RESEARCH
www.commprac.com
ISSN 1462 2815

DESIGN AND EVALUATION OF LIPOSOMAL CARRIERS FOR TARGETED DELIVERY OF siRNA IN CANCER THERAPY

Bikramaditya Mukherjee [1], Vipender Pratap Singh [2], Lalatendu Mohanty [3], Ranadheer Reddy Challa [4], Santosh Kumar Ranajit [5], Neelam Basera [6], Sonika Kalia [7] and Farah Deeba [8]*

[1] Associate Professor, Department of Biochemistry, Manipal Tata Medical College, Manipal Academy of Higher Education, Manipal, India.
[2] Postgraduate Juniour Resident, Clinical Biochemistry Department Uttar Pradesh University of Medical Sciences, Saifai, Etawah.
[3] Assistant Professor, Department of Pharmaceutical Sciences, HNB Garhwal University, Chauras Campus, PO-Kilkileswar, Via Kirtinagar, Uttarakhand.
[4] Research Scholar, Department of Pharmaceutical Sciences, School of Applied Sciences and humanities, Vignan's Foundation for Sciences, Technology and Research, Vadlamudi, Andhra Pradesh, India.
[5] Associate Professor, Centurion University of Technology and Management, Bhubaneswar, 752050, Odisha.
[6] Assistant Professor, School of Agriculture Graphic Era Hill University, Clement Town, Dehradun Uttarakhand Pincode- 248002.
[7] Assistant Professor, Department of Biotechnology, School of Applied and Life Sciences, Uttaranchal University, Dehradun, 248001.
[8] Assistant Professor, School of Pharmacy, Plot no 32, Sharda University, 34, Knowledge Park III, Greater Noida, Uttar Pradesh, India.
*Corresponding Author Email: farahdeeba..r@gmail.com

DOI: 10.5281/zenodo.13622770

Abstract

Precision medicine has advanced significantly with the design and testing of liposomal carriers for the targeted administration of small interfering RNA (siRNA) in cancer therapy. In light of the introduced exploratory information and ends, this study focused on the creation and portrayal of cationic liposomes containing 3,4-DMA lipids for productive siRNA transport and quality silencing in cancer cells. Liposomes were tried for size, zeta potential, polydispersity index (PDI), and DOTAP, Chol, and PEG colipids using electrophoretic light scattering and dynamic light scattering. In request to determine which lipoplex formulations were best at complexing with siRNA, we contrasted them with controls made with Lipofectamine 2000. These formulations were viewed as powerful in gel impediment and intracellular take-up in MDA-MB-231 cells. The liposomes' selectivity and biocompatibility towards cancer cells when contrasted with ordinary HEK 293 cells were affirmed by cytotoxicity study. Remarkably, these investigations showed how well C12-DMA liposomes containing PEG might advance the quietness of the surviving quality, a protein related with cancer cell endurance. These results suggest that the developed formulations may find application in targeted gene therapy, opening up new options for the development of cancer treatment strategies. In general, this study offers encouraging perspectives on the potential of liposomal carriers as a strong platform for targeted administration of siRNA in cancer therapy, opening the door to more efficient and individualized therapeutic approaches.

Keywords: Liposomal, SIRNA, Cancer, Therapy, DOTAP, Chol, Peg, Colipids, C12-Dma.

1.INTRODUCTION

Cancer, being one of the leading global killers, necessitates a never-ending quest for better treatments. Small interfering RNA (siRNA) has become a promising technology for gene silencing among the many ways since it may specifically target and downregulate the expression of oncogenes [1]. However, a number of issues, such as low bloodstream stability, restricted cellular uptake, and off-target effects that may cause inadvertent gene silence, make the therapeutic application of siRNA difficult [2].

The creation of effective distribution methods that are specifically targeted is essential to overcoming these challenges. Liposomes, which are spherical vesicles made of bilayers of phospholipid, have drawn a lot of interest as possible siRNA delivery vehicles [3]. Their power to modify the surface, biocompatibility, and ability to encapsulate hydrophilic and hydrophobic compounds make them excellent choices for targeted delivery systems [4].

Liposomes can be made to specifically detect and bind to receptors overexpressed on cancer cells by adding targeting ligands to their surface [5]. This makes it easier to target the tumor spot with siRNA [6]. The therapeutic efficacy of siRNA is enhanced while minimizing its adverse effects on healthy tissues by narrowing its focus to specific targets [7]. This study's main goal is to develop and assess liposomal carriers for the targeted administration of siRNA in cancer treatments.

For siRNA to be released under control and with great encapsulation efficiency and stability, liposomal formulations must be methodically optimized [8].The liposomes' physicochemical characteristics, which include their shape, surface charge, and particle size, are carefully defined because they are important factors that affect how well they function biologically. Moreover, liposome surface modification using targeting ligands being investigated to improve the liposomes' selectivity for cancer cells.

1.1 Background and Importance of Cancer Therapy

As the primary cause of morbidity and death worldwide, cancer continues to be one of the most urgent health issues [9]. Millions of new cases and fatalities from cancer are recorded each year, despite notable advances in medical research and technology. Conventional cancer therapies, such as radiation therapy, chemotherapy, and surgery, frequently have significant drawbacks, such as non-specific targeting, serious side effects, and the possibility of developing resistance. These drawbacks underscore the pressing need for novel therapeutic approaches that can offer more tailored and efficient treatment choices. Meeting this requirement is essential for raising patient survival rates and improving the lives of people afflicted with this crippling illness. Consequently, a key area of attention in the fight against cancer is the development of innovative, targeted, and less harmful medicines.

1.2 Potential of siRNA in Cancer Therapy

Small interfering RNA, often known as siRNA, is an essential component in the process of gene silence. It accomplishes this by destroying messenger RNA (mRNA) molecules, which in turn prevents the creation of particular proteins. Through the use of this technique, siRNA is able to accurately target and downregulate oncogenes. As a result of its specificity, siRNA is an effective instrument for selectively reducing the expression of genes that are associated with cancer without having an impact on healthy cells [10-14].

However, the therapeutic application of siRNA confronts a number of problems, such as its instability in the circulation, limited cellular uptake, and the danger of off-target effects, which can result in the silencing of genes that were not meant to be silenced. Getting over these obstacles is absolutely necessary in order to fully use the therapeutic potential of siRNA in the treatment of malignant malignancy.

2. MATERIALS AND METHODS

2.1 Materials

We bought the reagents as a whole and used them straightforwardly from the jug without filtering them. Things like fluorescent siRNA, human lasting siRNA, agarose, MTT tone, and penicillin/streptomycin hostile to microbial were obtained from Sigma-Aldrich in St. Louis, Missouri, US of America.

Alongside diethyl decarbonate (DEP), the following synthetics were additionally obtained from Sigma: L-glutamine, 3-(4,5-dimethylthiazol-2-ly)- 2,5-diphenyl-tetrazolium bromide (MTT), and an antimicrobial arrangement including penicillin G, streptomycin, and amphotericin B. The following ingredients were obtained completely from Avanti Polar Lipids, Inc. of Alabama, USA: 18:1 TAP, otherwise called dioleoyl-3-trimethylammonium propane (DOTAP), polyethylene glycol (PEG), and cholesterol (Chol). These high-performance liquid chromatography (HPLC) solvents were bought from S.D. Fine Manufactured Synthetics Ltd. of Mumbai, India. They comprised of ethyl acidic corrosive deduction, dichloromethane (DCM), dimethyl sulfoxide (DMSO), and sodium sulfate (Na2SO4). These fabricated materials were used for the reasons for the purging and workup processes.

2.2 Synthesis

The combination of the specified synthetic compounds required two cycles. You may find range information and detailed union instructions in Strengthening Materials. Complete examination of the composites was done using Fourier transform infrared (FTIR), hydrogen-1 centers nuclear alluring resonance (13C NMR), high-resolution mass spectroscopy (HRMS), and 1H nuclear magnetic resonance (NMR). The NMR spectra were affirmed using a Bruker 500 or 400 MHz framework (Bruker, Billerica, Mom, USA) using CDCl3 dissolvable. The outcomes were communicated in parts per million while comparing proton NMR compound developments with tetramethyl silane at a grouping of 0 ppm. To represent CDCl3 at 77.0 ppm, the carbon NMR compound movements were communicated as a ppm level.

The information was addressed using compound shift, assortment (s= singlet, d= doublet, dd= doublet of doublets, t= triplet, q= gathering of four), coupling constants (Hz), and combination.

The Exactive TM Orbitrap high-resolution mass spectrometer and Accela 600 UPLC framework were utilized by Ferro Fisher Logical of Houston, TX, USA, to record the HRMS information. The examples' liquefying points were estimated using a Polmon MP-96 Automelt softening point hardware, which is made by Polmon Instruments PVT LTD in Hyderabad, India. The proteins were separated by employing silica gel (100-200 lattice) section chromatography partition techniques. Infrared spectra were examined using an instrument made by Bruker (Billerica, Mama, USA) called an ALPHA FTIR spectrometer. Centimeters were used for the measurements of the thin film testing [15-58].

2.3 Plan, Method, and Spectral Information of Synthesized Cationic Lipids

2.3.1 The 3,4-DMA Lipid Synthesis Synthetic Scheme.

A grouping of synthetic cycles is utilized in the blend plan of 3,4-DMA lipid to fabricate the lipid's exceptional sub-atomic design. Picking and setting up the right beginning materials — which could incorporate unsaturated fats or their subsidiaries — is

normally the most vital phase simultaneously. These are then exposed to a controlled response with reagents like acyl chlorides or alkyl halides to deliver middle items. Purging techniques like recrystallization or segment chromatography are oftentimes utilized in ensuing stages to isolate and sanitize the expected lipid item. To check the construction and virtue of the integrated lipid, portrayal procedures including mass spectrometry and atomic attractive reverberation (NMR) spectroscopy are fundamental. The engineered method is expected to empower further uses of the 3,4-DMA lipid in different biomedical or specialized areas, as well as accomplishing high return while guaranteeing reproducibility and adaptability.

2.3.2 General Synthetic Methods for 3,4-DMA Lipid Synthesis

Shows for the Advancement of 3,4-DMA Lipid. Both Figure 1(a) and Figure 1(c) show the blend of Compound C. The $e procedure for developing medication C is displayed in Figure 1: Add (a) 1,4-DMA (1 mmol) and (b) potassium carbonate (4 mmol) to a 250 ml round-base cup that was at that point loaded up with ethyl acidic corrosive determination dissolvable (5 ml/gm). Mix the combination for 10 minutes at room temperature while the container was put in a torpid (N2 gas) climate. In this manner, 3 mmol of 1-bromo alkyne chain was introduced, and the blend was refluxed at 70°C for 48 hours. Following 48 hours, mass spectrometry was utilized to affirm the atomic weight and slender layer chromatography (tender loving care) was utilized to evaluate the response. (c) The blend was then weakened with water, vanished with dichloromethane, and got dried out with anhydrous sodium sulfate while under vacuum to eliminate any leftover solvents. From that point onward, methyl iodide and potassium carbonate were added, joined, and shaken for an entire day at room temperature. The response arrangement was inspected by tender loving care following 48 hours, and mass spectrometry was utilized to affirm the atomic weight. The combination was vacuum-vanished to eliminate any solvents and unreacted methyl iodide. The subsequent lipid was then separated with dichloromethane and weakened with water prior to being dried out in anhydrous sodium sulfate. The dissolvable had to dissipate utilizing a rotavap to create the last 3,4-DMA lipid quaternized item. The quarternized 3, 4-DMA lipid was filtered utilizing segment chromatography with ethyl acetic acid derivation/hexane filling in as the eluent. To depict this lipid, HRMS, IR, 13C NMR, and 1H NMR (remembered for the Benefit Data as scientific information 1.1) were employed.

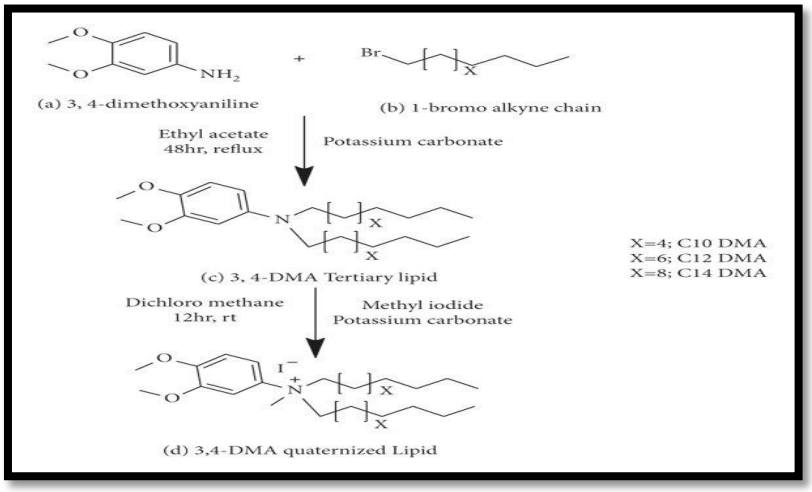

Figure 1: Plan for Producing Quaternized 3,4-DMA Lipid

2.4 The Creation of Formulations and Their Optimization

2.4.1 Preparation of Liposomes

- **Injectable Ethanol Process:** Liposomes containing 3,4-DMA lipid, PEG, DOTAP (dioleoyl-3-trimethylammonium propane), and cholesterol (Chol) were created using the ethanol infusion strategy. Ethanol was utilized to break up the 3,4-DMA lipid and cholesterol or lipid and PEG/DOTAP, which were then combined in 1:1 and 1:0.5 proportions, separately. To rapidly make liposomes, different lipid fixations (ranging from 1 mM to 0.1 mM) were blended in with deionized water. PEG was utilized with the DOTAP-liposome compound in a 1:0.5 proportion and cholesterol in a 1:1 proportion (compound to colipid).
- **Lipoplex Formation:** To construct lipoplexes, survivin siRNA was utilized. 50 ng of siRNA and 25 μM of liposomes were used for the investigations. A ZS90 model from Malvern, UK, was utilized to evaluate the size and zeta possibilities, with a liposome to siRNA charge proportion of 1:5. Shower sonication (POWERSONIC 405, New Delhi, India) was utilized to prevent liposome particles from clumping in request to ensure exact size examination.
- **Gel Retardation Assay:** Using a 2% agarose gel, the binding of siRNA with 3, 4-DMA liposomes was assessed. Using a 5:1 cationic lipid to siRNA proportion, 50 nanograms of siRNA were complexed with 25 μM DMA liposome in an all-out volume of 20 μL. For thirty minutes, the combination was incubated on a rotating shaker (Lab-200, Haryana, India). After the incubation period, each example got 2 μL of 6X loading color, and electrophoresis was run for 30 minutes at 100 V. Using a GelDoc Go Framework (Bio-Rad Research facilities, Hercules, CA, USA) with UV fluorescence identification and standard EtBr staining, the gel was shot.
- **Cell Culture and Compound Preparation:** The review's cell lines were procured from CSIR-IICT Hyderabad. Three cell lines were developed in DMEM (Himedia, Mumbai, India) enhanced with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 1% pen-strep anti-microbial arrangement, and human bosom cancer cell lines MDA-MB-231 and MCF7. The cells were kept in a 37°C climate with 5% CO2 and 95% mugginess. The compounds were synthesized in pure dimethyl sulfoxide (DMSO) and weakened in culture media, with working focuses ranging from 100 μM to 1000 μM and a stock centralization of 10 mM.

2.5 Cytotoxicity Studies

The MTT examine was utilized to assess the treated cell lines' essentialness. 5000 cells were refined per well in 96-well plates for the cytotoxicity tests. For 48 hours, the cells were exposed to various dosages of 3,4-DMA liposomes, ranging from 10 μM to 1 nM. Following the treatment period, each very much was loaded up with 90 μL of new sans serum medium and 10 μL of MTT reagent (5 mg/mL), and the plates were incubated for four hours at 37°C. Subsequent to adding 200 μL of DMSO to each well, the combination was incubated for ten minutes at 37°C. Absorbance at 570 nm was estimated using a Cooperative energy H1 Crossover Multi-Mode Peruser (BioTek Instruments, Winooski, VT, USA).

2.6 Intracellular Uptake Study

Using a 40x amplification confocal magnifying lens (model: FV1000, M/S Olympus, India), the intracellular take-up of siRNA by the cells was investigated. Six-well plates

were cultivated with generally 10^4-10^5 cells on coverslips covered with poly-L-lysine. Fluorescent siRNA alone, liposome + fluorescent siRNA, and Lipofectamine 2000 + fluorescent siRNA (10 µL for 50 nM of siRNA, according to maker's instructions) were the three circumstances that were examined. Following therapy, 4% PFA was utilized to fix the cells, and blue DAPI staining was applied for 20 minutes to highlight the cores. Red Cy5 was utilized to stamp the siRNA present in the cells. Using mounting media, coverslips were appended to glass slides and fixed with paraffin wax.

2.7 In Vitro Gene Silencing Efficiency

Using Western blotting, the viability of quality silencing was evaluated in vitro. Proteins can be isolated with this innovation according to their charge, size, and different properties. 10^5 to 2x10^5 cells were placed into each well of six-well cell culture plates. Different ideal amounts of lipoplexes were applied to the cells, which were created by combining 25 µM of liposome with 50 nM of siRNA at a 1:5 nitrogen/phosphate (N/P) charge proportion. Specifically, the treatments involved:

- (A) C12: Chol (1:1) + siRNA
- (B) C12: DOTAP: Chol (1:1:1) + siRNA
- (C) C12: DOTAP (1:1) + siRNA
- (D) Lipofectamine 2000 + siRNA
- (E) C12: PEG (1:0.5) + siRNA

Besides, a Lipofectamine: siRNA control was included, in which 10 µL of Lipofectamine was used for 50 nM of siRNA. Using ImageJ software (NIH, Bethesda, MD, USA), the thickness investigation of the Western smudge bands was done to evaluate the results.

3. DATA ANALYSIS AND RESULTS

3.1 Zeta Potential, Particle Size, and Polydispersity Index (PDI).

A Malvern Zetasizer (model: ZS90, Malvern Panalytical, Malvern, UK) was utilized to survey the size and charge of self-collected 3,4-DMA lipoplexes carrying siRNA. Photon connection spectroscopy and dynamic light scattering were utilized all the while. Tables 1-3 in the Beneficial Materials contain definite outcomes for every one of the three lipoplex formulations, along with matching size and charge diagrams in Figures S13 through S20.

Table 1: C10 3,4-DMA lipoplex size, charge, and PDI (C10 DMA liposome: siRNA).

Lipoplex Formulation	Size (nm)	PDI (%)	Zeta Potential (mV)
C10 : DOTAP (1:1)	511.3	29.3	57.2
C10 : DOTAP (1:1:1)	344.8	25.7	40.6
C10 : Chol (1:1)	355.1	26.8	22.2
C10 : PEG (1:0.5)	419.5	27.9	26.7

Table 2: The C12 DMA liposome, often known as siRNA, has the following characteristics: size, charge, and PDI.

Lipoplex Formulation	Size (nm)	PDI (%)	Zeta Potential (mV)
C12 : DOTAP (1:1)	232.8	28.5	29.4
C12 : DOTAP (1:1:1)	277.4	22.7	51.3
C12 : Chol (1:1)	311.6	26.2	28.7
C12 : PEG (1:0.5)	291.8	27.8	29.5

Table 3: C14 M 3,4-DMA lipoplex size, charge, and PDI (C14 DMA liposome: siRNA).

Lipoplex Formulation	Size (nm)	PDI (%)	Zeta Potential (mV)
C14 : DOTAP (1:1)	244.9	25.9	28.3
C14 : DOTAP (1:1:1)	351.0	31.9	51.2
C14 : Chol (1:1)	322.5	7.8	44.6
C14 : PEG (1:0.5)	341.5	31.6	35.6

3.2 In Vitro Studies

3.2.1 Gel Retardation Assay

Ethanol infusion was utilized to produce cationic liposomes with C10-3,4-DMA, C12-3,4-DMA, and C14-3,4-DMA lipids using DOTAP, Chol, and PEG as co-lipids. During the production of liposomes, the ratio of synthetic to co-lipid was kept at 1:1. After that, these liposomes were combined with siRNA at a 5:1 charge ratio and incubated at 37°C for an hour in order to generate lipoplexes [10]. The best complex formation was achieved using C12-3,4-DMA liposomes at 25 μM with PEG and 50 ng of siRNA at a 5:1 charge ratio, as shown in Figure 2(b). In contrast, when 50 ng of siRNA was added to C10-3,4-DMA liposomes, they were unable to form complexes at 25 μM. Similarly, C12-3,4-DMA liposomes combined with PEG were able to successfully complex siRNA.

3.2.2 MTT Cell Cytotoxicity Assay

Additional cytotoxicity tests were carried out using C12-DMA including different colipids on MCF7, MDA-MB-231, and HEK 293 cell lines, after gel retardation testing showed encouraging results regarding the effectiveness of complex formation (see Figure 2). When these lipoplexes were used instead of just liposomes or siRNA, the viability of the cells was significantly increased. As controls, MCF7, MDA-MB-231, and HEK 293 cell lines underwent further cytotoxicity testing. Gel retardation experiments were used to determine the effective complexation of lipoplexes containing C12-DMA and various colipids. When compared to liposomes or free siRNA alone, these complexes showed better biocompatibility.

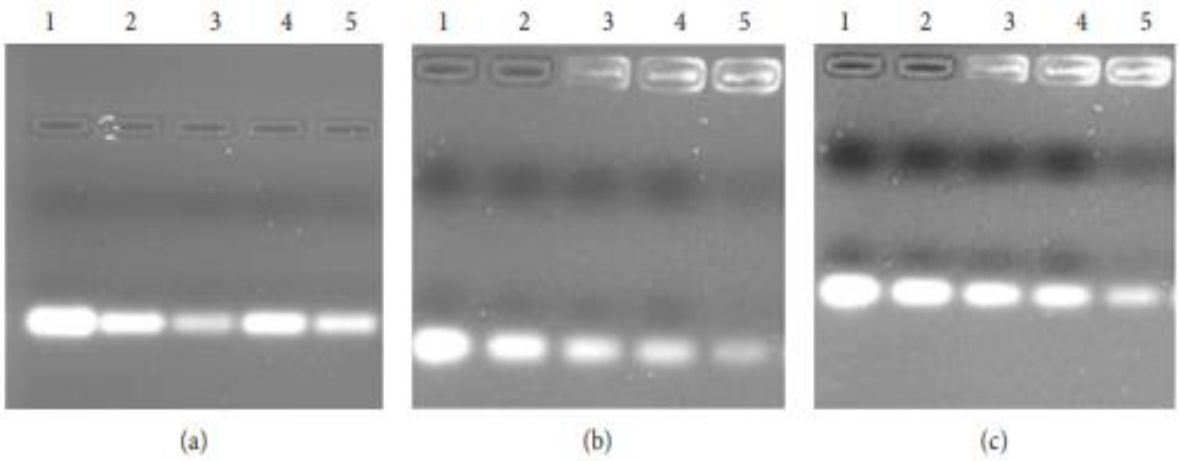

Figure 2: Profiles of the gel retardation assay for siRNA-containing lipoplex formulations (C10, C12, C14)

3.2.3 Intracellular Uptake Result

Figure 3 shows the intracellular uptake of siRNA by MDA-MB-231 cells based on confocal microscopy analysis. To create the lipoplex, a fluorescent siRNA solution (50 nM) was complexed with a C12-DMA liposome solution (25 μM). The siRNA was labeled with Cy-5, and DAPI was utilized as a nuclear counterstain. Remarkably, siRNA was successfully delivered into MDA-MB-231 cells at a concentration of 25 μM by the C12-DMA (1:0.5) liposomes without the need of lipofectamine or any other lipoplex agents. This indicates that siRNA was not taken up by the cells on their own.

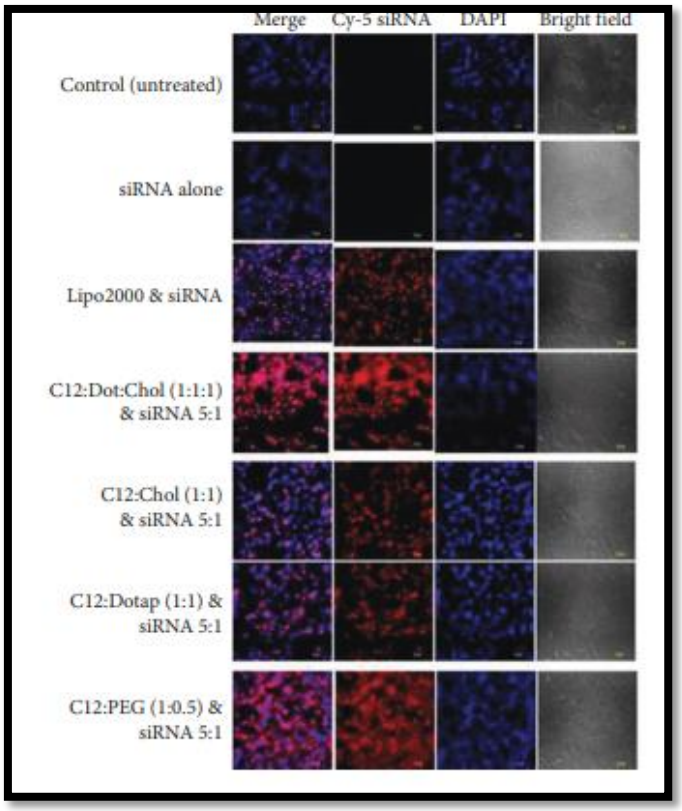

Figure 3: C12-DMA Cy-5siRNA Transfection Efficiency in Lipoplex vs. Lipo2000 Control on MDA-MB-231 Cells

3.2.4 Efficiency of In Vitro Gene Silencing.

The results of the Western blotting examination (refer to Figure 4) demonstrated a noteworthy decrease in survivin levels when several lipoplexes made with C12-DMA lipid, 25 µM of Speck, cholesterol (Chol), and PEG colipids, together with 50 nM of siRNA, were applied. In particular, MDA-MB-231 cells showed efficient inhibition of survivin expression when exposed to the C12-DMA (1:0.5) lipoplex.

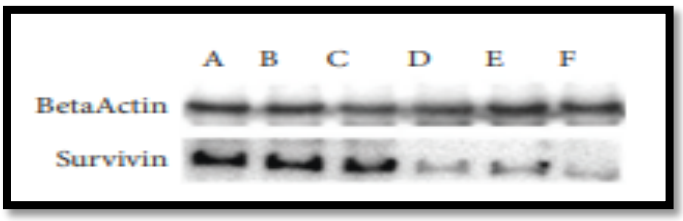

Figure 4: Western Blot: β-Actin-Based C12-DMA Lipoplexes vs Controls

Table 4: Findings from investigations on the cytotoxicity of several cell lines

Samples	Percent Viability (MDA-MB231)	Percent Viability (MCF7)	Percent Viability (HEK 293)
siRNA (50 nm)	99.25 ± 0.26	99.51 ± 0.19	98.71 ± 0.41
C12: DOTAP (1:1)	80.33 ± 0.71	88.95 ± 0.41	92.44 ± 0.18
C12: DOTAP (1:1:1)	89.44 ± 0.18	89.71 ± 0.18	96.51 ± 0.31
C12: Chol (1:1)	83.29 ± 0.86	90.95 ± 0.18	92.18 ± 0.33
C12 : PEG (1:0.5)	75.31 ± 0.53	95.77 ± 0.35	97.18 ± 0.20
C12: PEG (1:0.5) + siRNA (50 nm)	25.18 ± 0.59	32.61 ± 0.34	89.51 ± 0.29
C12: Chol (1:1:1) + siRNA (50 nm)	45.71 ± 0.41	51.86 ± 0.44	81.77± 0.34
C12: DOT:(1:1) + siRNA (50 nm)	51.31 ± 0.44	66.81 ± 0.15	82.31 ± 0.31
C12: Chol (1:1) + siRNA (50 nm)	58.99 ± 0.71	72.66 ± 0.18	83.31 ± 0.18
siRNA + lipofectamine 2000	65.44 ± 0.55	77.51± 0.31	71.68 ± 0.19

In request to find out how well C12 (1:0.5) liposomes conveyed surviving siRNA into bosom cancer cells, we contrasted them with Lipofectamine 2000. That's what the findings showed, in contrast with standard methods, C12-DMA liposomes containing PEG significantly increased the viability of intracellular siRNA administration. MTT tests checked the particular cytotoxicity of C12 (1:0.5) liposomes towards bosom cancer cell lines (MDA-MB-231 and MCF-7) over ordinary kidney cells (HEK 293), regardless of troubles in unambiguous cell targeting. Western smudge examination showed that C12-DMA liposomes stacked with DOTAP and cholesterol couldn't really diminish the declaration of surviving, though C12-DMA lipoplexes stacked with PEG were ready to bring down surviving levels in MDA-MB-231 cells, potentially making

them more defenceless to targeted apoptotic induction. These outcomes highlight the likely utility of C12-DMA liposomes as productive delivery vehicles for targeted siRNA in bosom cancer therapy.

4. DISCUSSION

After 3,4-DMA lipids were synthesized, characterized, and then liposome-formulated, important information about their potential as siRNA delivery vehicles became available. According to the results, the lipoplexes' size, zeta potential, and polydispersity index (PDI) were all within ranges that were ideal for efficient cellular absorption. With a size of 232.8 nm, a zeta potential of 29.4 mV, and a PDI of 28.5%, C12-DMA liposomes in particular showed ideal properties. Stable, consistently sized lipoplexes—which are essential for effective gene delivery—have these characteristics.

The effective complexation of siRNA with C12-DMA liposomes, especially when paired with PEG, was validated by the gel retardation test. The optimal complex formation and stability were demonstrated by this formulation, which is crucial for preventing siRNA degradation and guaranteeing its delivery to target cells. The lipoplexes' biocompatibility was confirmed by the MTT cytotoxicity assay, which demonstrated that at effective concentrations, they exhibited little toxicity towards both non-malignant (HEK 293) and carcinogenic (MCA-MB-231 and MCF7) cell lines. The effectiveness of C12-DMA liposomes in delivering siRNA into MDA-MB-231 cells was demonstrated by intracellular uptake assays conducted using confocal microscopy. Without the need for additional transfection agents like Lipofectamine 2000, the exact imaging made possible by the fluorescent tagging of siRNA confirmed its significant intracellular presence. This suggests that C12-DMA liposomes have the innate potential to promote siRNA release and cellular penetration.

The expression of survivin was significantly downregulated in MDA-MB-231 cells treated with C12-DMA lipoplexes, according to the results of the Western blot analysis for in vitro gene silencing efficiency. The efficacy of C12-DMA liposomes in delivering siRNA and accomplishing gene silencing is demonstrated by this outcome, which is important for therapeutic applications in the treatment of cancer. All things considered, the results indicate that C12-DMA liposomes, especially when prepared with PEG, offer a potential vector for siRNA administration in cancer treatment, combining biocompatibility, stability, and efficient gene silencing properties. To further investigate the safety and efficacy of these formulations in vivo, additional research is necessary.

5. CONCLUSION

liposomal carriers for targeted siRNA delivery in cancer therapy have shown great promise and problems. The development and testing of liposomal carriers for targeted siRNA delivery in cancer treatment has enhanced precision medicine. The experimental findings and conclusions led this work to produce and characterize cationic liposomes with 3,4-DMA lipids for cancer cell siRNA transport and gene silencing. Various lipoplex formulations were investigated for siRNA complexation against Lipofectamine 2000 controls. MDA-MB-231 cells showed gel retardation and intracellular absorption of various formulations. Cytotoxicity studies showed liposomes' selectivity and biocompatibility toward cancer cells over normal HEK 293 cells. This research demonstrated that C12-DMA liposomes with PEG can mute the

surviving gene, a cancer cell survival protein. These results show that targeted gene therapy may use the established formulations, expanding cancer treatment choices. This study suggests that liposomal carriers could be used to target siRNA in cancer therapy, enabling more efficient and personalized treatments. These findings emphasize the need of tailoring liposomal formulations for efficacy and safety, enabling targeted siRNA delivery to improve cancer therapy outcomes.

References

1) Abbasi, H., Rahbar, N., Kouchak, M., Khalil Dezfuli, P., & Handali, S. (2022). Functionalized liposomes as drug nanocarriers for active targeted cancer therapy: a systematic review. Journal of liposome research, 32(2), 195-210. https://www.tandfonline.com/doi/abs/10.1080/08982104.2021.1903035

2) Ashique, S., Almohaywi, B., Haider, N., Yasmin, S., Hussain, A., Mishra, N., & Garg, A. (2022). siRNA-based nanocarriers for targeted drug delivery to control breast cancer. Advances in Cancer Biology-Metastasis, 4, 100047. https://www.sciencedirect.com/science/article/pii/S2667394022000211

3) Chowdhury, N., Chaudhry, S., Hall, N., Olverson, G., Zhang, Q. J., Mandal, T., ... & Kundu, A. (2020). Targeted delivery of doxorubicin liposomes for Her-2+ breast cancer treatment. Aaps Pharmscitech, 21, 1-12. https://link.springer.com/article/10.1208/s12249-020-01743-8

4) Eloy, J. O., Petrilli, R., Raspantini, G. L., & Lee, R. J. (2018). Targeted liposomes for siRNA delivery to cancer. Current Pharmaceutical Design, 24(23), 2664-2672. https://www.ingentaconnect.com/content/ben/cpd/2018/00000024/00000023/art00004

5) Kabilova, T. O., Shmendel, E. V., Gladkikh, D. V., Chernolovskaya, E. L., Markov, O. V., Morozova, N. G., ... & Zenkova, M. A. (2018). Targeted delivery of nucleic acids into xenograft tumors mediated by novel folate-equipped liposomes. European Journal of Pharmaceutics and Biopharmaceutics, 123, 59-70. https://www.sciencedirect.com/science/article/pii/S0939641117306240

6) Kara, G., Calin, G. A., & Ozpolat, B. (2022). RNAi-based therapeutics and tumor targeted delivery in cancer. Advanced drug delivery reviews, 182, 114113. https://www.sciencedirect.com/science/article/pii/S0169409X22000035'

7) Khabazian, E., Vakhshiteh, F., Norouzi, P., Fatahi, Y., Dinarvand, R., & Atyabi, F. (2022). Cationic liposome decorated with cyclic RGD peptide for targeted delivery of anti-STAT3 siRNA to melanoma cancer cells. Journal of Drug Targeting, 30(5), 522-533. https://www.tandfonline.com/doi/abs/10.1080/1061186X.2021.1973481

8) Liang, Z., Du, L., Zhang, E., Zhao, Y., Wang, W., Ma, P., ... & Zhen, Y. (2021). Targeted-delivery of siRNA via a polypeptide-modified liposome for the treatment of gp96 over-expressed breast cancer. Materials Science and Engineering: C, 121, 111847. https://www.sciencedirect.com/science/article/pii/S0928493120337668

9) Mendes, L. P., Sarisozen, C., Luther, E., Pan, J., & Torchilin, V. P. (2019). Surface-engineered polyethyleneimine-modified liposomes as novel carrier of siRNA and chemotherapeutics for combination treatment of drug-resistant cancers. Drug delivery, 26(1), 443-458. https://www.tandfonline.com/doi/abs/10.1080/10717544.2019.1574935

10) Nikam, R. R., & Gore, K. R. (2018). Journey of siRNA: clinical developments and targeted delivery. Nucleic acid therapeutics, 28(4), 209-224. https://www.liebertpub.com/doi/abs/10.1089/nat.2017.0715

11) Obeid, M. A., Aljabali, A. A., Alshaer, W., Charbe, N. B., Chellappan, D. K., Dua, K., ... & Tambuwala, M. M. (2021). Targeting siRNAs in cancer drug delivery. In Advanced drug delivery systems in the management of cancer (pp. 447-460). Academic Press. https://www.sciencedirect.com/science/article/pii/B9780323855037000274'

12) Saraf, S., Jain, A., Tiwari, A., Verma, A., Panda, P. K., & Jain, S. K. (2020). Advances in liposomal drug delivery to cancer: An overview. Journal of drug delivery science and technology, 56, 101549 https://www.sciencedirect.com/science/article/pii/B9780323855037000274

13) Skupin-Mrugalska, P. (2019). Liposome-based drug delivery for lung cancer. In Nanotechnology-Based Targeted Drug Delivery Systems for Lung Cancer (pp. 123-160). Academic Press. https://www.sciencedirect.com/science/article/pii/B978012815720600006X

14) Subhan, M. A., & Torchilin, V. P. (2019). Efficient nanocarriers of siRNA therapeutics for cancer treatment. Translational Research, 214, 62-91. https://www.sciencedirect.com/science/article/pii/S1931524419301392

15) Yadav, D. N., Ali, M. S., Thanekar, A. M., Pogu, S. V., & Rengan, A. K. (2022). Recent advancements in the design of nanodelivery systems of siRNA for cancer therapy. Molecular Pharmaceutics, 19(12), 4506-4526. https://pubs.acs.org/doi/abs/10.1021/acs.molpharmaceut.2c00811

16) Bonlawar, J., Setia, A., Challa, R.R., Vallamkonda, B., Mehata, A.K., Vaishali, , Viswanadh, M.K., Muthu, M.S. (2024). Targeted Nanotheransotics: Integration of Preclinical MRI and CT in the Molecular Imaging and Therapy of Advanced Diseases. *Nanotheranostics*, 8(3), 401-426. https://doi.org/10.7150/ntno.95791.

17) Pasala, P. K., Rudrapal, M., Challa, R. R., Ahmad, S. F., Vallamkonda, B., & R., R. B. (2024). Anti-Parkinson potential of hesperetin nanoparticles: *in vivo* and *in silico* investigations. *Natural Product Research*, 1–10. https://doi.org/10.1080/14786419.2024.2344740

18) Suseela, M. N. L., Mehata, A. K., Vallamkonda, B., Gokul, P., Pradhan, A., Pandey, J., ... & Muthu, M. S. (2024). Comparative Evaluation of Liquid-Liquid Extraction and Nanosorbent Extraction for HPLC-PDA Analysis of Cabazitaxel from Rat Plasma. *Journal of Pharmaceutical and Biomedical Analysis*, 116149. https://doi.org/10.1016/j.jpba.2024.116149

19) Chakravarthy, P.S.A., Popli, P., Challa, R.R. *et al.* Bile salts: unlocking the potential as bio-surfactant for enhanced drug absorption. *J Nanopart Res* **26**, 76 (2024). https://doi.org/10.1007/s11051-024-05985-6

20) Setia, A., Vallamkonda, B., Challa, R.R., Mehata, A.K., Badgujar, P., Muthu, M.S. (2024). Herbal Theranostics: Controlled, Targeted Delivery and Imaging of Herbal Molecules. *Nanotheranostics*, 8(3), 344-379. https://doi.org/10.7150/ntno.94987.

21) Dhamija P, Mehata AK, Tamang R, Bonlawar J, Vaishali, Malik AK, Setia A, Kumar S, Challa RR, Koch B, Muthu MS. Redox-Sensitive Poly(lactic-*co*-glycolic acid) Nanoparticles of Palbociclib: Development, Ultrasound/Photoacoustic Imaging, and Smart Breast Cancer Therapy. Mol Pharm. 2024 May 5. doi: 10.1021/acs.molpharmaceut.3c01086. Epub ahead of print. PMID: 38706253.

22) Eranti, Bhargav and Mohammed, Nawaz and Singh, Udit Narayan and Peraman, Ramalingam and Challa, Ranadheer Reddy and Vallamkonda, Bhaskar and Ahmad, Sheikh F. and DSNBK, Prasanth and Pasala, Praveen Kumar and Rudrapal, Mithun, A Central Composite Design-Based Targeted Quercetin Nanoliposomal Formulation: Optimization and Cytotoxic Studies on MCF-7 Breast Cancer Cell Lines. Available at SSRN: https://ssrn.com/abstract=4840349 or http://dx.doi.org/10.2139/ssrn.4840349

23) Setia A, Challa RR, Vallamkonda B, Satti P, Mehata AK, Priya V, Kumar S, Muthu MS. Nanomedicine And Nanotheranostics: Special Focus on Imaging of Anticancer Drugs Induced Cardiac Toxicity. Nanotheranostics 2024; 8(4):473-496. doi:10.7150/ntno.96846. https://www.ntno.org/v08p0473.htm

24) Pasala, P. K., Rcaghupati, N. K., Yaraguppi, D. A., Challa, R. R., Vallamkond, B., Ahmad, S. F., ... & DSNBK, P. (2024). Potential preventative impact of aloe-emodin nanoparticles on cerebral stroke-associated myocardial injury by targeting myeloperoxidase: In Supporting with In silico and In vivo studies. *Heliyon*.

25) Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-s498.

26) Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).

27) Bhandari S, Chauhan B, Gupta N, et al. Translational Implications of Neuronal Dopamine D3 Receptors for Preclinical Research and Cns Disorders. *African J Biol Sci (South Africa)*. 2024;6(8):128-140. doi:10.33472/AFJBS.6.8.2024.128-140

28) Tripathi A, Gupta N, Chauhan B, et al. Investigation of the structural and functional properties of starch-g-poly (acrylic acid) hydrogels reinforced with cellulose nanofibers for cu2+ ion adsorption. *African J Biol Sci (South Africa)*. 2024;6(8): 144-153, doi:10.33472/AFJBS.6.8.2024.141-153

29) Sharma R, Kar NR, Ahmad M, et al. Exploring the molecular dynamics of ethyl alcohol: Development of a comprehensive model for understanding its behavior in various environments. Community Pract. 2024;21(05):1812-1826. doi:10.5281/zenodo.11399708

30) Mandal S, Kar NR, Jain AV, Yadav P. Natural Products As Sources of Drug Discovery: Exploration, Optimisation, and Translation Into Clinical Practice. African J Biol Sci (South Africa). 2024;6(9):2486-2504. doi:10.33472/AFJBS.6.9.2024.2486-2504

31) Kumar S, Mandal S, Priya N, et al. Modeling the synthesis and kinetics of Ferrous Sulfate production: Towards Sustainable Manufacturing Processes. African J Biol Sci (South Africa). 2024;6(9):2444-2458. doi:10.33472/AFJBS.6.9.2024.

32) Revadigar RV, Keshamma E, Ahmad M, et al. Antioxidant Potential of Pyrazolines Synthesized Via Green Chemistry Methods. African J Biol Sci (South Africa). 2024;6(10):112-125. doi:10.33472/AFJBS.6.10.2024.112-125

33) Sahoo S, Gupta S, Chakraborty S, et al. Designing, Synthesizing, and Assessing the Biological Activity of Innovative Thiazolidinedione Derivatives With Dual Functionality. African J Biol Sci (South Africa). 2024;6(10):97-111. doi:10.33472/AFJBS.6.10.2024.97-111

34) Mandal S, Bhumika K, Kumar M, Hak J, Vishvakarma P, Sharma UK. A Novel Approach on Micro Sponges Drug Delivery System: Method of Preparations, Application, and its Future Prospective. Indian J of Pharmaceutical Education and Research. 2024;58(1):45-63.

35) Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: a Pharmaceutical Perspective. Community Practitioner, 21(2), 29–37. https://doi.org/10.5281/zenodo.10642768

36) Singh, M., Aparna, T. N., Vasanthi, S., Mandal, S., Nemade, L. S., Bali, S., & Kar, N. R. (2024). Enhancement and Evaluation of Soursop (Annona Muricata L.) Leaf Extract in Nanoemulgel: a Comprehensive Study Investigating Its Optimized Formulation and Anti-Acne Potential Against Propionibacterium Acnes, Staphylococcus Aureus, and Staphylococcus Epidermidis Bacteria. Community Practitioner, 21(1), 102–115. https://doi.org/10.5281/zenodo.10570746

37) Khalilullah, H., Balan, P., Jain, A. V., & Mandal, S. (n.d.). Eupatorium Rebaudianum Bertoni (Stevia): Investigating Its Anti-Inflammatory Potential Via Cyclooxygenase and Lipooxygenase Enzyme Inhibition - A Comprehensive Molecular Docking And ADMET. Community Practitioner, 21(03), 118–128. https://doi.org/10.5281/zenodo.10811642

38) Mandal, S. Vishvakarma, P. Pande M.S., Gentamicin Sulphate Based Ophthalmic Nanoemulgel: Formulation and Evaluation, Unravelling A Paradigm Shift in Novel Pharmaceutical Delivery Systems. Community Practitioner, 21(03), 173-211. https://doi.org/10.5281/zenodo.10811540

39) Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: A Pharmaceutical Perspective. Community Practitioner, 21(2), 29–37. https://doi.org/10.5281/zenodo.10642768

40) Singh, M., Aparna, T. N., Vasanthi, S., Mandal, S., Nemade, L. S., Bali, S., & Kar, N. R. (2024). Enhancement and Evaluation of Soursop (Annona Muricata L.) Leaf Extract in Nanoemulgel: a Comprehensive Study Investigating Its Optimized Formulation and Anti-Acne Potential Against Propionibacterium Acnes, Staphylococcus Aureus, and Staphylococcus Epidermidis Bacteria. Community Practitioner, 21(1), 102–115. https://doi.org/10.5281/zenodo.10570746

41) Gupta, N., Negi, P., Joshi, N., Gadipelli, P., Bhumika, K., Aijaz, M., Singhal, P. K., Shami, M., Gupta, A., & Mandal, S. (2024). Assessment of Immunomodulatory Activity in Swiss Albino Rats Utilizing a Poly-Herbal Formulation: A Comprehensive Study on Immunological Response Modulation. Community Practitioner, 21(3), 553–571. https://doi.org/10.5281/zenodo.10963801

42) Mandal S, Vishvakarma P, Bhumika K. Developments in Emerging Topical Drug Delivery Systems for Ocular Disorders. Curr Drug Res Rev. 2023 Dec 29. doi: 10.2174/0125899775266634231213044704. Epub ahead of print. PMID: 38158868.

43) Abdul Rasheed. A. R, K. Sowmiya, S. N., & Suraj Mandal, Surya Pratap Singh, Habibullah Khallullah, N. P. and D. K. E. (2024). In Silico Docking Analysis of Phytochemical Constituents from Traditional Medicinal Plants: Unveiling Potential Anxiolytic Activity Against Gaba, Community Practitioner, 21(04), 1322–1337. https://doi.org/10.5281/zenodo.11076471

44) Pal N, Mandal S, Shiva K, Kumar B. Pharmacognostical, Phytochemical and Pharmacological Evaluation of Mallotus philippensis. Journal of Drug Delivery and Therapeutics. 2022 Sep 20;12(5):175-81.

45) Singh A, Mandal S. Ajwain (Trachyspermum ammi Linn): A review on Tremendous Herbal Plant with Various Pharmacological Activity. International Journal of Recent Advances in Multidisciplinary Topics. 2021 Jun 9;2(6):36-8.

46) Mandal S, Jaiswal V, Sagar MK, Kumar S. Formulation and evaluation of carica papaya nanoemulsion for treatment of dengue and thrombocytopenia. Plant Arch. 2021;21:1345-54.

47) Mandal S, Shiva K, Kumar KP, Goel S, Patel RK, Sharma S, Chaudhary R, Bhati A, Pal N, Dixit AK. Ocular drug delivery system (ODDS): Exploration the challenges and approaches to improve ODDS. Journal of Pharmaceutical and Biological Sciences. 2021 Jul 1;9(2):88-94.

48) Shiva K, Mandal S, Kumar S. Formulation and evaluation of topical antifungal gel of fluconazole using aloe vera gel. Int J Sci Res Develop. 2021;1:187-93.

49) Ali S, Farooqui NA, Ahmad S, Salman M, Mandal S. Catharanthus roseus (sadabahar): a brief study on medicinal plant having different pharmacological activities. Plant Archives. 2021;21(2):556-9.

50) Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: An Analysis Of The Medicinal Properties Of The Plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.

51) Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.

52) Mandal S, Vishvakarma P, Mandal S. Future Aspects And Applications Of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. European Journal of Molecular & Clinical Medicine.;10(01):2023.

53) Chawla A, Mandal S, Vishvakarma P, Nile NP, Lokhande VN, Kakad VK, Chawla A. Ultra-Performance Liquid Chromatography (Uplc).

54) Mandal S, Raju D, Namdeo P, Patel A, Bhatt AK, Gupta JK, Haneef M, Vishvakarma P, Sharma UK. Development, characterization, and evaluation of rosa alba l extract-loaded phytosomes.

55) Mandal S, Goel S, Saxena M, Gupta P, Kumari J, Kumar P, Kumar M, Kumar R, Shiva K. Screening of catharanthus roseus stem extract for anti-ulcer potential in wistar rat.

56) Shiva K, Kaushik A, Irshad M, Sharma G, Mandal S. Evaluation and preparation: herbal gel containing thuja occidentalis and curcuma longa extracts.

57) Vishvakarma P, Kumari R, Vanmathi SM, Korni RD, Bhattacharya V, Jesudasan RE, Mandal S. Oral Delivery of Peptide and Protein Therapeutics: Challenges And Strategies. Journal of Experimental Zoology India. 2023 Jul 1;26(2).

58) Mandal, S., Tyagi, P., Jain, A. V., & Yadav, P. (n.d.). Advanced Formulation and Comprehensive Pharmacological Evaluation of a Novel Topical Drug Delivery System for the Management and Therapeutic Intervention of Tinea Cruris (Jock Itch). Journal of Nursing, 71(03). https://doi.org/10.5281/zenodo.10811676