Formulation and Repurposing of Mefloquine loaded Nanoemulsion against A549 -Lung carcinoma cells with the comparison on HaCaT cells

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Research Article

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Abstract

Combining multiple anticancer agents in a nanocarrier can result in a formula with a low dose and few undesirable side effects.The purpose of the study was to formulate Garlic oil-loaded Mefloquine and Tamoxifen (TQ) in an oil-based nanoemulsion and evaluate its potential for inhibiting growth of lung cancer cells and normal skin cells.The antimalarial drug Mefloquine was repurposed using garlic oil nanoemulsion, which demonstrated better anti-cancer effects against A549 cell lines than Tamoxifen loaded nanoemulsion. The Mefloquine-loaded garlic nanoemulsion significantly reduced cell viability and promoted apoptosis in A549 cells in cytotoxic experiments. Physicochemical characterization, drug release tests, and cytotoxic studies were used to compare the drug-loaded nano emulsions. Mefloquine indicated less variance in hydrodynamic size, with a value of 1.01 ±0.13nm, than Tamoxifen-loaded nanoemulsion. Mefloquine loaded nanoemulsion showed better-sustained release, lower coarsening and constant colour stability. n simulated intestinal fluid, the drug release study of Mefloquine loaded nanoemulsion is 53.5% at 12 hours and Tamoxifen loaded nanoemulsion is 26.3 % at 8 hours (SIF). The percentage of cell viability of Mefloquine loaded Garlic oil nanoemulsion and Tamoxifen loaded Garlic oil nanoemulsion against lung cancer cells was 75.65 % and 64.35 %, respectively (A549). In normal cells, the cell viability of Mefloquine loaded Garlic oil nanoemulsion was lower than that of Tamoxifen loaded Garlic oil nanoemulsion. The findings imply that the Mefloquine-loaded Garlic oil nanoemulsion could be used as a nanotherapeutic carrier to target cancer cell without hampering the normal cells.

Highlights

- Ultrasonic emulsification of **Mefloquine** and **Tamoxifen** loaded ***Allium sativum*** essential oil was formulated.
- Drug release kinetics has demonstrated sustained release in **simulated intestinal fluid (SIF)** when compared with **simulated body fluid (SBF)**.
- An enhanced **anticancer activity** of Mefloquine loaded nanoemulsion was observed against Tamoxifen (chemotherapeutic agent).
- Cytotoxicity studies of mefloquine loaded nanoemulsion was evaluated against **human lung cancer cell line (A549) and** observed to be less toxic to HaCaT cell lines.

1. Introduction

Cancer causes uncontrolled cell division in the body, interfering with the operations of tissues, cells, and organs [1]. The cells can rapidly proliferate, invade tissue, and spread to other parts of the body to form new tumours; a process known as metastasis [2]. Most cancers can be averted by avoiding risk factors such as excessive cigarette use, alcohol consumption, a poor diet, and lack of physical activity[3]. Cancer can be decreased with early identification and follow-up methods such as radiotherapy, chemotherapy, and surgery [4]. Conventional anti-cancer drug formulations target the biggest tissues and cells while harming normal cells. Some new ways for repurposing pharmaceuticals in nanocarriers have shown useful in recent investigations. Essential oil-based nanoemulsion outperforms all other nanocarriers due to its eco-friendly nature, which ensures efficacy without side effects and is biologically safe[5]. As a result, the conventional medicine is repurposed by dissolving it in an appropriate essential oil utilising the nanoemulsion process in order to make a formulation, it will easy-to-target cancer cell[6].

Due to the hydrophobic character of the respective oil system and the need to construct a soluble nature in order to apply an emulsification technique, the essential oil-based nanoemulsion formulation is appropriate[7]. Emulsions are a mixture of two immiscible liquids with a droplet size of 20–200 nanometres [8]. Homogenization, microfludization, and ultrasonic emulsification are only a few of the high-energy processes used. Nanoemulsion is a heterogeneous phase that combines oils and surfactant Smix in varying ratios[9]. Furthermore, based on their surfactant volume and external force, these two liquid phases used to alter due to their surface tensions with the use of ultrasonication[10]. Repurposing existing drugs into Nanoemulsion serves as a drug delivery mechanism[6].

Antimalarial drugs, have extended half-lives and the active component in the drug is effective, so they could be a good choice for repurposing[11]. Due to its extended shelf life, low restriction concentrations, and good dosage tolerance, Mefloquine has been demonstrated to be beneficial against breast cancer, glioblastoma, liver cancer, and lung cancer[12]. Mefloquine has been demonstrated to be more effective, and it is currently being researched for use against a variety of illnesses as well as other broad-spectrum uses[13]. Tamoxifen, a common medicine, belongs to the antioestrogen family and it has a mild antiestrogen action and suppresses the activity of estrogen (a feminine hormone) in the breast[14]. The cytochrome P450 enzyme is the active ingredient in its metabolism, which takes place in the liver. antiestrogens limit the effects of estrogen hormones in the human body and it inhibits the growth of cancers that rely on estrogen as a key growth agent[15].

The use of ultrasonic emulsification techniques to repurpose drugs into nanoemulsions using an oil system and surfactant could increase drug reusability[16]. Exploiting existing medications with nanoemulsion-based drug delivery has proven to be one of the most effective

methods for developing novel drugs. The purpose of the study is to create a Mefloquine and Tamoxifen-loaded garlic oil nanoemulsion, perform physiochemical studies, evaluate drug release kinetics, and test the efficacy against lung cancer cells and normal skin cells to compare toxicity.

2. Chemicals And Methodology

2.1 Chemicals

Garlic Oil, Mefloquine Hydrochloride and Tamoxifen citrate were bought from Sigma Aldrich, India, Cremophore EL from HI Media, India, Brij35 from SRL, India, and ultra-pure water from Cascada™ bio water system, Pall Corporation, USA with resistivity 18.2 MΩ cm was used for the preparation of all solutions. The chemicals required for maintaining the A549 cell line are DMEM, FBS, Trypsin, Antibiotic Penicillin-streptomycin, MTT assay kit, Trypan blue from HiMedia, India and Phosphate buffer solution from Gibbico, India. The chemicals required for release studies are Monobasic phosphate, NaOH, $CaCl_2$, KCl, $MgSO_4$, $NaCl_2$, $NaHPO_4$, Saccharose, Hepes, Nitrocellulose membrane all purchased from SRL and HiMedia, India. All the above chemicals are of analytical grade.

2.2 Cell Culture

The A549 cell line, HaCaT cell line was bought from NCCS (National Centre for Cell Science) Pune, India. The A549 cell line was maintained by DMEM media supplement with 8–10% fetal bovine serum (FBS), 50–100µg/mLof antibiotics pen-strep at 37ºc, 5% CO_2 with humidified atmosphere. The cell line was maintained and monitored on a regular basis to ensure that it was healthy enough for further research.

2.3 Methodology

2.3.1 Smix(Preparation of Surfactants

Two different surfactants were used to formulate the drug-loaded nanoemulsion. According to their HLB value, two non-ionic surfactants Cremophore EL and Brij35 were prepared by combining their HLB values. Among all the alternative ratios, the final ratio 14.1 is carried out further, and a stock solution of 10% w/v Smix is created[17].

2.3.2 Mefloquine and Tamoxifen standard Graph

The λmax of Mefloquine and Tamoxifen (2µg.ml^{-1}) was calculated from its adsorption spectrum in an ultraviolet spectrophotometer (Hitachi U-2910, Japan) with a solvent (Methanol) for reference and blank. Mefloquine and Tamoxifen were produced as a stock solution with a concentration of 2 mg/ml and diluted (1–10 µg.ml^{-}1). The maximum absorbance of Mefloquine is 284nm, while the maximum absorbance of tamoxifen is 248nm. The detection and quantification limits are calculated using the formula below (LOD = Limits of Detection, LOQ = Limits of Quantification).[18].

LOD= $3.3 XSDofintercept / Slope$

LOQ = 10 $XSDofintercept / Slope$

2.3.3 Mefloquine and Tamoxifen Solubility

Excess Mefloquine and Tamoxifen were added to Garlic essential oil and vortexes for 15 minutes[19]. Following the observation, the sample was shaken for 72 hours in an orbital shaker to dissolve Mefloquine and Tamoxifen in Garlic oil. The undissolved Mefloquine and Tamoxifen were separated from the oil using centrifugation at 3000g rpm (spin win, Tarson product Pvt. Ltd)[20] .The physical solubility was next examined, and undissolved Mefloquine in the form of a pellet was discovered. A UV spectrophotometer was used to determine the dissolution concentrations of Mefloquine and Tamoxifen in the supernatant absorbance at 284nm and 248nm, respectively. Finally, a drug-loaded nanoemulsion concentration of 2mg/mL was chosen as the optimum concentration for future characterization and application[21].

2.3.4 Nano emulsion Formulation

2.3.4.1 Ternary Phase Diagram (TPD)

TPD was created in the same way but with a few tweaks[22]. At room temperature, the TPD was built using the water titration method. Milli Q was added 100–200µlat a time, stirring for 5 minutes at 500 rpm (Remi 2MLH). The turbid/clear/milky appearance of coarse emulsion. The TPD was created with clear nanoemulsion in view.

2.3.4.2 Preparation of Nanoemulsion-

Garlic essential oil (5%), the Smix of two surfactants Cremophore EL, Brij35, and the drug Mefloquine Hydrochloride and Tamoxifen Citrate (loaded or free set of emulsion) were used to produce the drug Mefloquine Hydrochloride and Tamoxifen Citrate (loaded or free set of emulsion). The formulation was an oil-in-water nanoemulsion, with the oil and Smix thoroughly mixed for at least 10–15 minutes before adding the water, and water added to the mixed solution of oil and Smix using a titration approach[23]. After formation, it was sonicated for 10 minutes at 20kHZ with a 40 percent amplitude using an ultrasonicator emulsifier (Sonics, VIbra cell line, USA) [10]. The sample container was placed in an ice bath during the ultrasonication procedure to keep the temperature at 10ºC and avoid heat energy[24]. Before being utilised in characterisation investigations, a clear nanoemulsion was kept at room temperature overnight to observe phase separation[5]. In Garlic oil, the final concentrations of Mefloquine hydrochloride and Tamoxifen Citrate were 2mg.ml^{-}1. The droplet size, and stability of the formulated nanoemulsion were analysed, and the nanoemulsion was then used in in vitro studies.

2.3.5 Characterization of Nano emulsion

The list of nano emulsions formulated were characterized by the below mentioned methods:

2.3.5.1 Determination of Droplet size and Zeta potential

The DLS was used to determine the droplet size of nanoemulsion and drug-loaded nanoemulsion (SZ-100, Horiba, Japan). Before final reading, all clear formulations following the overnight process were diluted in a 1:4 ratio with water to reduce the various scattering effects and all of the results were checked in triplicate. The electrophilic mobility of the droplets was assessed using a laser temperature [25]. The droplet size was measured using Doppler frequency shifts in scatter laser light, from which the zeta-potential was measured.

2.3.5.2 Stability of Formulated Nano-emulsion

The kinetics of both drug-loaded emulsions were evaluated by centrifugation at 1400g rpm for 30 minutes[26]. The clear nanoemulsion with a decreased surfactant ratio were labelled as optimized nanoemulsion and analysed for stability for 1 to 6 months at room temperature.

2.3.5.3 Physiochemical properties of Nano emulsion

The drug-loaded nanoemulsion as well as its physiochemical properties were observed. The pH of the emulsions was measured with a pH metre (Mark VI, Systronic, Ahmedabad, India). The turbidity of the emulsions was measured at 600 nm using a UV spectrophotometer (U2910, Hitachi) with MilliQ as a blank reference[27]. A conductivity meter measures the conductivity (CM180, Elico, Hyderabad, India).

2.3.6 Drug Release Kinetics

The kinetics of Mefloquine and Tamoxifen release were measured in simulated intestinal fluid (SIF) and simulated body fluid (SBF). As previously reported, the two-body fluids were produced using the USA pharmacopoeia. The Franz-diffusion equipment was used to aid in the release of the drug[28]. There were two types of compartments in the setup: a donor compartment and a 5 ml recipient compartment that were both kept at ambient temperature and a cellulose nitrate dialysis membrane separated two compartment. For the first 1 hour in a 15-minute interval, the release of drug-loaded nanoemulsion in the respective fluid was measured at 284 nm and 248 nm, and then for the next 12 hours (max value)[29]. To keep the overall amount of the fluid constant, the samples were taken from the recipient compartment and replaced with freshly prepared fluid.

2.3.7 Morphology study of cell line (A549 & HaCaT)

The cell lines were cultured with 70% – 90% confluency through(Bright field Microscope) for carrying out the cell counting and cytotoxicity studies[30].

2.3.8 Cytotoxicity Assay

$$\text{Cellviability} = \frac{[\text{Mean absorbance of untreated cells (control)} - \text{Mean absorbance of treated cells}]}{\text{Mean absorbance of untreated cells (control)}} \times 100$$

Using the previously mentioned technique, the effects of changing the concentration value of mefloquine and tamoxifen in loaded nanoemulsion and drug free nanoemulsion were analysed[7]. MTT Assay kit (3-(4,5, methylthiazol-2-yl)-,5 diphenyl-2H-Tetrazolium bromide) from Hi-media, India, was used to assess the viability of the A549 and HaCaT cell lines. For a period of 12 h and 24 h, different quantities of Mefloquine and Tamoxifen loaded nanoemulsion, as well as free Garlic oil nanoemulsion as a control, were tested. The MTT in PBS solution (MTT (5mg/ml) was added to the culture plate after a particular time period [3]. The plate will be covered with aluminium foil (MTT light-sensitive) and incubated at 37º C for at least 4–5 hours after adding the MTT solution to the well[31]. It was studied under a phase-contrast

microscope after the incubation time, and purple formazan was apparent with dead cells. We applied washing buffer (Hi-media, India) 100µl of 100% to each well after washing with PBS and incubated for 4 hours at room temperature to dissolve the purple formazan. The absorbance was then measured at (570) nm and (630) nm with a BIO RAD ELISA microplate absorbance spectrophotometer. The measurement was carried out three times for each well, and the mean value, standard deviation, and standard error were calculated[32].

2.3.9 Statistical Analysis

The statistical analysis occurred using a graph pad (prism V.5.01, GraphPad software inc, CA). The data from MTT assays were calculated into mean value, ±SD for each different value, as well as SE. The drug release kinetics analysis was done by Microsoft office excel 2019 software.

3. Results And Discussion

3.1 Nanoemulsion Formulation

3.1.1Ternary Phase Diagram (TPD)

The three-component system comprising water, Smix of Chremophore EL, Brij 35, and Garlic oil is depicted in this ternary phase diagram. Ten nanoemulsion formulations were chosen and characterized, each with a different concentration of Garlic oil and Smix in Figure-S1 (supplementary file). With water weight concentrations ranging from 0 to 100%, a continuous single-phase nanoemulsion area was seen across the oil. In Fig. 1, the nanoemulsion region is the area inside the frame. The nanoemulsion points are plotted, the area referring to the nanoemulsion region and the outer area referring to multiphase turbid regions.

3.1.2 Nanoemulsion Formulation and Physiochemical properties-

Different ratios of oil and surfactant were used to make nanoemulsions. The droplet sizes of the nanoemulsion are shown in Fig. 2. Cremophore EL (HLB 14–16) and Brij 35 (HLB − 16) were chosen as surfactants for an oil-in-water emulsion because of their high hydrophilic-lipophilic balance. Smix (a mixture of both surfactants) has an HLB value of 14.1, which is required for an oil-in-water emulsion [33]. Furthermore, both surfactants are non-toxic and can be employed on human cancer and skin cell lines [34]. The average droplet size was found to be smaller when the surfactant concentration in the nanoemulsion preparation was increased.With a reduced surfactant content, the garlic oil nanoemulsions are stable above the ratio of 1:2[33]. When centrifuged at 4000 rpm for 30 minutes, it displayed phase separation, indicating that they are. Unstable Figs. 2(A) and 2(B) demonstrate the droplet size and polydispersity index (PDI) of both drug-loaded formulations (Mefloquine and Tamoxifen). Garlic oil nanoemulsion formulation's mean hydrodynamic diameter and PDI values are 17.9 ± 1.43nm and 0.372nm, respectively. Mefloquine and Tamoxifen loaded garlic oil nanoemulsions with mean hydrodynamic diameters and polydispersity index values are 15.5 ± 1.73 and 0.213nm, 19.5 ± 0.238, and 0.08nm respectively. In table − 1, the physiochemical characteristics are listed.

Following the stability experiments, the 1:3 ratio in Mefloquine loaded garlic nanoemulsions and the 1:4 ratio in Tamoxifen loaded garlic oil nanoemulsions have been further described, and applications are carried out. The Mefloquine loaded garlic oil nanoemulsion has a zeta potential of -1.5 ± 0.12 and the Tamoxifen loaded garlic oil nanoemulsion has a zeta potential of -2.3 ± 0.01. Mefloquine loaded garlic oil nanoemulsion has a pH of 5.36 ± 0.14 and Tamoxifen loaded garlic oil nanoemulsion has a pH of 6.08 ± 0.13. The turbidites of both drug-loaded nanoemulsions have 0.011 ± 0.004 and 0.032 ± 0.00. The conductivities of Mefloquine and Tamoxifen-loaded garlic nanoemulsions have 0.19 ± 0.05, and 0.16 ± 0.19, respectively.

After centrifugation at 4,000 rpm for 25–30 minutes, the nanoemulsion with a lower surfactant value shows phase separation, indicating its instability in the ratios 1:1 and 1:2. In comparison to Tamoxifen-loaded nanoemulsion, there were considerable changes in mean droplet size diameter from around 15.5 to 17.1 nm, 13.5 to 16.6 nm, and 12.7 to 14.2 nm in Mefloquine loaded nanoemulsions Figure-3 (A)&(B).

3.2 Drug Release Kinetics

Figure-4(A)&(B) shows that in simulated body fluid, Tamoxifen-loaded nanoemulsion showed 1–5% after 3 h, while Mefloquine loaded nanoemulsion showed 10–15% after 3 hours. In the first 3 hours, the stimulated intestinal fluid showed greater sustained release of 5–10% in Tamoxifen-loaded nanoemulsion and 15–20% in Mefloquine loaded nanoemulsion. Mefloquine-loaded nanoemulsions remained in stimulated intestinal fluid for 12 h until they reached saturation (60–70%). The Tamoxifen-loaded nanoemulsion continued to release until (30–35%) after 8 hours of sustained release. Mefloquine and Tamoxifen were released in very small quantities as a control. Mefloquine and Tamoxifen were released in Garlic oil in stimulated intestinal fluid, with R 2(coefficient determination) values of 0.989 and 0.9576 in Figure:S2 (supplementary file).

3.3 Cytotoxicity Assay-

We calculated the cell viability of Mefloquine and Tamoxifen loaded garlic oil nanoemulsion on human lung cancer cells (A549) to be 62 ± 8.6 and 82 ± 0.6 after 12 h of incubation and 35 ± 1.7µl/mL and 52 ± 1.6µl/mL after 24 h in Fig. 5(A) & (B). By exposing cultured cells to varying concentrations of both drugs loaded nanoemulsions (1-100µl/mL) for 12 h and 24 h. The cell viability data shows that the Mefloquine-loaded nanoemulsion effectively inhibited the proliferation of A549 cells in a dose and time-dependent manner (12h-24h). After 24 hours of incubation, Mefloquine-loaded nanoemulsion with a 25µl/mL of low concentration suppressed cell growth more than Tamoxifen-loaded nanoemulsion. After 12 hours of incubation in both drugs, the cell was suppressed by 100µl/mL of high concentration.

Figures 6 (A) & (B) show the administration of a garlic oil-loaded nanoemulsion containing Mefloquine and Tamoxifen to HaCaT cultured (Human skin cells) for 12 h and 24 h respectively. For 12 h incubation, the cell viability of the Mefloquine loaded garlic oil nanoemulsion treated group is 68.3µl/mL, while Tamoxifen is 40.06µl/mL. The cell viability of the 24 h treated group with both the drug-loaded garlic oil nanoemulsion is 40 ± 0.3µl/mL and 15 ± 2.3µl/mL, respectively. Low concentrations (25µl/mL) of Mefloquine loaded garlic oil nanoemulsion considerably inhibited cell proliferation after 24 h of incubation, whereas low concentrations (25µl/mL) of Tamoxifen loaded garlic oil nanoemulsion strongly inhibited cell proliferation after 12 hours of incubation. At 24 h, the high concentration (100µl/mL) Mefloquine loaded garlic oil nanoemulsion reduced cell inhibition, but the low concentration (25µl/mL) Tamoxifen loaded garlic oil nanoemulsion increased cell inhibition.

4. Discussion

The ternary phase diagram with the oil-in-water nanoemulsion area is given in fig-1. As the surfactant concentration increases, the water concentration decreases, as mentioned in the study. As a result, the droplet size was lowered and the turbidity was reduced[5]. The turbidity of an emulsion is determined by particle concentration and particle size. Our ternary phase diagram showed that the formulations are homogeneous and had low viscosity, making them ideal for ultrasonication to create nanoemulsion[22].

This is consistent with the findings of, the addition of surfactant to a nanoemulsion system induced the interfacial film to condense and solidify, resulting in smaller droplet sizes. Nanoemulsion stability against coalescence is influenced by the nature of the surfactant and its concentration in an aqueous solution. Our findings are similar to an earlier study that utilizing a low oil: surfactant ratio can result in small droplet sizes. Droplet growth may occur after the formation of both drug-loaded and drug-free nanoemulsions due to the Ostwald ripening/coalescence process, which is dependent on surfactant and oil composition value and the drug efficacy in the oil.[35] Because there is insufficient surfactant to adequately stabilize the newly generated interface in the surfactant poor regime, the rate of droplet coalescence is high. Because the increased surfactant can stabilize more interfacial areas, a moderate increase in surfactant concentration induces a significant drop in particle size because the extra surfactant is able to stabilize more interfacial area[36].

Polydispersity ensures consistency in droplet size stability in the formulation. Low polydispersity values may explain droplet sizes in the micron range. Droplet size homogeneity suffers as a result of high polydispersity. Important findings published by [22] include the spherical form of nanoemulsion and its range of (15–70)nm, as demonstrated by AFM and TEM (transmission microscopy). Because of repulsive forces formed between droplets against flocculation and coalescence,[23] found that increasing the surface charge improves the stability of nanoemulsions significantly.

UV-Spectroscopy is used to examine the turbidity of both drug-loaded nanoemulsions.. The findings revealed that as the concentration of surfactant was increased, the turbidity of nanoemulsions reduced due to a reduction in droplet size. According to the findings, the nanoemulsion becomes transparent as the surfactant concentration increases. Our findings are comparable to those in the prior research[24].

The stability of nanoemulsions is important in most commercial biomedical activities. As a result, we investigated the influence of storage duration on the stability of formed nanoemulsions using various ratios, as shown in Fig. 4. Some droplet development may be caused by coalescence or Ostwald ripening[7]. In biomedical applications, however, minor changes in droplet diameter are relatively stable[27].

Stimulated intestinal Fluid produced greater sustained release than stimulated body fluid in both drug-loaded nanoemulsions. As shown in graphs 5 (A) & (B), the release of drug-loaded nanoemulsion was more consistent and better than Tamoxifen-loaded nanoemulsion. The release rate is proportional to the drug concentration in the dosage form. According to, [17] the release research is based on a first-order model.

The MTT assay revealed that the drug-loaded garlic oil nanoemulsion effectively suppressed the growth of both the cell line in a dose and time-dependent manner[7]. Mefloquine-loaded nanoemulsion had a stronger inhibitory effect on A549 lung cancer cells and Tamoxifen-loaded nanoemulsion had a stronger inhibition effect on HaCaT cells[37]. When compared to a Mefloquine-loaded nanoemulsion, cell growth

suppression is advised. The tamoxifen nanoemulsion rapidly penetrates the ordinary cell membrane, resulting in cell death[38]. As a result of their rapid induction of cell proliferation, Tamoxifen-loaded formulations were found to be harmful to HaCaT (normal skin cells).

.As a result, nanoemulsion therapy may effectively stop lung cancer cells from proliferating by inducing apoptosis. Based on previous findings from the anticancer effect of quinone-based groups, autophagy is an ATP-dependent process[38]. As a result, autophagy may assist in this type of crosstalk, hallmark apoptosis without directly triggering cell death[39]. In cancer cells, the nanoemulsion loaded with the drug showed a considerable increase in the number of autophagy vesicles as compared to untreated cells[40]. Due to the participation of apoptosis and autophagy activation in the nanoemulsion mechanism of action, with a direct contribution of both processes to cell death, targeting the molecular mechanism underlying the nanoemulsion loaded quinone-based medication killing cancer cells compared to normal cell lines[41]. This finding is consistent with previous findings derived from apoptosis analysis, which revealed effective apoptotic induction[38]. In other words, the combination treatment loaded within Nanoemulsion autophagy appears to stimulate the apoptotic pathway and contribute simultaneously, though not cooperatively, to cell death[30]. In agreement with this data, nanoemulsion can deliver Mefloquine with increased apoptosis induction, which suppresses lung cancer cell proliferation[42]. When the medicinal herb garlic, rich in Allicin, is combined with Mefloquine, introduced through a nanoemulsion delivery system, it has a synergistic effect on molecular mechanism studies such as cell morphology and targeting pathway studies.

5. Conclusion

Our findings with drug-loaded nanoemulsions imply that knowledge of conventional medicinal systems can be exploited to bio prospecting, identify, develop, and exploiting novel safe, and effective medicine sources. It is high in medicinal components and can be exploited as a therapeutic molecule and clinically certified antimalarial drug Mefloquine repurposed for cancer cell lines treatment. In the constructed antimalarial and chemotherapeutic drug-loaded nanoemulsion, the oil: surfactant ratio, droplet size, and turbidity were all increased. In comparison to the Tamoxifen-loaded nanoemulsion, the Mefloquine-loaded nanoemulsion showed sustained release in SIF (Stimulated Intestinal Fluid) than SBF (Stimulated Body Fluid).

This preliminary research supports the use of our nanoemulsion formulation as a complementary and alternative medicine (CAM) for the treatment of lung cancer using a reverse pharmacological approach Garlic oil nanoemulsion reduces cancer cell viability and cytotoxicity in culture, depending on the concentration and interaction time. Future study into Mefloquine-loaded nanoemulsions could aid in the development of specialized therapeutic applications in cancer cells, and also the discovery of previously undisclosed cell death and autophagy pathways. The anticancer properties of garlic oil, Mefloquine, and nanoemulsions combination were found to be more effective than the well-known chemotherapeutic medication Tamoxifen. It produced less collateral damage to non-cancerous cells than tamoxifen (HaCaT). More research into the mechanism that underpins this formulation will be conducted. The findings suggest that the nanoemulsion could be a viable option for administering the combination therapy of Mefloquine-loaded Garlic, perhaps lowering the effective anticancer dose of medicines used to treat lung cancer. To better understand the molecular processes that this formulation triggers to improve anticancer activity, more research is needed. The molecular and phenotypic variability of human lung carcinomas makes developing successful treatment regimens difficult. Their highly variable response to therapy, including plant-derived remedies, can be observed, and their acquired drug resistance poses a hurdle. As a result, nanoemulsion-based combination therapy, which mixes a variety of plant-derived anticancer medicines with traditional chemotherapy, should be investigated. Multiple signalling pathways in cancer cells may be harmed, perhaps delaying treatment resistance. More research is needed to generate new nano-based combination medicines that contain plant-derived compounds with well-validated anticancer properties to increase therapy options in clinical oncology.

Declarations

Credit Author Statement

Priyadarshini Mohapatra: Investigation, Methodology, Visualization, Formal analysis, Writing-Original Draft.

Amitava Mukherjee: Formal analysis, Resources.

N. Chandrasekaran: Conceptualization, Methodology, Supervision, Project administration, Writing- Review and editing.

1.Declaration-

Hereby, I Dr. N. Chandrasekaran consciously assure that for the manuscript "**Formulation and Repurposing of Mefloquine loaded Nanoemulsion against A549 -Lung carcinoma cells with the comparison on HaCaT cells**" the following is fulfilled:

1) This material is the authors' own original work, which has not been previously published elsewhere.

2) The paper is not currently being considered for publication elsewhere.

3) The paper reflects the authors' own research and analysis in a truthful and complete manner.

4) The paper properly credits the meaningful contributions of co-authors and co-researchers.

5) The results are appropriately placed in the context of prior and existing research.

6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.

7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content..

2. Ethics approval and consent to participate

'Not applicable'

3. Consent for publication

We, give our consent for the publication of identifiable details, which can include

Photograph (s) and/or videos and/or case history and/or details within the text ("Material") to be published by in Journal and Article **"Nano scale Research Letters".** I have discussed this consent form with Dr. Amitava Mukherjee and Ms. Priyadarshini Mohapatra, who are co-authors of this paper.

4. Availability of data and material

'Not applicable'

5. Competing interests

The authors involved reveal no sources of conflict of interest.

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7. Authors' contributions

Priyadarshini Mohapatra: Investigation, Methodology, Visualization, Formal analysis, Writing-Original Draft.

Amitava Mukherjee: Formal analysis, Resources.

N. Chandrasekaran: Conceptualization, Methodology, Supervision, Project administration, Writing- Review and editing.

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Tables

Table 1. Physiochemical Characteristics of Standardized Tamoxifen and Mefloquine loaded formulation of Garlic Nano emulsion

EMULSIONS	Tamoxifen loaded (mean ± SD)				Mefloquine-loaded (mean ± SD)			
Concentration of NE	Zeta Potential(mv)	Conductivity ($\mu S.cm^{-1}$)	pH	Turbidity (OD at 600nm) AU	Zeta Potential(mv)	Conductivity	pH	Turbidity (OD at 600nm) AU
1:3	-2.3±0.01	0.19±0.05	6.08±0.13	0.032±0.0	-1.5±0.12	0.16±0.19	5.36±0.14	0.011±0.004
1:4	-2.9±0.00	0.21±0.02	6.14±0.05	0.046±0.13	-1.8±0.06	0.18±0.23	5.65±0.02	0.024±0.005
1:5	-2.4±0.00	0.22±0.04	6.08±0.09	0.50±0.12	-2.2±0.08	0.21±0.12	5.87±0.09	0.041±0.007

Figures

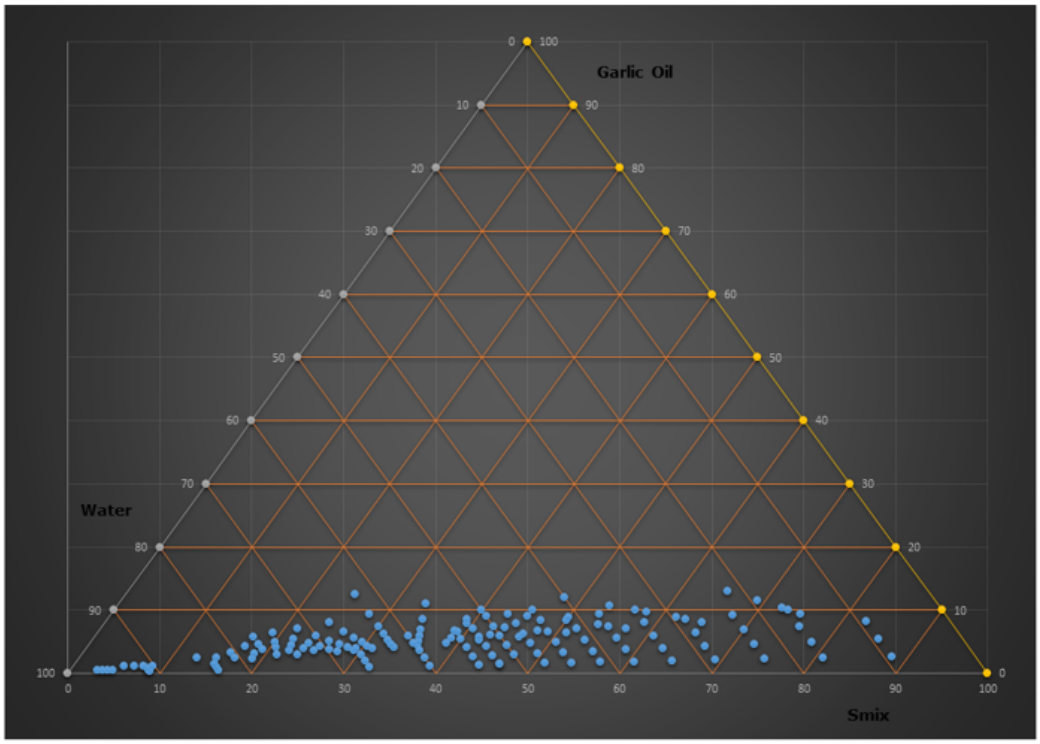

Figure 1

(Construction of Ternary phase diagram)

Ternary phase diagram construction of Nano emulsion: Oil, Smix (Cremophore EL and Brij 35), Mili Q.

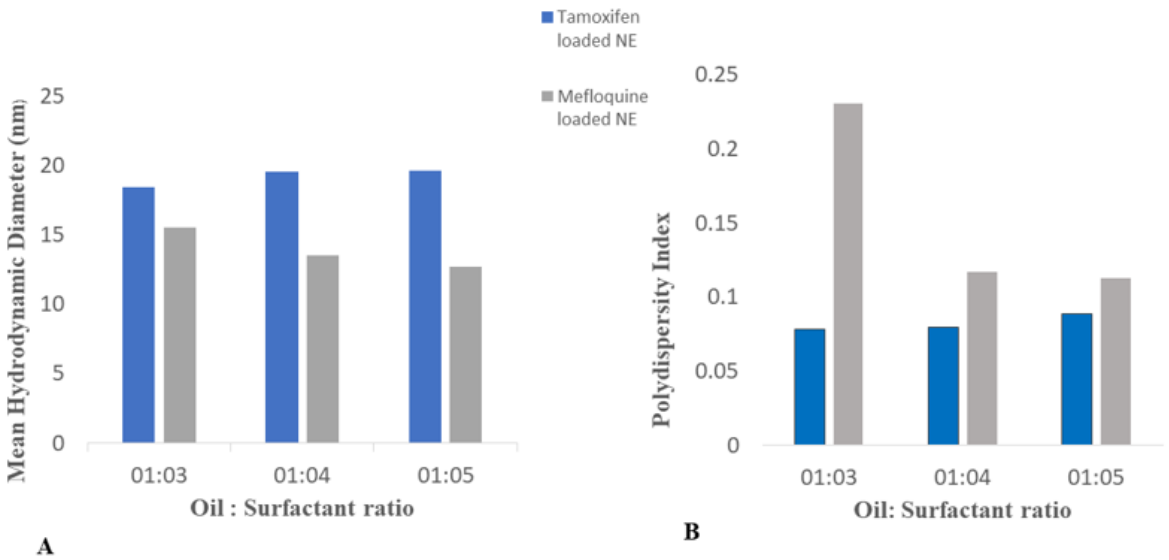

Figure 2

(DLS and PDI Values)

(A) Mean hydrodynamic diameter values of different both drug loaded nanoemulsion ratios. (1:3,1:4,1:5) all the values presented as mean ±standard deviation; n=3

(B) Polydispersity index values of different both the drug loaded nanoemulsion ratios. (1:3,1:4,1:5) all the values presented as mean ±standard deviation; n=3

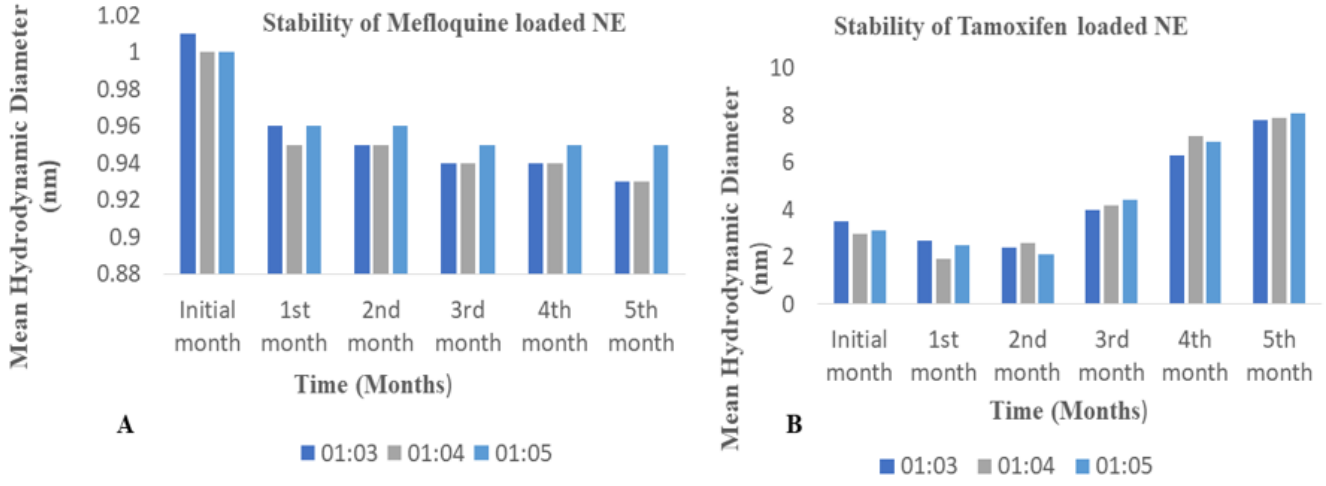

Figure 3

(Stability value)

(A)Long term Stability analysis of Mefloquine -loaded Nano emulsion and (B) Tamoxifen loaded nanoemulsion (long term stability purpose) all the values presented as mean ±standard deviation; n=3

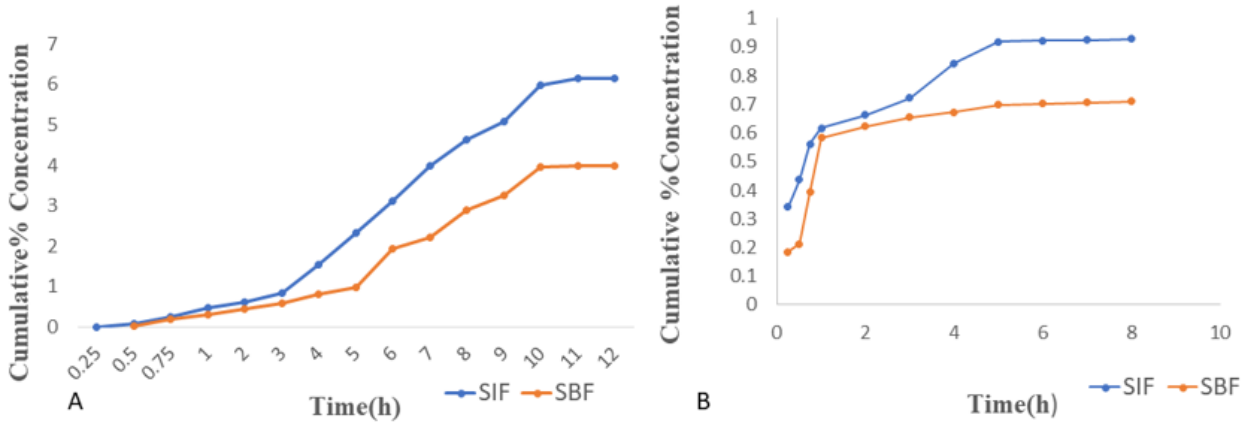

Figure 4

(Release Kinetics of Mefloquine)

(A)Release profile of garlic nanoemulsion loaded Mefloquine Hydrochloride in Simulated Intestine and body fluids(B) Release profile of garlic nanoemulsion loaded Tamoxifen citrate in Simulated Intestine and body fluids All the values presented as mean ±standard deviation; n=3

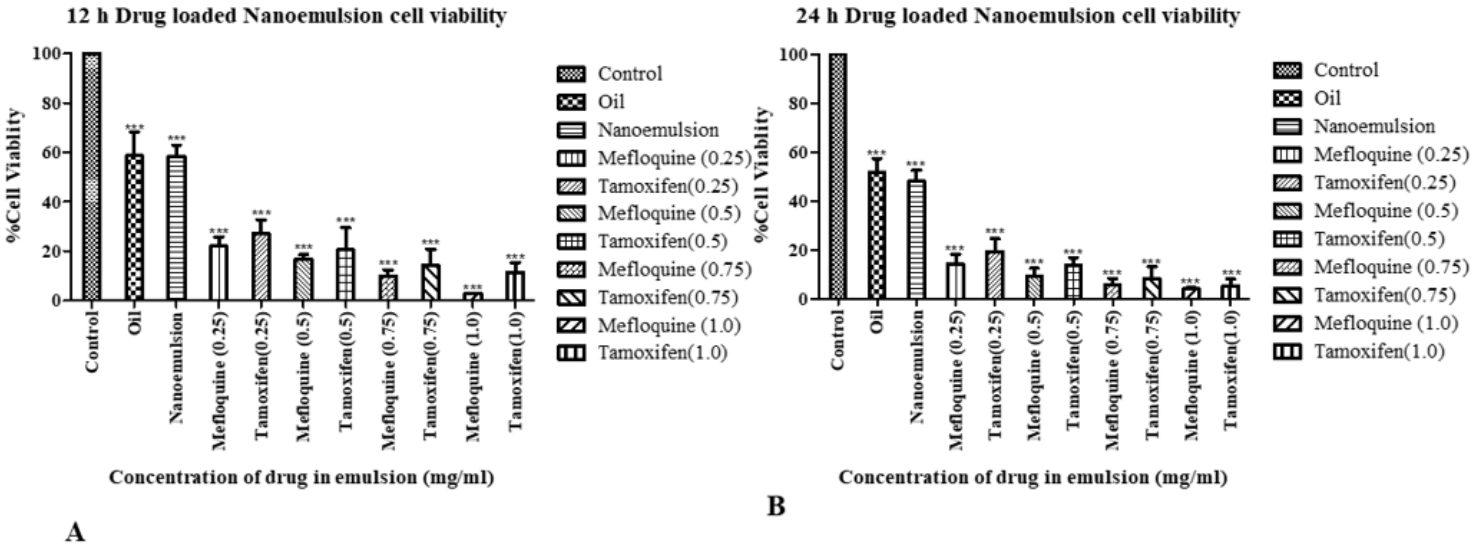

Figure 5

(Cytotoxicity studies)

Cytotoxicity of Mefloquine and Tamoxifen loaded garlic oil nano emulsion Nano emulsion with 1:3 (v/v) ratio of loaded oil and surfactant on A549 lung cancer cell lines. Image (A) 12h interaction of (B) 24h interaction of Mefloquine loaded garlic oil nanoemulsion and tamoxifen loaded. (Concentration value of Mefloquine loaded garlic oil nano emulsion increased order) All the values presented as mean ±standard deviation; n=3

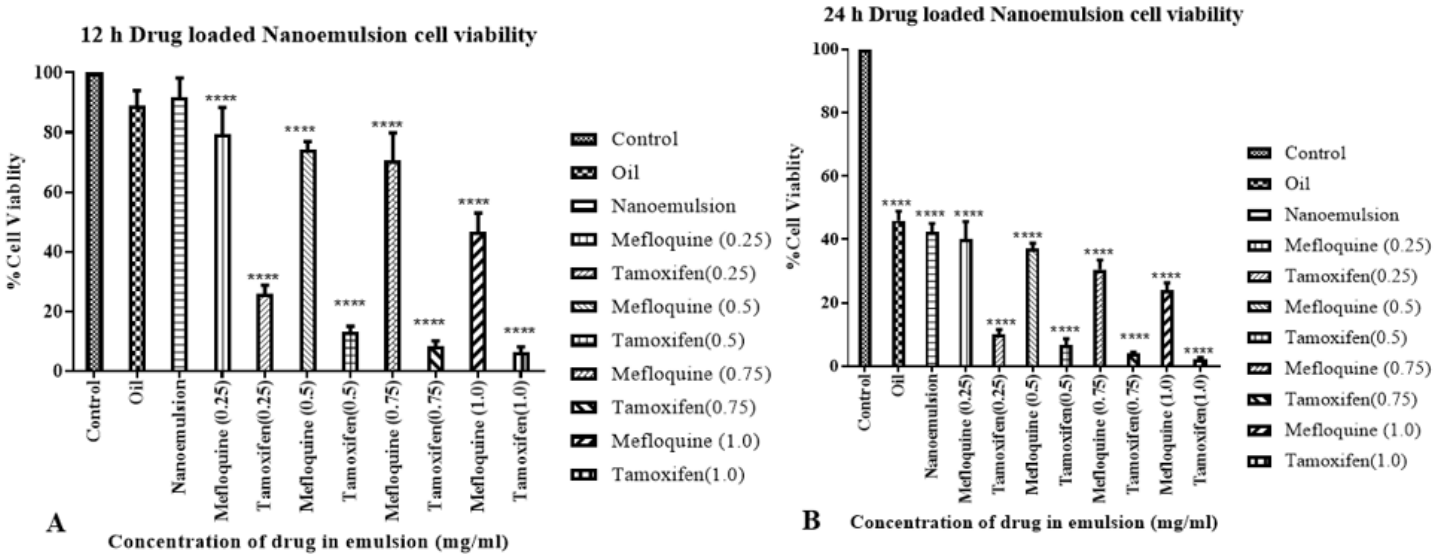

Figure 6

(Cytotoxicity studies)

Cytotoxicity of Mefloquine and Tamoxifen loaded garlic oil nano emulsion Nano emulsion with 1:3 (v/v) ratio of loaded oil and surfactant on HaCaT (Human skin cells). Image (A) 12h interaction of (B) 24h interaction of Mefloquine loaded garlic oil nanoemulsion and tamoxifen loaded. (Concentration value of Mefloquine loaded garlic oil nano emulsion increased order) All the values presented as mean ±standard deviation; n=3

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