 million by 2030.[2] The cancer progression impairs the normal biological process of healthy cells which is achieved by the invasion of nearby tissues and metastasize to distant tissues.[1] Breast cancer is one of the leading causes of cancer-related death among females in the world. Cancer that develops from breast tissue is Breast cancer. The major sign of breast cancer includes lump in the breast, change in breast shape, discharge of fluid from the nipple, or scaly patch in the skin. As the disease progresses, there can be pain in the bone, lymph nodes swell, or shortness of breath.[3,4] It accounts for 23% of all recently occurring cancers and represents 13.7% of all cancer deaths in women worldwide. All around the world, the incidence

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of breast cancer shows varied rates. The rates are low in less-developed countries and greatest in the more-developed countries. Breast cancer is related to age with only 5% of all breast cancers occur in women under 40-year old.[4,5]

For many decades, natural plants are utilized to counteract and treat different diseases. These herbal means are currently being investigated as a possible hotspot for the invention and development of a lead compound in most cancers prohibition. Many novel medicinal bioactive compounds had been selected for epidemiological, preclinical, and early medical research for the prohibition and remedy of assorted sorts of carcinoma. These amazing bioactive compounds, which are very treasured inside the aversion and remedy of carcinoma act through different, signaling molecules and routes.[6]

Phenolic compounds have received increasing interest in this regard owing to their potential antioxidant activity. Evidence suggests that oxidative stress plays a key role in cancer occurrence. Phenolic compounds play a protective role against oxidative stress through their radical scavenging ability and lipid peroxidation lowering potential.[7] Sesamol (2H-1,3-Benzodioxol-5-ol), is a natural phenolic compound and a major lignan isolated from sesame seeds (*Sesamum indicum*) and sesame oil (SO).[8,9] Raw sesame seeds contain a small amount of Sesamol while it is produced from the decomposition of Sesamolin during roasting of sesame seeds.[10] It is a white crystalline solid which is sparingly soluble in water and miscible with most oils.[11] The therapeutic potential of Sesamol was investigated intensively, and there is compelling evidence that Sesamol possesses antioxidant, anti-mutagenic, anti-inflammatory, and chemopreventive properties.[8,9]

Though the phenolic compounds rich in Sesamol induces growth arrest and apoptosis in cancer cells.[12] However, their pharmaceutical significance is restricted because of poor bioavailability. The poor bioavailability of Sesamol is because of the poor aqueous solubility. The oral bioavailability of Sesamol was found to be 35.5% ± 8.5.[13,14] Attributable to their pharmaceutical limitations, newer strategies have been attempted aiming to mitigate problems associated with the effective oral delivery of Sesamol and to boost their anticancer potential.

Lipid-based formulations (LBFs) are one of the efficient technologies to improve aqueous solubility, and thus to improve the bioavailability of lipophilic drug molecules.[15] Among the LBFs, self-nano emulsifying drug delivery systems (SNEDDS) have received great attention, as an approach for the enhancement of oral bioavailability of poorly water-soluble drugs.[16] The SEDDS is an isotropic mixture of oils and surfactants with or without cosolvents, which upon introduction into the aqueous media spontaneously forms oil-in-water nanoemulsion with only gentle agitation like GI motility.[17] SNEDDS not only can improve the delivery of the insoluble drug, but they may also provide an improved enzymatic and chemical stability while at the same time have a bigger interfacial area for absorption and enhance oral bioavailability upon administration.[18]

Hence, in the present study, an attempt has been made to optimize and develop Sesamol-loaded SNEDDS, assess its physiochemical properties, and evaluate the *in vitro* cytotoxic effect of Sesamol-loaded SNEDDS toward breast cancer cell line (MCF-7).

MATERIALS AND METHODS

Materials

Sesamol was procured from Avra Synthesis Private Ltd, Hyderabad, Telangana, Isopropyl myristate (IPM), Isopropyl Palmitate (IPP), Caprylic capric triglycerides (CCTG), Polyethylene glycol-400 (PEG) has been procured from Loba Chemicals Pvt. Limited (Mumbai), SO (local purchase), Span-80, and Tween-80 were procured from HiMedia Laboratories Pvt. Ltd., India, Breast Cancer Cell line (MCF-7) has been procured from NCCS, Pune. All other ingredients were of analytical grade. Double distilled water was used for all experiments.

Methods

Solubility studies

The solubility of Sesamol in oils, surfactants, and cosurfactants was determined by the equilibrium solubility method. An excess amount of Sesamol was added to 2 mL of selected oils, surfactants, and cosurfactants separately in stoppered vials and mixed with the help of a vortex mixer. The vials were kept at 30°C in an isothermal shaker for 48 h to succeed in equilibrium then centrifuged at 3000 rpm for 10 min. The supernatant was filtered through a 0.22 µm filter and analyzed for the drug concentration.[19]

Construction of pseudo-ternary phase diagrams

The selected oil, surfactant, cosurfactant based on solubility studies were used to develop the pseudo-ternary phase diagrams using the phase titration method. The various surfactant–cosurfactant (S_{mix}) ratios were prepared using different proportions of surfactant and cosurfactant to fulfill the hydrophilic Lipophilic Balance (HLB) value requirement for the formation of a transparent clear solution. A series of oil/S_{mix} mixtures were prepared and titrated with water to identify the nanoemulsion region. The total water consumed was noted in terms of w/w and during titration oil–S_{mix} ratio and observations were made for phase clarity. These values were used to determine the boundaries of the nanoemulsion region corresponding to the selected value of oil and S_{mix} ratio.[20]

The physical state of the nanoemulsion was marked on a pseudo-three component phase diagram with one axis representing aqueous phase, the other representing oil, and the third representing a mixture of surfactant and cosurfactant at fixed volume ratios (S_{mix} ratio). From each phase diagram constructed, different formulations were selected from the nanoemulsion region so that a single dose of the drug could be easily incorporated into the oil phase.[21]

Characterization and evaluation of self nano-emulsifying drug delivery system

Identification of in situ nano-emulsification

Each liquid formulation (0.2 ml) containing oil-S_{mix} in the different ratios was introduced into 300 ml of distilled water in a glass beaker under gentle agitation using a magnetic stirrer at 37°C. Tendency to form nanoemulsion was identified as "Grade A" when the formulation spread easily and form a clear solution and it was sorted as "Grade B, C, D" when there is milky, poor or no emulsion is formed. All the mixtures were stored at ambient temperature for further use.[22]

Measurement of self-emulsification time

For the determination of emulsification time, 1 ml of the formulation was added to the 900 ml of distilled water which was maintained under mild agitation and temperature condition of 37°C ± 0.5°C. The study was performed using USP dissolution test apparatus II. The time required to disperse the system completely and form clear nanoemulsion was recorded as emulsification time.[23,24]

Droplet size analysis and zeta potential determination

The formulations identified as "Grade A" (that indicated self-emulsification and nonprecipitation) were subjected to droplet size analysis and zeta potential measurement. The droplet size and zeta potential were measured using a Malvern zeta sizer (Malvern, UK). The

Zeta potential of each SNEDDS was determined to access the stability of the formulation.[25]

Formulation development (Drug loading)

All the formulations identified as qualified after passing the established criteria were selected for drug loading.

As a general rule for drug loading, firstly drug was dissolved in oil at 35°C–40°C. Upon dissolution of the drug in a carrier oil, preheated Span 80 and Tween 80 were added one by one at 35°C–40°C under unidirectional vigorous agitation using a vortex mixer until a homogeneous product formed.[26,27]

Drug content estimation

Sesamol from preweighed SNEDDS was extracted by dissolving in 20 mL chloroform. Sesamol content was analyzed for drug quantification using a UV-Visible spectrophotometer at 294 nm.[26]

Cloud point

Optimized formulations were evaluated for cloud point value. Formulations were diluted with distilled water in the ratio of 1:100 w/v. Diluted formulations were placed in a water bath with a gradual increase (2°C/min) in temperature (from 25°C to 80°C). The temperature at which sudden appearance of cloudiness as seen visually was measured as the cloud point. To check the reproducibility of the experiment, diluted formulations were cooled and heated once again.[23-25]

Thermodynamic stability study

A thermodynamic stability study was done to find the instability of the system. Each selected Sesamol-loaded SNEDDS undergoes five heating-cooling cycles and freezes–thaw cycles.[26]

In vitro drug release studies

The *in vitro* release study of optimized SNEDDS was performed using USP dissolution apparatus Type II (Shimadzu). Formulations were filled in a dialysis bag and used for the study. The performance of the developed formulation was compared with pure drugs. The study was conducted in simulated gastric fluid and simulated intestinal fluid (pH 6.8 buffers). The samples were withdrawn at 10, 20, 30, 40, 50, 60, 120, and 240 min, and the drug content was estimated using a UV-Visible spectrophotometer at 294 nm. The study was conducted in triplicate.[27,28]

Accelerated stability study

Stability studies of the optimized formulations were carried out for 6 months under three different storage conditions, namely refrigerator (4°C ± 2°C), room condition (25°C ± 2°C and 60% ± 5% RH), and humidity chamber (40°C ± 2°C and 75% ± 5% RH). The samples were analyzed for their drug content after the specific time intervals of 0, 0.5, 1, 2, 3, and 6 months.[29]

Transmission electron microscopy

Emulsion globules of optimized Sesamol SNEDDS formulations (phase code IPM 05 and CCTG 09) were visually observed by negative staining electron microscopy using a freshly glow discharged carbon-coated grid of 400 mesh.

Screening for antioxidant activity by 2,2′-diphenyl-1-picrylhydrazyl method

The antioxidant potential of Sesamol nanoformulation was assessed by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay. In this assay when DPPH reacts with an antioxidant compound, which can provide hydrogen, it is reduced. This can be observed by a color change from deep violet to light yellow. Different concentrations of the test sample (1 ml) were added to 1 ml of a methanolic solution containing DPPH. The resulted mixture was vigorously agitated and allowed to stand for 30 min. After that, absorbance was taken at 517 nm using a UV-Visible spectrophotometer.[30] Ascorbic acid was used as the reference standard. The radical scavenging activity (RSA) was estimated as the % of DPPH decolorization, using the equation:

% of DPPH decolorization = (Absorbance of standard-Absorbance of sample/Absorbance of standard) ×100

The results were obtained from the average of three independent experiments and are expressed as mean % RSA ± standard deviation and as mean IC_{50} value.

Cell viability assay

3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed to determine cell viability after exposure of cancer cells (MCF-7 cells) to finalized Sesamol nanoformulation i.e., SNEDDS. Varying concentrations of the formulation were added to a 96-well plate containing about 10,000 cells/100 ml/well and incubated for 24 h. In each well, 20 ml of MTT solution (5 mg/ml in phosphate-buffered saline [PBS]) was added and further incubated for 2–3 h at 37°C. After centrifugation at 2500 rpm, the supernatant was removed and 100 ml of dimethylsulfoxide was added into each well, incubated for 15 min, and analyzed spectrophotometrically at 517 nm.[31] The antineoplastic effectiveness of Sesamol nanoformulation was compared with standard marketed preparation (i.e., Tamoxifen tablet).

Reactive oxygen species activity assay

Reactive oxygen species (ROS) generation in the MCF-7 cell line was studied by microscopic fluorescence imaging by subjecting to various concentrations of Sesamol. Cells were seeded as described above for the MTT assay. Cells were then exposed to 20 mM, 40 mM, and 80 mM concentrations of Sesamol for 12 h and incubated with 2, 7-Dichlorodihydrofluorescein diacetate (DCFH-DA) (10 mM) for 30 min at 37°C. The reaction mixture was aspirated and replaced with 200 ml of PBS in each well. The plate was kept on the shaker for 10 min at room temperature in the dark. An inverted fluorescent microscope (Nikon ECLIPSE Ti-S, Japan) was used to visualize the intracellular fluorescence of cells and to capture images. For quantitative ROS analysis, cells (16104per well) were re-seeded in 96-well black bottom culture plate and allowed to adhere for 24 h in a CO_2 incubator at 37uC. Cells were incubated with DCFH-DA (10 mM) for 30 min at 37uC. Fluorescence intensity was measured by the multiwall microplate reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Values were expressed as the percentage of fluorescence intensity relative to the control wells.[30,31]

RESULTS

Solubility

The solubility of Sesamol was assessed in different oils alone and combination with surfactant and cosurfactant. The solubility of the Sesamol was found to be highest in triglycerides (CCTG and SO) and fatty esters (IPM and IPP) when compared to mineral oil (paraffin oils).

Ternary phase diagram

Based on solubility studies, IPM, IPP, CCTG, and SO were selected for the oil phase, Span 80, and Tween 80 were selected as the surfactant, and PEG 400 was taken as cosurfactant for the construction of the ternary

phase diagram. Carrier oils and their RHLB values are presented in Table 1.

The ternary plot explored for self-emulsification of IPM/IPP and CCTG/SO is presented in Figure 1. Ternary plots were further explored with magnifying values using surfactant and cosurfactant. The area to be explored for nano-emulsification is demonstrated in Figure 2.

Identification of *in-situ* nano-emulsification

After the preparation of formulations, the nano-emulsification zone was identified by manual assessment through visual observation. The combinations were sorted based on the formation of nanoemulsion on mild agitation.

As illustrated from Table 2, the ternary phases identified as translucent were further studied for the effect of cosurfactant on the nanoemulsification of the formulation. For this purpose, PEG400 was used in various ratios with surfactant mix maintaining the concentration of carrier oil as constant. Results are depicted in Table 3.

The *in situ* nanoemulsification of these alternate combinations was studied further and sorted in the same way as described above. Transparent formulations (designated as Grade A) were subjected for further evaluation including Self-emulsification time, Particle size analysis, and Zeta potential measurement.

Measurement of self-emulsification time

As represented in Table 4, the range of self-emulsification of SNEDDS was found between 12.00 ± 1.00 s to 70 ± 1.00 s. Formulations taking less than 30 s for emulsification were subjected to further studies.

Emulsion droplet size analysis

Formulations having a particle size < 100 nm were rejected because with the decrease in droplet size the capability of drug loading decreases. The droplet sizes of formulations are given in Table 4 and demonstrated in Figure 3. After Sesamol was incorporated, the droplet sizes of the optimized formulations increased. The zeta potential of optimized formulations is depicted in Figure 4. All the optimized formulations exhibited zeta potentials around-15 mV, which indicated the stability of SNEDDS.

Thermodynamic stability study

Each system was observed to be clear, without phase separation, creaming, cracking, and cloudiness at every freeze-thaw cycle. Thus, all the optimized formulations were stable.

Accelerated stability study

After an accelerated stability study of 6 months, it was observed that there is no significant decrease in drug content. During the entire 6 months, all the formulations showed desirable stability.

In vitro drug release study

As illustrated from Figure 5, IPM 05 formulation showed maximum drug release as compared to pure drug and other optimized formulations at 240 min. It also follows a controlled release pattern during drug release.

Table 1: Carrier oils and their required hydrophilic lipophilic balance values

Carrier oils	RHLB value
CCTG	11.0
Sesame oil	7.0
IPM	11.5
IPP	11.5

RHLB: Required hydrophilic lipophilic balance, CCTG: Caprylic capric triglyceride; IPM: Isopropyl myristate; IPP: Isopropyl palmitate

Table 2: Screening of combinations based on the formation of nano-emulsion on mild agitation

Appearance	Designation	Phase code
Transparent	Grade A	IPM05, IPM09, IPM14 IPP09, IPP14, IPP20 CCTG09
Translucent	Grade B But to be further studied with a cosurfactant	IPM20 IPP05 CCTG08 SO08
Opaque	Grade C	-
Transparent with oil separated	Grade D	-

Table 3: Effect of cosurfactant on the nano-emulsification of the formulation

Appearance	Designation	Phase code
Transparent	Grade A	IPM20 (80:20) IPP05 (60:40), IPP05 (40:60) CCTG08 (80:20), CCTG08 (60:40) SO08 (80:20)
Opaque	Grade B	-

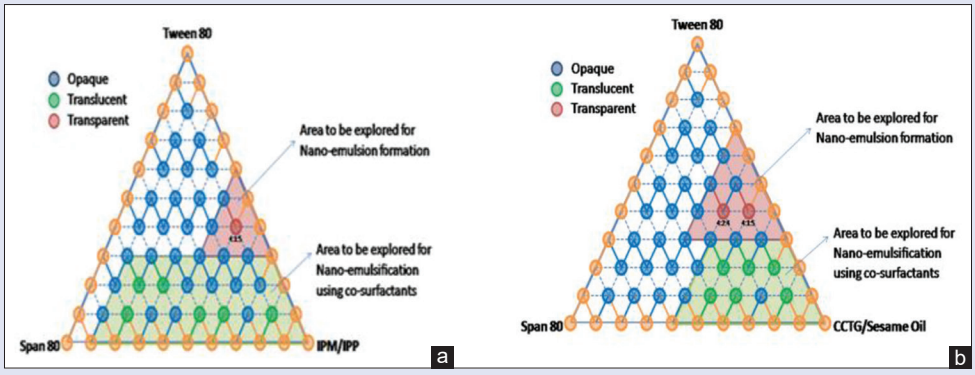

Figure 1: Ternary plot showing area of Isopropyl myristate/Isopropyl palmitate and Caprylic Capric triglycerides/Sesame oil which shows area of self-emulsification and area to be explored for self-emulsification

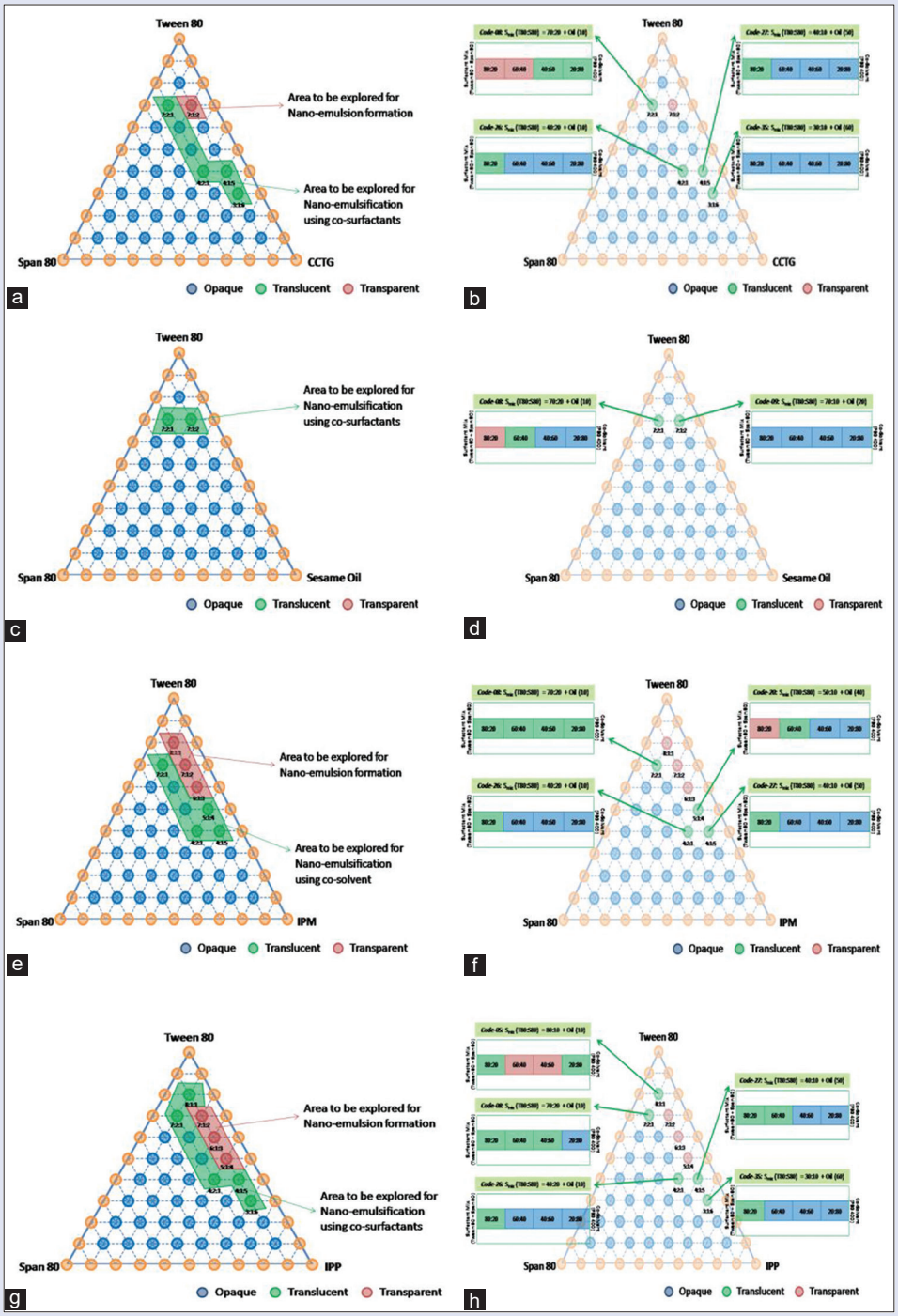

Figure 2: Ternary plots explored further with magnifying values using surfactant and cosurfactant in depicted in (a) Caprylic capric triglycerides, (c) Sesame, (e) Isopropyl myristate, (g) Isopropyl palmitate, and (b, d, e, f and h) shows area to be explored for nanoformulation using poly-ethylene glycol-400 as cosurfactant using fixed mix and cosurfactant ratio (20:80, 40:60, 60:40, and 80:20)

Transmission electron microscopy

TEM images of formulation demonstrate the spherical nature of SNEDDS as shown in Figure 6. The mean diameter of the particle was approximately 116.5 nm. IPM 05 phase code showed more appropriate results as compared to CCTG 09. IPM 05 phase code has globule more spherical as compared to CCTG 09 which showed that free drug is present in very less amount in IPM 05. Hence, IPM 05 was further characterized based on a cell line study.

Antioxidant activity by 2,2'-diphenyl-1-picrylhy drazyl assay

As depicted in Figure 7, the DPPH study showed that there is no significant difference between Sesamol nanoformulation and ascorbic acid.

Cell viability and alteration in cells morphology

The MTT data showed in Figure 8 illustrated that with increasing concentration of Sesamol nanoformulation, percentage cell viability

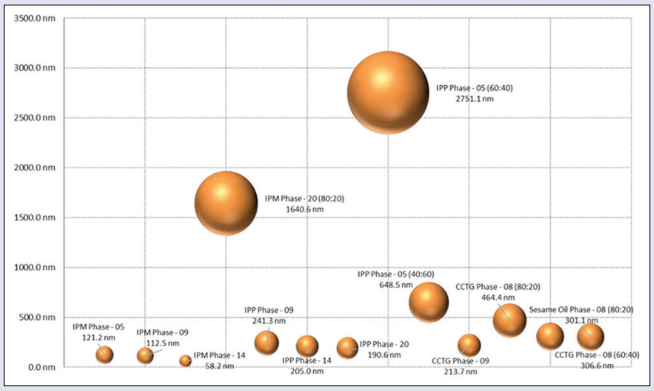

Figure 3: Droplet size of optimized formulation

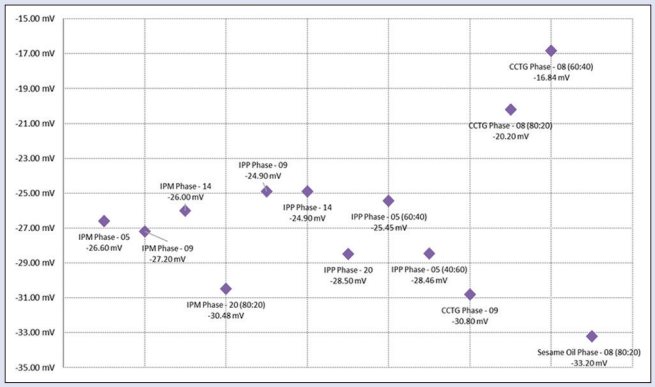

Figure 4: Zeta potential of optimized formulations

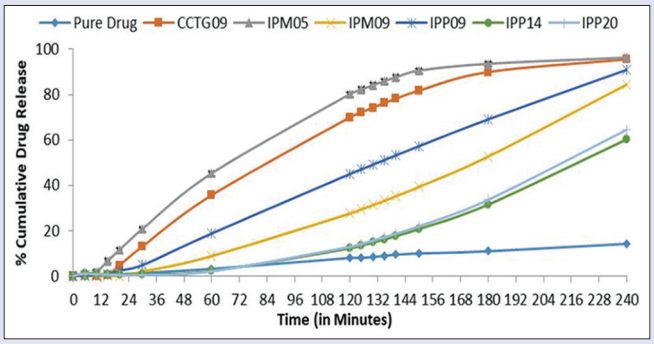

Figure 5: Cumulative drug release profile of optimized formulations

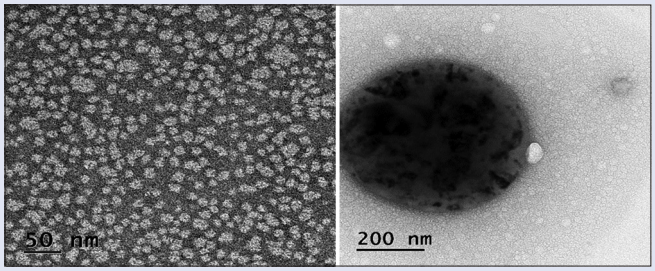

Figure 6: TEM images of the optimized self nanoemulsifying drug delivery system formulation Isopropyl myristate 05

decreases. As compared to control, the test and standard showed a significant difference ($P < 0.05\%$). However, test and standard had no significant difference. Cell morphology images [Figure 9] also revealed the same pattern. No death of cell has been observed in control whereas Sesamol nanoformulation (test) and Tamoxifen standard formulation showed comparable results. The cytotoxicity test indicates the potential of Sesamol SNEDDS formulation in inhibiting the growth of the test cells.

Reactive oxygen species activity assay

As illustrated from Figure 10a and b data, Sesamol formulation at 20, 40, and 80 µg/ml doses significantly enhanced the ROS intensity in a dose-dependent manner in respect to the untreated cells in MCF-7 cells of breast cancer. The quantitative percentage of DCF fluorescence demonstrated that Sesamol formulation at 20, 40, and 80 µg/ml concentrations were encouraging ($P < 0.05$) as compared to untreated MCF-7 cells.

DISCUSSION

Sesamol is a natural phenolic compound and a major lignan isolated from sesame seeds (*S. indicum*) and SO.[10] The therapeutic potential of Sesamol was investigated intensively, and there is compelling evidence that Sesamol acts as a metabolic regulator that possesses antioxidant, antimutagenic, and chemopreventive properties. Various studies have reported that Sesamol exerts potent anticancer effects.[8] Hence, in this modest attempt, the potency of a self-nanoemulsifying drug delivery system was successfully investigated to provide an effective system for the delivery of Sesamol. Developed Sesamol-loaded SNEDDS was studied for *in vitro* cytotoxic effect toward breast cancer cell line (MCF-7).

Table 4: Phase code isopropyl myristate 05, isopropyl myristate-09, isopropyl palmitate-09, isopropyl palmitate-14, isopropyl palmitate-20, and caprylic capric triglyceride 09 has been selected for drug loading based on droplet size and time of emulsification

Oil	Phase code	Droplet size (nm)	Time of emulsification (s)
IPM	Phase - 05	121.2	16±1.00
	Phase - 09	112.5	15±1.00
	Phase - 14	58.2	12±1.00
	Phase - 20 (80:20)	1640.6	50±1.00
IPP	Phase - 09	241.3	26±1.00
	Phase - 14	205.0	20±1.00
	Phase - 20	190.6	17±1.00
	Phase - 05 (60:40)	2751.1	70±1.00
	Phase - 05 (40:60)	648.5	38±1.00
CCTG	Phase - 09	213.7	24±1.00
	Phase - 08 (80:20)	464.4	34±1.00
	Phase - 08 (60:40)	306.6	29±1.00
Sesame oil	Phase - 08 (80:20)	301.1	29±1.00

CCTG: Caprylic capric triglyceride; IPM: Isopropyl myristate; IPP: Isopropyl palmitate

For formulating SNEDDS, the solubility of the drug in different oils is an essential step for the nanoemulsion formulation. So before starting the phase diagram, one must have to select the oil, surfactant, and cosurfactant in which the drug shows maximum solubility, to be in the desired solubility range.[15,16] The solubility of Sesamol was assessed in different oily phases alone and combination with surfactant and cosurfactant. The solubility of the Sesamol was found to be highest in IPM, IPP, CCTG, and SO. The surfactant chosen must be able to lower interfacial tension to a very small value to aid the dispersion process during the preparation of the nanoemulsion.[17] Safety is a major determining factor in choosing a surfactant as large amounts of

surfactants may cause GI irritation. Nonionic surfactants are less toxic than ionic surfactants. An important criterion for the selection of the surfactants is that the required HLB value. The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water.[17,19,20] Span 80 and Tween 80 were selected because these are liquid at room temperature, easily processable, easily available, and are less costly. The HLB value of Span 80 is 4.3 whereas, for Tween 80, it is 15.[18] Therefore, these two surfactants are effectively covering the required HLB of all oil carrier candidates. Transient negative interfacial tension and the fluid interfacial film are rarely achieved by the use of a single surfactant, usually necessitating the addition of a cosurfactant. The presence of cosurfactants decrease the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsion over a wide range of composition. PEG-400 was chosen as a cosurfactant.[16,20]

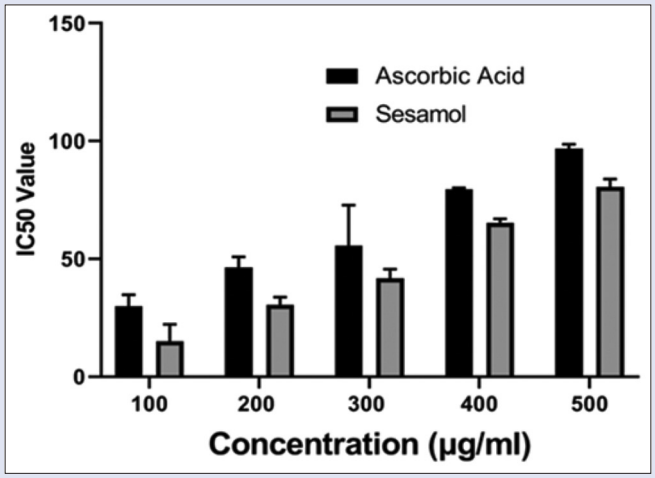

Figure 7: Figure demonstration IC 50 value of test and standard at different concentrations. Statistical analysis at ($P < 0.05$) shows that there is no significant difference

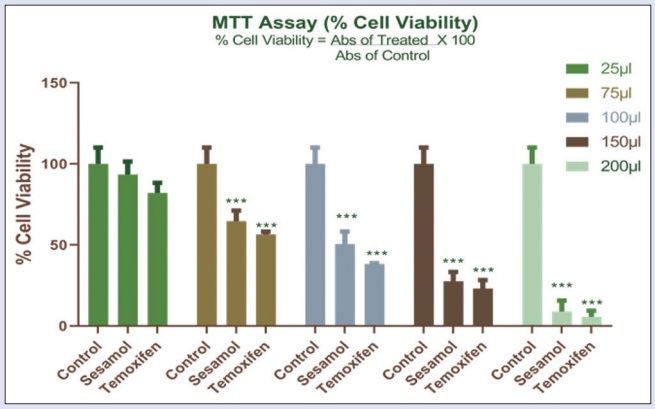

Figure 8: Histogram demonstrating cell viability of control, Sesamol, and Tamoxifen (Standard) formulations at different concentrations on MCF-7 cell lines

SNDDS were prepared by phase titration method with the optimized composition of the oil phase, S_{mix} (surfactant and cosurfactant mixture), and deionized water as an aqueous phase. The pseudo-ternary phase diagrams were drawn to determine the S_{mix} ratio and its ratio with the oil phase, which provides the region for the development of suitable SNEDDS. After taking an observation, pseudo ternary phase diagrams were constructed based on the observations marked during titration.[22-25] Phase diagrams were constructed separately for each ratio of S_{mix} prepared so that o/w nanoemulsion regions could be identified. The ternary plots were explored for self-emulsification of IPM/IPP and CCTG/SO [Figure 1]. Ternary plots were further explored with magnifying values using surfactant and cosurfactant [Figure 2]. After building the backbone of the nanoemulsion delivery system, different formulations were selected at a different point from the phase diagram justifying the drug dose considering the drug solubility in the oils phase.[25,27] All formulations were evaluated based on self-emulsification efficacy, droplet size, zeta potential, thermodynamic stability, surface morphology, *in vitro* release studies of optimized Sesamol-loaded SNEDDS, antioxidant activity by DPPH assay, *in vitro* cell viability and alteration in cells morphology, and intracellular ROS production in MCF-7 cells.

Self-emulsification of the formulation is among the most important attributes for the design of this formulation. After intake, the formulation should undergo emulsification on its own with the gastric fluid without getting precipitated.[18] Self-emulsification measurement helps in assessing the emulsification efficiency of the formulations. It also regulates the rate of release of the drug. Formulations taking <30 s for emulsification were subjected to further studies.[26]

Droplet size is the most important attribute in the case of emulsion, as reduced droplet size increases the stability of the emulsion, the solubility of the drug and is a necessity for increasing the bioavailability of the drug. Small droplet size also increases the interfacial surface area which results in fast drug absorption.[25] The droplet size for the optimized formulation should range between 100 nm and 250 nm. Formulations having a particle size <100 nm were rejected because with the decrease in droplet size the capability of drug loading decreases. Zeta potential helps in identifying charges on oil globules in an emulsion. Greater zeta potential value (negative or positive) indicates strong repulsive forces between the globules which prevents the coalescence of globules.[24,27] All selected formulations exhibited zeta potentials in the range, which signify the stability of SNEDDS.

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant, and water, with no phase

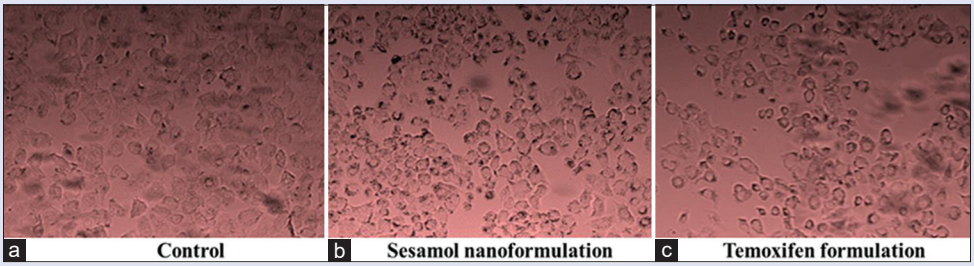

Figure 9: Figure (a, b and c) demonstrating cell viability at $P < 0.05$ of Sesamol and Tamoxifen (standard formulation)

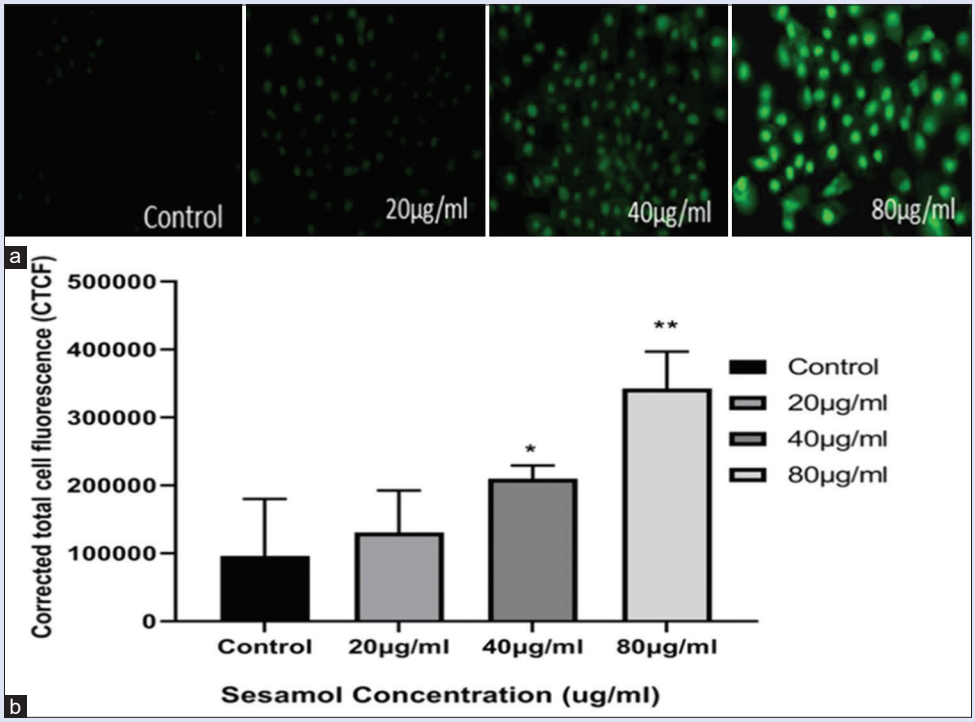

Figure 10: Sesamol induces intracellular reactive oxygen species in MCF-7 cells (a) Photomicrograph showing intracellular reactive oxygen species production in MCF-7 cells after being treated with selected effective doses, namely 20, 40, and 80 µg/ml of Sesamol formulation. (b) Histogram displaying the percentage of intracellular Reactive oxygen species generation at 20, 40, and 80 µg/ml of Sesamol formulation in MCF-7 cells

separation, creaming, or cracking. Thus, the selected formulations were subjected to different thermodynamic stability by using heating-cooling cycle, centrifugation, and freeze-thaw cycle stress tests. All the optimized formulations were cleared by the stress test.[26]

From the *in vitro* drug release study, it was observed that fatty esters are more capable of entrapping drugs and facilitating dissolution. However, CCTG 09 showed comparative dissolution to IPM05. Formulations with a higher concentration of tween 80 and a lesser concentration of span 80 results in a better drug release pattern. Both IPM05 and CCTG09 have tween 80 at a concentration of 80% and 70%, respectively, whereas both have span 80 at a level of just 10%.[27,28] Data obtained from the stability studies also showed that the selected SNEDDS remains stable over 6 months of storage period at 25 ± °C/60 ± 5% RH, as there was no phase separation or creaming seen in the SNEDDS ($P > .05$), respectively.[29]

The antioxidant potential of Sesamol nanoformulation was assessed by DPPH assay. Based on the data treatment, i.e., calculation of IC_{50} values and comparison with ascorbic acid, Sesamol was found to be an efficient scavenger and pointing toward the potential of Sesamol to be developed as a possible therapeutic.[30]

The cellular morphology and MTT cell viability assay show that the control cells experienced no toxicity and remained healthy. The cells treated with Sesamol-loaded SNEDDS experienced morphological changes as revealed by the photomicrograph [Figure 9]. The study suggested that Sesamol loaded-SNEDDS potentially inhibits the MCF-7 cells proliferation in a dose-dependent manner.[31]

The ROS production plays a significant role in the apoptosis-induced cell death and, therefore, DCFH-DA staining was performed to determine the level of oxidative stress in MCF-7 cells. ROS are responsible for the destabilization of mitochondrial membrane and activation of signal molecules that trigger the discharge of apoptotic cells and the resultant increase in the cytotoxic effect. The study findings appeared to be closely relevant to our findings [Figure 10]. The augmentation in fragmentation of apoptotic nuclei and their accumulation in intracellular ROS production showing that developed SNEDDS have potent efficacy against (MCF-7 cells) human breast cancer, without damaging normal cells.[30,31]

CONCLUSION

The optimized formulation containing Sesamol as a drug and Span 80 and Tween 80 as surfactant and cosurfactant were successfully developed with an increased solubility and dissolution rate. *Ex vivo* studies and cytotoxicity assay proven that Sesamol-loaded SNEDDS had better permeation and potential anticancer efficacy against MCF-7 cells human breast cancer due to higher bioavailability and greater penetration.

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Conflicts of interest

There are no conflicts of interest.

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