10. SUMMARY AND CONCLUSION

In the present work, we have designed two nanoparticulate formulations, Polymer Lipid Hybrid Nanocarriers and Mesoporous Silica Nanoparticles for efficient and targeted delivery of two anticancer drugs widely used for treatment of breast cancer, namely fulvestrant (FLV) and exemestane (EXE). Though various other nano delivery platforms have been research upon for both these drugs, this was the first instance where in polymer lipid hybrid nano carriers and mesoporous silica nanoparticles with folate conjugation have been formulated for targeted delivery of both these drugs to breast cancer cells.

Fulvestrant, a newer anticancer agent is utilized in management of progressive breast cancer. It acts as complete antagonist unlike SERMs like tamoxifen, and due to being non-targeted, it presents various side effects, that are related to estrogen deficiencies such as weight gain, thromboembolic problems, neutropenia, leukaemia, anaemia. Some fewer common side-effects observed are vulvovaginal dryness, pelvic pain, and vaginitis. This negative aspects of fulvestrant is the major hindrance for its efficiency against breast cancer and therefore it demands the development of new formulation which is targeted and have control release efficiency.

Exemestane is approved by the USFDA for hormone dependent breast cancer treatment in postmenopausal females It is orally active potent irreversible steroidal aromatase inactivator which behaves as a false substrate for the aromatase enzyme and binds to its active site and reduces the synthesis of estrogen. However, oral clinical applications of EXE is limited because of its low aqueous solubility (80 μg/mL), high lipophilicity, poor oral bioavailability (5%) and rapid first pass metabolism. Therefore, to improve the clinical effectiveness of EXE prolonged circulation and targeted delivery are highly desired.

Quercetin is naturally occurring dietary flavonoid which has been found to have anticancer activity and application in the treatment of breast cancer. Quercetin shows a wide range of biological and pharmacological effects including antioxidative, anticancer, anti-inflammatory, antidiabetic, hepatoprotective and anti-obesity activities. Among polyphenols, quercetin has been shown as one of the most potent antioxidants. It can inhibit several enzymes that produce oxidative species such as xanthine oxidase, NADP and phosphate oxidase. It has been shown that quercetin inhibits cell proliferation as it may induce apoptosis and/or cell cycle arrest (either G2/M arrest or G1 arrest. In fact, the ability of this polyphenol to interfere with various target molecules identified as hallmarks of cancer, renders it as a multi-target key molecule in

different types of tumours. It prevents the resistance towards the FLV and EXE by inhibition of PI3KCA enzyme.

The rationale of the present work was to develop novel drug delivery systems for anti-neoplastic drugs for targeted delivery and improvement of both cellular uptake and efficacy of drug for the treatment of breast cancer. The existing formulation of **Fulvestrant** is depot formulation, which has some significant problems such as its inability to reach steady state plasma concentration, its route of administration, its termination of therapy and its metabolism. Similarly, for **Exemestane** its low solubility and bioavailability is matter of concern. Targeted, biodegradable polymer-based nanoparticles in the treatment of cancer can show selective accumulation of drug at tumor site and increase the drug concentration due to their nano dimension and thus lead to a reduction in the incidence of side effects of the anti-cancer agents. The addition of quercetin in MSNs can provide synergistic effect by pro-oxidation and pro-apoptosis of tumor cells. The addition of **targeting moieties** such as Folic acid can improve the specificity of the nanoparticles towards target, resulting in increase in intratumoral concentration of drug and decreasing its side effects.

POLYMER LIPID HYBRID NANOCARRIERS (PLHNCs)

Polymer lipid hybrid nanocarriers , provides a unique drug delivery platform in which the biocompatibility and surface modification, like liposomes can be achieved using lipid composition (DOPE:SPC:DSPE-PEG2000) and the structural integrity with mechanical stability is provided by polymeric (PLGA) core. Delivery of fulvestrant and exemestane targeted PLHNCs to the cancer cells via intravenous route directs passive tumour targeting by Enhanced Permeability and Retention effect (EPR) owing to the nanosized of the particles (< 200 nm) and reduction in side effects due to off- targeted toxicity can be achieved by sustained release of small amount of drug at regular intervals at the site of action from the Nanoparticles. The project aims to develop a develop targeted delivery systems namely Polymer Lipid Hybrid Nanocarriers and Mesoporous Silica Nanoparticles with surface modifications to target the formulation to breast cancer cells. The developed nanoparticles will be able to release the loaded drugs within tumor microenvironment and thereby reduce the toxicity associated with conventional anticancer therapy.

FULVESTRANT PLHNCs

Analytical techniques were developed to estimate pure fulvestrant, fulvestrant in PLHNCs, Dissolution media (Phosphate buffer saline pH 7.4, phosphate buffer pH 6.6 and acetate buffer pH 5.5). Calibration curve of fulvestrant was prepared by direct UV estimation. The calibration was plotted by measuring the absorbance at 280 nm (λmax), calibration curve was prepared for fulvestrant in Acetonitrile, Methanol, Tetrahydrofuran, Phosphate buffer pH 7.4, phosphate buffer pH 6.6 and acetate buffer 5.5 separately and the methods were validated for accuracy, precision, LOD and LOQ. For estimation Phospholipid content in formulation by Stewart method, calibration of total phospholipid was prepared in chloroform. For estimation of fulvestrant in biological samples, calibration curve of fulvestrant was developed by HPLC method for plain drug, in rat plasma for pharmacokinetic studies.

Different methods were screened for preparation of PLHNCs such as two-step method, double emulsion solvent evaporation and single step nanoprecipitation method to formulate PLHNCs. From this, single step nanoprecipitation with little modification followed by extrusion was found to formulate PLHNCs of desired characteristics. Preliminary studies were performed to define the ranges of formulation as well as process parameters. Preliminary studies also played significant role in determination of amount of cationic lipid needed to achieve maximum entrapment efficiency of fulvestrant. It was confirmed that the 30:50:20 ratio of DOPE:SPC – 3: DSPE PEG2000 has enough capacity to form a lipid layer onto the polymeric surface. Further increase in lipid ratio doesn't necessarily increases the encapsulation efficiency. Total seven factors (polymer concentration (mg/mL), lipid/polymer percentage (%), drug input percentage (%), stirring speed (RPM), stirring time (h), sonication time (S) and extrusion cycle (Nos) were selected for Placket-Burman screening study. From those, three factors (i.e., concentration of polymer, lipid to polymer ratio and drug input) were selected to further optimize the design space using Box-Behnken design.

QbD enabled design expert software suggested an optimized batch having composition of polymer concentration (8mg/ml), lipid to polymer ratio (15%) and drug input percentage (11%) which possessed predicted size of 118 nm and 79.84% entrapment efficiency and the same batch was formulated to validate the results and particle size was found to be 122.2 ± 3.8 nm with the PDI of 0.045 ± 0.003 and Zeta potential was found to be 28.3 ± 1.28 mV which is due to presence of cationic lipid i.e., DOPE. The entrapment efficiency was determined using

Ultracentrifuge to separate entrapped and unentrapped drug. The % Entrapment efficiency was found to be 82.13 ± 2.52 % (n =3) in the optimized formulation.

The transmission electron microscopy (TEM) was performed to characterize PLHNCs structure with negative staining by uranyl acetate. The thickness of the ring is less than 20 nm, which equals the thickness of DOPE monolayer plus a DSPE-PEG_{2000} shell. The PLGA core was found to be dense indicating its presence inside vesicles. The average size was found to be 133.4 nm through TEM. Surface visualization and shape of the vesicle was confirmed by SEM and PLHNCs were found to spherical in the shape with the size of 130 nm approximately.

In vitro drug release studies were performed in phosphate buffer pH 7.4, phosphate buffer pH 6.6 and acetate buffer pH 5.5 for fulvestrant loaded PLHNCs and it showed sustained release pattern. Fulvestrant portrayed pH dependent release at pH 5.5 it shows highest release as compared to phosphate buffer pH 7.4. The order of release from fulvestrant PLHNCs at different pH media was pH 7.4 to pH 5.5: 8.84% < 11.24% < 15.62%. Estimation of residual solvent was checked by Gas chromatography. The USP guidelines suggests that acetonitrile is class II solvent and the limit for PDE (Permitted Daily Exposure) is 4.1 mg/day equivalent to 410 ppm. From the data of residual solvent, it was confirmed that acetonitrile present in the final optimised batch of PLHNCs was within the limits as per USP guidelines of residual solvents.

Cell line studies were performed to assess the cytotoxicity, cellular uptake of formulation on MDA MB 231 and MCF – 7 breast cancer cell lines. MTT assays of fulvestrant suspension, blank PLHNCs, F-PLHNC and FA FLV PLHNC were performed to assess the effect of lipid and polymer on cell cytotoxicity. All the blank formulations were found to be non-toxic to cell lines indicating safety of formulation components. From the images of confocal microscopy obtained for cellular uptake studies it can be concluded that the PLHNCs could successfully carry fulvestrant into cytosol. Successful engulfment into cell using PLHNCs depict that they are easily up taken by the cells through endocytosis due to their cationic characteristic. Chemosensitization of MDA MB 231 cells was confirmed by performing and comparing MTT assays of Fulvestrant suspension, F-PLHNC, and FA FLV PLHNC and it was found that FA FLV PLHNC was having IC_{50} value of 0.55 µM against 1.23 µM for FLV suspension after 72 hrs., indicating 2.23-fold reduction. From the cell migration assay it was concluded that both FLV PLHNC and FA FLV PLHNC have enhanced anti migratory effect of the fulvestrant,

which could provide a great tool for the shrinkage of the tumors and regression of tumor metastasis.

The in vivo pharmacokinetic parameters were calculated and it was found that the maximum $t_{1/2}$ value of 12 days was observed with FA FLV PLHNC compared to 16.94 hours with fulvestrant suspension after intravenous administration. Eventually, there was an increment in the AUC for PLHNCs compared to the AUC of Fulvestrant suspension. FA FLV PLHNC showed 23.2 times higher AUC values compared with AUC values of fulvestrant suspension after intravenous administration. The Tmax values for FA F-PLHNC was 38 hours compared to fulvestrant suspension which has only 3 hours, thereby confirming the maintenance of effective drug concentration with F-PLHNC in blood for prolonged period compared to FLV suspension.

The in vivo anticancer activity of various formulations was checked against chemically induced tumor in Sprague Dawley rats and compared with control groups: Normal control (no treatment, no tumor), model control (no treatment but tumor), Standard control (tumor + tamoxifen), Drug control (tumor + fulvestrant suspension), and test controls with different formulation. A significant change ($p<0.001$) in the weight of different treatment group rats was observed as compared to model control. Drug control and Standard control were found to reduce the weight due to side effect, whereas FLV PLHNC and FA FLV PLHNC were found to maintain the initial weight. According to the Kaplan Meier survival curve, all the model control rats died after 12 weeks. The rats treated with standard control showed 50 % survival and treated with fulvestrant suspension showed 66.67% survival, those treated with FLV PLHNCs showed 83.33% survival whereas the animals treated with FA FLV PLHNCs showed 100% survival during treatment (6 weeks).

Stability studies were carried out for lyophilized PLHNCs at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for three months and at long term conditions (5°C ± 3°C) up to 6 months. During stability monitoring, no significant differences ($p<0.05$) were observed in particle size, assay, and zeta potential of lyophilized PLHNCs stating the storage in the form of lyophilized formulation at refrigerated conditions.

EXEMESTANE PLHNCs

Analytical techniques were developed to estimate pure exemestane, exemestane in PLHNCs, Dissolution media (Phosphate buffer saline pH 7.4, phosphate buffer pH 6.6 and acetate buffer pH 5.5). Calibration curve of exemestane was prepared by direct UV estimation. The

calibration was plotted by measuring the absorbance at 243 nm (λmax), calibration curve was prepared for exemestane in Acetonitrile, Methanol, Phosphate buffer pH 7.4, phosphate buffer and acetate buffer 5.5 and the methods were validated for accuracy, precision, LOD and LOQ. Phospholipid content in formulation was estimated by Stewart method, calibration of total phospholipid was prepared in chloroform. For estimation of exemestane in biological samples, calibration curve of exemestane was developed by HPLC method for plain drug, in rat plasma for pharmacokinetic studies.

Different methods were screened for preparation of PLHNCs such as two-step method, double emulsion solvent evaporation and single step nanoprecipitation method to formulate PLHNCs. From this, single step nanoprecipitation with little modification followed by extrusion was found to formulate PLHNCs of desired characteristics. Preliminary studies were performed to define the ranges of formulation as well as process parameters. Preliminary studies also played significant role in determination of amount of cationic lipid needed to achieve maximum entrapment efficiency of exemestane. It was confirmed that the 30:40:30 ratio of DOPE:P90G: DSPE PEG_{2000} has enough capacity to form a lipid layer onto the polymeric surface. Further increase in lipid ratio doesn't necessarily increases the encapsulation efficiency. Total seven factors (polymer concentration (mg/mL), lipid/polymer percentage (%), drug input percentage (%), stirring speed (RPM), stirring time (h), sonication time (S) and extrusion cycle (Nos) were selected for Placket-Burman screening study. From those, three factors (i.e., concentration of polymer, lipid to polymer ratio and drug input) were selected to further optimize the design space using Box-Behnken design.

QbD enabled design expert software suggested an optimized batch having composition of polymer concentration (4mg/ml), lipid to polymer ratio (30%) and drug input percentage (18%) which possessed predicted size of 117.9 nm and 84.06 % entrapment efficiency and the same batch was formulated to validate the results and particle size was found to be 120.8 ± 2.38 nm with the PDI of 0.045 ± 0.003 and Zeta potential was found to be 6.89 ± 0.86 mV which was due to presence of cationic lipid i.e., DOPE. The entrapment efficiency was determined using Ultracentrifuge to separate entrapped and unentrapped drug. The % Entrapment efficiency was found to be 86.84 ± 3.57 % (n =3) in the optimized formulation.

The transmission electron microscopy (TEM) was performed to characterize PLHNCs structure with negative staining by uranyl acetate which stains DOPE and the lipids conjugated with PEG to enhance their electron density, resulting in dim ring surrounding the PLGA core.

The thickness of the ring is less than 20 nm, which equals the thickness of DOPE monolayer plus a DSPE-PEG_{2000} shell. The PLGA core was found to be dense indicating its presence inside vesicles. The average size was found to be 129.6 nm through TEM. Surface visualization and shape of the vesicle was confirmed by SEM and PLHNCs were found to spherical in the shape with the size of 140 nm approximately.

In vitro drug release studies were performed in phosphate buffer pH 7.4, phosphate buffer pH 6.6 and acetate buffer pH 5.5 for exemestane loaded PLHNCs and it showed sustained release pattern. Exemestane portrayed pH dependent release at pH 5.5 it showed highest release as compared to phosphate buffer pH 7.4. The order of release from exemestane PLHNCs at different pH media was from pH 7.4 to pH 5.5 Estimation of residual solvent was checked by Gas chromatography. The USP guidelines suggest that acetonitrile is class II solvent and the limit for PDE (Permitted Daily Exposure) is 4.1 mg/day equivalent to 410 ppm. From the data of residual solvent, it was confirmed that acetonitrile present in the final optimised batch of PLHNCs was within the limits as per USP guidelines of residual solvents.

Cell line studies were performed to assess the cytotoxicity, cellular uptake of formulation on MDA MB 231 and MCF – 7 breast cancer cell lines. MTT assays of exemestane suspension, blank PLHNCs, EXE PLHNC and FA EXE PLHNC were performed to assess the effect of lipid and polymer on cell cytotoxicity. All the blank formulations were found to be non-toxic to cell lines indicating safety of formulation components. From the images of confocal microscopy obtained for cellular uptake studies it can be concluded that the PLHNCs could successfully carry exemestane into cytosol. Successful engulfment into cell using PLHNCs depict that they are easily up taken by the cells through endocytosis due to their cationic characteristic. Chemosensitization of MCF – 7 cells was confirmed by performing and comparing MTT assays of Exemestane suspension, EXE PLHNC, and FA EXE PLHNC and it was found that FA EXE PLHNC was having IC_{50} value of 3.24 µM against 4.76 µM for EXE suspension after 72 hrs., indicating 1.47-fold reduction. From the cell migration assay it was concluded that both E-PLHNC and FA-E-PLHNC have enhanced ant-migratory effect of the exemestane, which could provide a great tool for the shrinkage of the tumors and regression of tumor metastasis.

The in vivo pharmacokinetic parameters were calculated and it was found that the maximum $t_{1/2}$ value of 7 days was observed with FA EXE PLHNC compared to 5.21 hours with exemestane suspension after intravenous administration. Eventually, there was an increment in

the AUC for PLHNCs compared to the AUC of Exemestane suspension. FA EXE PLHNC showed 5.59 times higher AUC values compared with AUC values of exemestane suspension after intravenous administration. The Tmax values for FA EXE PLHNC was 31.87 hours compared to exemestane suspension which has only 2.24 hours, thereby confirming the maintenance of effective drug concentration with EXE PLHNC in blood for prolonged period compared to EXE suspension.

The in vivo anticancer activity of various formulations was checked against chemically induced tumor in Sprague Dawley rats and compared with control groups: Normal control (no treatment, no tumor), model control (no treatment but tumor), Standard control (tumor + tamoxifen), Drug control (tumor + exemestane suspension), and test controls with different formulation. A significant change ($p<0.001$) in the weight of different treatment group rats was observed as compared to model control. Standard control and Drug control were found to reduce the weight due to side effect, whereas EXE PLHNC and FA EXE PLHNC were found to maintain the initial weight. According to the Kaplan Meier survival curve EXE PLHNCs showed 83.33% survival and FA EXE PLHNCs showed 100 % up to the course of treatment which suggested the improvement of efficacy of EXE with NPs compared EXE suspension.

Stability studies were carried out for lyophilized PLHNCs at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for three months and at long term conditions (5°C ± 3°C) up to 6 months. During stability monitoring, no significant differences ($p<0.05$) were observed in particle size, assay, and zeta potential of lyophilized PLHNCs stating the storage in the form of lyophilized formulation at refrigerated conditions.

MESOPOROUS SILICA NANOPARTICLES (MSNs)

First, basic skeleton of Mesoporous silica nanoparticles was fabricated based on modified Stober template-based synthesis. Thereafter, the surfactant template was removed by acid solvent reaction method. The surfactant free mesoporous silica nanoparticles were further used for post synthetic surface modification. Amination was done on the MCM – 41 MSNs by using APTES. Functionalization offers advantages like sustained release. The amination of nanoparticles also served as strong platform for further functionalization with folic acid. The initial zeta potential before functionalization was -22.7 mV, on surface functionalization with amine group the zeta potential shifted towards positive i.e., +26.5 mV, further on folic acid conjugation the zeta shifted towards negative -17.1 mV. The particle size for blank mesoporous silica nanoparticles was found to be 54.5 nm.

The surface area and porosity estimation from the nitrogen sorption studies gave a clear trend of maximum surface are and pore size obtained for MCM-41 nanocarriers. On drug loading when compared to bare carriers there was reduction in surface area due to engulfment of drug in its pores. The BET and BJH surface areas were highest for bare MCM-41 with value of 1229.12 m^2/g and 1386.56 m^2/g respectively. The trend was same for BET and BJH both the surface areas. The SEM and TEM images of the fabricated MSNs were proof of spherical and uniform morphology and intact hexagonal structure of MSNs. After complete characterization of the synthesized MSNs, drug loading was performed by solvent immersion method. The solvent used was methanol which was easily evaporated giving a facile way of obtaining drug loaded MSNs.

The Plackett-Burman study design has been implemented for screening of various formulation and process related parameters i.e., surfactant concentration (%) (factor A), Silica source concentration (%) (factor B), TEA concentration (w/w) (factor C), Ethanol concentration (%) (factor D) stirring speed (RPM) (factor E), stirring time (h) (factor F) and stirring temperature (°C) (factor G) and its impact on particle size, %yield and surface area. These parameters were assessed to be of high importance in consideration with other factors based on different trials. Based on the results of the primary factor screening design, three variables (i.e., surfactant concentration, silica source concentration and stirring temperature) were selected for further optimization using Box-Behnken design.

FULVESTRANT LOADED MSNs

Fulvestrant loaded MSNs have followed the sustained release kinetics (Figure 6.24a). From the three pH conditions, the highest release curve was observed in pH 5.5, which suggested maximum release of the drug in cancer cells. Release of the fulvestrant from the MSNs in the different media was observed to be in decreasing order of pH 5.5 > pH 6.6 > pH 7.4, which indicates the least drug release in plasma and blood. The sustained release of fulvestrant was achieved owing to the presence of drug in MSN pores. The release of fulvestrant suspension was found to be completed within 24 hours, indicating the need for dose administration frequently. There was no significant difference in the drug release pattern in different pH conditions.

Cell line studies were performed to assess the cytotoxicity, cellular uptake of formulation on MDA MB 231 and MCF – 7 breast cancer cell lines. MTT assays of fulvestrant suspension, FMSN, QMSN, FQMSN were performed. From the images of confocal microscopy obtained

for cellular uptake studies it can be concluded that the MSNs could successfully carry fulvestrant into cytosol. Successful engulfment into cell using MSNs depict that they are easily up taken by the cells through endocytosis due to their cationic characteristic. Chemosensitization of MDA MB 231 cells was confirmed by performing and comparing MTT assays of Fulvestrant suspension, FMSN, and FQMSN and it was found that FQMSN was having IC_{50} value of 0.59 µM against 2.07 µM for FLV suspension after 72 hrs., indicating 3.51-fold reduction. From the cell migration assay it was concluded that QMSN, FMSN and FQMSN have enhanced ant-migratory effect of the fulvestrant, which could provide a great tool for the shrinkage of the tumors and regression of tumor metastasis.

The in vivo pharmacokinetic parameters were calculated and it was found that the maximum $t_{1/2}$ value of 7 days was observed with FQMSN compared to 12.61 ± 0.54 hours with fulvestrant suspension after intravenous administration. Eventually, there was an increment in the AUC for MSNs compared to the AUC of Fulvestrant suspension. FQMSN showed 20.05 times higher AUC values compared with AUC values of fulvestrant suspension after intravenous administration. The Tmax values for FQMSN was 34.18 hours compared to fulvestrant suspension which had only 3.62 hours, thereby confirming the maintenance of effective drug concentration with FQMSN in blood for prolonged period compared to FLV suspension.

The survival time for different experimental rats inoculated with MNU were observed after inoculation. All the rats were monitored up to 12 weeks after first tumor palpitation and then sacrificed. The data of survival study postulated that the nanoparticles prolonged the survival of the animals, as the samples treated with standard and drug control showed death of 50 % animals (3 animals) within the course of treatment (6 weeks), whereas with FMSN there was death of only 13.33 % of animals (1 animal). The animals treated with FQMSN, showed no death, and had 100 % survival up to weeks, though 1 animal died after 2 weeks of discontinuing the treatment. So, it can be said that FQMSN showed 100 % survival rate during treatment as opposed to standard and drug control that had only 50 % survival rate.

EXEMESTANE LOADED MSNs

Exemestane loaded MSNs have followed the sustained release kinetics. From the three pH condition, the highest release was found in pH 5.5, which suggested maximum release of drug in cancer cells. Release of exemestane from the MSNs in the different media was observed to be in decreasing order of pH 5.5 > pH 6.6 > pH 7.4, which indicates least drug release in plasma and blood. From the kinetic model fitting analysis, it was concluded that for exemestane and

quercetin co-loaded MSNs, the best fit model was Korsmeyer Peppas model with the R^2 value of 0.9926, with the n value of 0.892, which is consistent with the drug release by anomalous transport or non-Fickian diffusion that involves two phenomena: drug diffusion and relaxation of the polymer matrix.

Cell line studies were performed to assess the cytotoxicity, cellular uptake of formulation on MDA MB 231 and MCF – 7 breast cancer cell lines. MTT assays of exemestane suspension, EMSN, QMSN, EQMSN were performed. From the images of confocal microscopy obtained for cellular uptake studies it can be concluded that the MSNs could successfully carry exemestane into cytosol. Successful engulfment into cell using MSNs depict that they are easily up taken by the cells through endocytosis due to their cationic characteristic. Chemosensitization of MDA MB 231 cells was confirmed by performing and comparing MTT assays of Exemestane suspension, EMSN, and EQMSN and it was found that EQMSN was having IC_{50} value of 2.08 µM against 5.68 µM for EXE suspension after 72 hrs., indicating 2.73-fold reduction. From the cell migration assay it was concluded that QMSN, EMSN and EQMSN have enhanced ant-migratory effect of the exemestane, which could provide a great tool for the shrinkage of the tumors and regression of tumor metastasis.

The in vivo pharmacokinetic parameters were calculated and it was found that the maximum $t_{1/2}$ value of 5 days was observed with EQMSN compared to 5.92 ± 0.24 hours with exemestane suspension after intravenous administration. Eventually, there was an increment in the AUC for MSNs compared to the AUC of Exemestane suspension. EQMSN showed 2.44 times higher AUC values compared with AUC values of exemestane suspension after intravenous administration. The Tmax values for EQMSN was 37.41 hours compared to exemestane suspension which has only 3.12 hours, thereby confirming the maintenance of effective drug concentration with EQMSN in blood for prolonged period compared to EXE suspension.

The survival time for different experimental rats inoculated with MNU were observed after inoculation. Rats treated with standard and drug control showed 50% survival. The samples treated with QMSN showed 66.67% survival which is 1.33 times more compared to its standard counterpart and standard drug sample. The sample with EMSN showed 83.33% survival which is 1.66 times more compared to standard and drug control. EQMSN showed 100% survival which was 1.20 times more than that of EMSN, whereas 2 times more than that of the standard and model control.

CONCLUSION

In current investigations, folic acid conjugated Polymer lipid hybrid nanocarriers and folate conjugated and dual drug loaded Mesoporous silica nanoparticles were developed for the delivery folate receptor targeted delivery of fulvestrant and exemestane respectively. The results suggested that the developed folate conjugated PLHNCs and folate conjugated quercetin co loaded mesoporous silica nanoparticles with fulvestrant and exemestane have potential to target the breast cancer cell and reduce their toxicity towards normal cells. The formulations showed sustained release of drug and the pharmacokinetic studies also supported the prolonged drug release action. The biodistribution studies for folate conjugated nanoparticles showed increased concentration of drugs within tumor cells. The in vivo anticancer activity carried out on chemical induced rat tumor model showed reduction in overall tumor burden and increased the survival rate of animals. Thus based on the obtained results it can be said that the formulated nanoparticles were capable of showing ligand responsive intracellular drug release which may help to enhance the efficacy of anticancer treatment and reduce undesirable side effects.