12.1. Introduction

Endometrial carcinoma -a tumour originating in the endometrium- is the most common gynaecologic malignancy. Globally, endometrial cancer is the sixth most common cancer in women merely the second to cervical cancer for the frequency among gynaecological cancers. The major risk factors include age, unopposed oestrogen exposure, hormone replacement therapy and obesity. EC is a disease that primarily affects postmenopausal women, with the average age of 60 at the time of diagnosis and the age-specific incidence increases steeply from 50 years. However, up to 25% of women are pre-menopausal at the time of diagnosis. If diagnosed and treated at an early stage whilst confined to the uterus, EC carries an excellent prognosis with high curability.

Current management options available for EC are medical, surgical, radiological, and genetic modalities. Surgical procedure includes hysterectomy (removal of the uterus). This operation includes removing the uterus, fallopian tubes, and ovaries. Lymph nodes from the pelvis and around the aorta may also be removed (a pelvic and para-aortic lymph node dissection (LND)) and/or sampled to examined for cancer spread. Depending on the spread of the cancer, other treatments, such as radiation and/or chemotherapy are recommended. The invasive surgical procedure has its own complications. Hysterectomy is not an appropriate option for women who wants to have pregnancy or wants to avoid surgery. If the cancer is not benign, then surgery may not be helpful, and so chemotherapy or other treatments may be used.

Chemotherapy does play an important role in un-resectable metastatic disease, surgically resected advanced disease, and recurrent disease. The role of chemotherapy in high-risk, early stage disease is also currently being studied. Multimodality therapy is also recommended presently for type II histologies because these tumours are considered more aggressive with a higher incidence of extra-uterine disease.

PTX and CBP is a more favourable regimen to use for advanced/metastatic or recurrent EC because of its comparable response rates and less toxicity. The response rate of

PTX/CBP in this setting is roughly 40% to 62% and overall survival is approximately 13 to 29 months. PTX/CBP is generally better tolerated than other agents and is the preferred regimen for most patients. Single-agent therapy options may be considered if multi-agent chemotherapy regimens are not tolerated or contraindicated.

Currently PTX is available in the form of solution for injection. In which solubility of PTX is enhanced with a mixture of 50:50 Cremophor EL® and ethanol. For administration, it must be further diluted 5 to 20 fold with normal saline or 5% dextrose solutions before intravenous infusion. In particular poly-oxyethylated castor oil is biologically and pharmacologically active and leaches plasticizers from standard intravenous tubing, releasing di-(2-ethylhexyl) phthalate (DEHP). Its infusion produces histamine release with consequent well-described hypersensitivity reactions, including anaphylaxis and neutropenia. Carboplatin possess good solubility in water (14mg/ml). On account of its water solubility, various IV injectable formulations available in the market. Various nano-carrier based systems i.e. Nanoparticles, Liposomes, Nanocapsules etc, are studied in order to achieve tumour targeting and to reduce systemic side effects. However, all these preparations are IV administered and thus associated with more chemotherapeutic side effects i.e. Low blood counts, Alopecia, Peripheral neuropathy, Arthralgias and myalgias, Abnormal ECG, Weakness and fatigue etc. Hence, for both the drugs, vaginal delivery can prove to be a promising approach targeting the drug to the site of action. Past studies have reported that drugs when administered intravaginally, targeted to the uterus where their tissue concentration is augmented and systemic absorption is decreased which limits the circulating level and side effects. This phenomenon is known as first uterine pass effect. Drugs loaded in nano vesicles reduces tissue exposure of the drug of interest and also aid in drug transport. The nano sized vesicles with ultradeformable membrane further facilitate drug transport through biological membranes to a greater extent. Hence these delivery systems could be of importance to target the uterus as site of action for EC through FUPE and EPR effect. Despite the variety of formulations for intravaginal therapy like creams, gels, tablets, suppositories, their efficacy is often limited by a poor retention at the site of action,

leakage and messiness. A prolonged vaginal residence time can be achieved by the use of Intravaginal Rings (IVR).

The present study was aimed to target the drugs used for the management of EC i.e. PTX and CBP to uterus by vaginal route to achieve maximum therapeutic effect by avoiding the systemic absorption to reduce the side effects of drugs and provide a safe, economic and patient friendly therapy for EC.

12.2. Analytical methods

HPLC methods for quantitative estimation of PTX and CBP were developed and validated. HPLC system (LC-20AT/SPD-20A; Shimadzu, Japan) with Supelco® C18 column (Sigma-Aldrich, India) was used.

For PTX – mixture of acetonitrile and water [ratio, 60:40 v/v] was used as mobile phase at a flow rate of 1 mL/min. The chromatogram was generated at 227 nm wavelength and calibration plot was generated with linearity range of 2 to 10 µg/ml and R^2 value of 0.9995.

For CBP – mixture of acetonitrile and water [ratio, 20:80 v/v] was used as mobile phase at a flow rate of 1 mL/min. The chromatogram was generated at 230 nm wavelength and calibration plot was generated with linearity range of 2 to 18 µg/ml and R^2 value of 0.9996.

12.3. Preformulation Studies and Preliminary Development

Identification of both the drugs was performed using FTIR and DSC analysis. Drug-excipient compatibility was also performed using DSC to ensure safe and stable formulation development. Preliminary trials were carried out to formulate ultradeformable vesicles. Preliminary trials were conducted to address formulation challenges associated with the lipophilic drug PTX and hydrophilic drug CBP. Selection of lipids and other excipients best suited for individual drug formulation was done by performing various experimental trials. Crystallization of PTX was thoroughly understood by performing various experiments. Processing techniques were discovered to reduce the crystallization of PTX in order to ameliorate its entrapment

in formulation. Methods to improve entrapment of CBP were studied and successfully implemented in the formulation development. Various process parameters affecting critical quality attributes of the formulations were studied and the preferred ranges/levels derived.

12.4. Formulation Development- Paclitaxel UDNVs

PTX loaded UDNVs formulation was prepared using thin film hydration method. A mixture of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC; Tg: 55 °C) and 1,2-Diacyl-sn-glycero-3-phosphocholine, Egg (EggPC Tg: −5 to −15 °C) was used as bilayer components together with sodium deoxycholate (SDC) as edge activator. When stored between the glass transition temperatures (Tg) of the two lipids, the lipid with higher Tg forms a gel state while lipid with lower Tg forms a liquid crystal state. The presence of several gel and liquid crystal phase in lipid bilayers improves entrapment of hydrophobic drugs and stabilize vesicular system by restricting lateral movement and aggregation. The quality by design (QbD) approach was employed for the development of the formulation. Based on the scientific, therapeutic, industrial and regulatory aspects, quality target product profile (QTPP) elements and their targets were laid down. Similarly, based on the prior knowledge, literature review and experiment trials, three response variables viz., %EE, Deformability and Vesicle size were focused for screening of CMAs and CPPs using Plackett-Burman design. Minitab® 16.1.1 statistical software was used for screening of various factors. Based on the results of screening design two CQAs viz., %EE, and Deformability were finalized to be focused during optimization of the formulation. Design-Expert® 7.0.0 was used to optimize the formulation using 3^3 Box-Behnken response surface design. Batches were prepared, evaluated for CQA and data obtained were entered in software for statistical processing. The software suggested quadratic model. The model terms with p-value less than or equal to 0.05 (α-level) were considered as significant while removal of insignificant model terms (p-value > 0.1) was done using backward elimination to simplify the model equations. ANOVA of both the CQAs showed high correlation coefficients (R^2) value indicating better prediction of responses by the model. A good

agreement of Predicted R^2 with adjusted R^2 also supported the prediction potential of the model. Diagnostic plots further demonstrated the normal distribution of data, constant variance and easy prediction of values from the model. Numerical optimization for achieving minimum maximum entrapment and deformability resulted optimization solutions with composite desirability of 0.966. The optimized composition showed %EE of 91.27 ± 1.26 and deformability of 25.61 ± 1.18 which falls within 95% confidence interval. The optimized formulation was freeze dried in medical grade silicone tubing as vesicular gel to form intravaginal rod inserts.

12.5. Formulation Development- Carboplatin UDNVs

CBP loaded UDNVs formulation was prepared using thin film hydration method. A mixture of 1,2-Diacyl-sn-glycero-3-phosphocholine, hydrogenated (HSPC; Tg: 52 °C) and 1,2-Diacyl-sn-glycero-3-phosphocholine, Soy (SPC; Tg: −20 to −30 °C) was used as bilayer components together with sodium deoxycholate (SDC) as edge activator. The combination of lipids in the bilayer that resulted in to separate two phases, a gel phase and a liquid-crystalline phase. The coexistence of these two immiscible phases in the membrane was said to create discontinuous regions in the bilayer, which reduced the movement and aggregation of the drug, resulting in increased stability of the formulation. The quality by design (QbD) approach was employed for the development of the formulation. Based on the scientific, therapeutic, industrial and regulatory aspects, quality target product profile (QTPP) elements and their targets were laid down. Similarly, based on the prior knowledge, literature review and experiment trials, three response variables viz., %EE, Deformability and Vesicle size were focused for screening of CMAs and CPPs using Plackett-Burman design. Minitab® 16.1.1 statistical software was used for screening of various factors. Based on the results of screening design two CQAs viz., %EE, and Deformability were finalized to be focused during optimization of the formulation. Design-Expert® 7.0.0 was used to optimize the formulation using 3^3 Box-Behnken response surface design. Batches were prepared, evaluated for CQA and data obtained were entered in software for statistical processing. The software suggested quadratic model. The model terms with

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12.6. Formulation Characterization

The optimized formulations of PTX and CBP were characterized for drug entrapment, loading, deformability, vesicle size, zeta potential, morphological analysis by TEM and in vitro drug release. The developed formulations were also characterized for their tissue permeation behaviours ex vivo. The optimized PTX formulation showed vesicle size and zeta potential of 214.2 ± 4.22 nm and -34.3 ± 2.32 mV respectively. The optimized CBP formulation showed vesicle size and zeta potential of 293.7 ± 3.17 nm and -28.1 ± 1.54 mV respectively. The drug loading was 8.92 ± 0.12 % w/w and 8.22 ± 0.24 % w/w for PTX and CBP formulations respectively. The deformability index was 25.61 ± 1.18 and 89.13 ± 3.44 for PTX and CBP formulations respectively. TEM analysis revealed that both the formulations contained discrete, spherical and uniform vesicles with size in agreement with that obtained by dynamic light scattering. The PTX loaded UDNVs formulation was able to sustain drug release for 144 h while more than 95% of plain drug suspension was released within 18 hr. CBP loaded UDNVs formulation was able to sustain drug release for 120 h while almost all drug from the plain drug solution was released within 8 hr. Various mathematical models were applied to define pattern of drug release where both PTX and CBP loaded formulation showed zero order drug

release with R^2 value of 0.985 and 0.991 respectively. The total amount of drug penetrated through the vaginal membrane after 24 h were 1460.75±45.16 & 257.45±31.73 µg/cm^2 in the case of PTX-UDNVs and Plain drug suspension respectively. The value of Jss for PTX-UDNVs was 1.6 times higher than that of plain drug. Moreover, the % drug permeation after 24 h was 5.7 times high than that of plain drug. The total amount of drug penetrated through the vaginal membrane after 24 h were 1109.24±92.8 & 164.07±38.83 µg/cm^2 in the case of CBP-UDNVs and Plain drug solution respectively. The value of Jss for CBP-UDNVs was 5.9 times higher than that of plain drug. Moreover, the % drug permeation after 24 h was 6.8 times high than that of plain drug.

12.7. Biocompatibility and Safety Assessment

Histopathology study using goat vaginal tissue was performed to observe the pathological changes in the linings of vaginal mucosa in contact with the drugs and drug loaded formulations, administered intra vaginally to target the uterus. Results revealed no signs of irritancy or damage to the mucosal tissue upon contact with the UDNVs formulations of both the drugs.

The haemolytic toxicity of the developed formulations of PTX and CBP was assessed qualitatively and quantitatively with comparison to that of marketed formulations of both the drugs. The qualitative and quantitative results revealed significantly lower haemolysis for the developed formulation of CBP and PTX as compared to the marketed formulations of respective drugs. The results indicate that the developed formulation are more safe and biocompatible to that of marketed formulations.

12.8. Cell line studies

In vitro cytotoxicity studies (MTT Assay) were performed on MCF-7 cell line at different concentrations (0.001, 0.01, 0.1, 1, 10 µg/ml) of drug loaded UDNVs formulations and marketed formulations at three different incubation times (12, 24 and 48 hours). The results revealed that the UDNVs formulations of both the drugs were more effective in causing cell toxicity than the marketed formulations of respective drugs. The IC50

values of both the formulation were lower than that of marketed formulations indicating that lower dose of UDNVs formulation is required to inhibit cell growth than the marketed drug formulations. The quantitative cellular uptake studies revealed significantly larger mean fluorescence intensities than the plain dye solution representing augmented intracellular uptake of the developed formulations.

12.9. In-Vivo Biodistribution and Efficacy study

In vivo biodistribution studies were carried out to examine the uterine targeting efficiency of the developed formulation using ^{99m}Tc labelling. Radiochemical purity of labelled PTX was found to be 96.5 ± 1.2 % even after 48 h of incubation. The results indicate that the PTX was successfully labelled using ^{99m}Tc. The dissociation of the ^{99m}Tc and the CBP from the complex was only 23.62 % which was reduced to 2.55 % at the end of 3 h incubation time indicative of lack of suitability of CBP for bio distribution studies. The results of radioactivity after incubation of 48 h revealed that % radiolabelling of 94.6 ± 1.3 and 91.9 ± 1.5 exhibited by PTX-^{99m}Tc in normal saline and serum respectively. The outcomes of the in vitro stability studies are indicative of its suitability to use for in vitro studies. DTPA Transchelation Study demonstrated that the labelling efficiency of the radio labelled complex did not alter significantly in the presence of DTPA indicating the higher binding affinity of technetium with PTX. When ^{99m}Tc-PTX loaded UDNVs were administered via vaginal route, maximum amount of drug was found in uterus even after 48 hours with no distribution in any other organs of the body. In the case of ^{99m}Tc-PTX plain drug administration, comparatively very less amount was able to retain in the uterus after 48 hours. The quantitative systemic absorption study showed that the systemic absorption was considerably lower in the case of ^{99m}Tc-PTX loaded UDNVs formulation compared to plain drug when administered though intravaginal route.

The efficacy of the developed formulations was evaluated on the EC induced rabbit model to establish the effectiveness of developed formulations. Two different rabbits, induced with EC were administered PTX-UDNVs and CBP-UDNVs loaded intravaginal rod (IVR) inserts. After four weeks of the treatment with PTX-UDNVs and CBP-UDNVs,

the lumens of the uterine horns in both the groups of rabbits were observed clear of cellular mass indicating regression of the induced EC.

12.10. Stability Studies

Three months short term stability study of developed UDNVs formulations and IVRs of both the drugs was performed at two different stability conditions i.e. refrigerated (2-8 °C) and at Room Temperatures (25 -30 °C) as per ICH Q1A(R2) guidelines. The UDNVs formulations were monitored for % Entrapment efficiency (%EE), Deformability (%) and mean vesicle size while the IVRs were evaluated for in vitro drug release during stability. No significant change in these parameters were observed during three months of storage supporting the stability of developed formulations.

12.11. Conclusion

PTX-UDNVs and CBP-UDNVs were successfully prepared and optimized to have maximum drug entrapment and deformability using Plackett-Burman screening design and 3^3 Box-Behnken response surface design. In vitro characterization revealed spherical shape, uniform size distribution, desired zeta potential and prolonged drug release over 144 h and 120 h for PTX and CBP loaded UDNVs respectively. Ex vivo studies revealed augmented tissue permeation. Histopathology and haemolytic toxicity studies supported biocompatibility and safety of the developed formulations. Biodistribution study by Gamma Scintigraphy revealed the preferential uptake of liposomes by the uterus when the formulation was administered by vaginal route. Cell toxicity studies revealed higher % cell inhibition, lower IC50 values and significantly high quantitative uptake for drug loaded UDNVs compared to the marketed formulations. In vivo biodistribution studies using Gamma Scintigraphy revealed preferential uptake of drug loaded UDNVs by uterus when administered through vaginal route while negligible distribution in blood. In vivo studies in rabbit model showed maximum regression of induced EC for drug loaded UDNVs IVR administered through vaginal route. The developed formulations were found stable during short term stability studies at refrigerated and room temperature.

In a nutshell, uterine targeting of drugs via – a non-invasive – vaginal route seems to be a promising approach for the treatment of disorders related to uterus like EC. The uterine tissue concentrations of the administered drug transcends the systemic distribution after vaginal administration favouring a dose reduction to elicit the therapeutic action and thereby circumvention of the systemic side effects. Extended research involving preclinical and clinical trials may further prove the potential of the formulation in drug targeting to the uterus via vaginal route.