INTRODUCTION

Ovarian Cancer (OC) is a cancer that begins in the ovaries. Ovaries are the female reproductive gland each about a size of grape located on the either side of the uterus. There are more than thirty different types of ovarian cancers which are classified based on the type of cells from which they are originated. Most commonly occurring OCs are that occurs from the abnormal growth of epithelial cells, stromal cells and germ cells of the ovary. From the data of American Cancer Society, in 2018, there will be approximately 22,240 new cases of ovarian cancer diagnosed and 14,070 ovarian cancer deaths in the US. The treatments of the ovarian cancer include surgery, chemotherapy, immunotherapy and radiotherapy. Among the chemotherapeutic drugs, Paclitaxel is a first line drug which is approved by the FDA for the treatment of OC. It is a naturally occurring diterpenoid isolated from *Taxus bravifoliais* (1). It acts by stabilizing the microtubules of the cell where it binds to tubulin, and interfere with normal function of microtubule growth. It inhibits late G2 or M phase of the cell cycle and thereby inhibits cell replication. It is a first line drug for the treatment of all forms of ovarian cancer but suffers from the disadvantage of poor water solubility and nonspecific biodistribution which causes serious side effects to the human body. Owing to its low water solubility, it was formulated in a mixture of cremophore EL and ethanol (50:50% v/v), a marketed combination known as “Taxol” which suffers from serious side effects like allergic reactions, neutropenia, and neuropathy to the patients. Therefore it is the need of the hour to develop some alternative formulations of PTX which are free from cremophore EL. Genistein (Gen) (4', 5, 7-trihydroxyisoflavone) is a naturally occurring soy

isoflavonoid, possessing anticancer, antiproliferation, antioxidant-like properties which makes it suitable to be used as anticancer medicine for the treatment of various cancers including genital and ovarian cancer (1). Genistein is well known in the prevention of Poly cystic ovarian disease (PCOD). In spite of its strong antineoplastic activities, Genistein somehow, did not find the place in the market due to its flaws for human use (2). It is sensitive to heat, light, and oxidation and also suffers from less oral bioavailability due to poor water solubility. Numerous studies had revealed that bioavailability profile of poorly water-soluble drugs could be controlled by their entrapment in nano-colloidal systems. Accordingly, a number of systems have been investigated for Gen and PTX formulation; however, the trails were unsuccessful because of the insufficient solubilizing capacity and poor stability of the formulations. So, there is a strong demand of the time to have some formulation system, which will be able to deliver a maximum of the administered dose to the target site with least side effects. To circumvent the difficulties of the currently existing system of the drug, nanoformulation systems can provide a better way out.

Nanotechnology is being applied extensively to provide targeted drug therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology. Both the lipid based and polymer based nano drug delivery systems proved to be highly efficient for the delivery of chemotherapeutics drugs. They act by Enhanced Permeation and Retention effect (EPR) as they get escape from the capillaries through pores in the cancer tissues due to their nano size. The lipids employed to prepare lipid nanoparticles are usually physiological lipids (biocompatible and biodegradable) so, that drugs can be delivered at the required site of action with the

controlled release with low acute and chronic toxicity. Among the lipid formulation, the solid matrix containing nanoformulations are more popular. Two types of solid matrix formulations can be prepared namely, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). NLCs had been introduced at the end of the 1990s by R.H. Muller (2, 3). They are the solid matrix lipid formulations, formulated by the blend of spatially different solid as well as liquid lipids which adds more imperfections in the molecule to accommodate more amount of drug. A small amount of liquid lipid is added to the formulation usually 70:30 or 60:40, solid lipid to liquid lipid ratio. Regardless of the presence of liquid lipids, NLC matrix is solid at room temperature (2-6). They are much stable then the SLNs due to the formation of imperfect crystal lattice will be formed on storage which will not lead to the drug leakage. Poly (lactic co glycolic) acid (PLGA) is one of the most widely used biodegradable, non toxic polymer which is certified as safe by GRAS (Generally regarded as safe) (7-9). In the past decade, PLGA nanoparticles had shown their potential in improving the non specific biodistribution and thus improved the oral bioavailability of therapeutic agents of different classes.

As the methods used for the formulation of NLCs are complex and are greatly influenced by the variables involved, optimization of critical parameters by conventional methods is not possible. However, the use of most advanced principles of design of experiments (DoE), reduces the number of trials to a larger extent still the runs needed to perform are significantly high and poses the burden of higher cost of materials and workforce required to carry out this work. The various steps of DoE include: (a) define objective (b) identify critical quality attributes (c) screening of most

influencing factors (d) experimental design (e) analysis of responses (f) optimization process and (g) validation of methodology (10, 11). Among the various experimental designs, Placket-Burman (PB) factorial design was chosen for preliminary screening of factors owing to its ability to choose most influential factors with fewer runs. Further, the optimization of the best formulation was carried out by response surface methodology (RSM) based Box Behnken Design (BBD) followed by desirability approach based numerical optimization technique.

In this perspective, the aim of the present study was to develop the nanoformulations (Lipid based nanostructured lipid carriers; NLCs & PLGA based polymeric nanoparticles) of Paclitaxel and Genistein and evaluate their potential for the treatment of ovarian cancer both in combination and as single entity so that the toxicity and hypersensitivity reactions caused by paclitaxel can be prevented. We hypothesized that by combination of phytoestrogen (Gen) with the semisynthetic drug (PTX), as the dose of PTX will be reduced, the dose related toxicity of PTX will be reduced. Further, due to sustained release characteristics of nanoformulations, dosing frequency may be reduced, causing more patient compliance. Reduction in dose of PTX will provide safe and effective treatment of ovarian cancer.

The study was divided into 2 parts: Nanostructured lipid carriers (NLCs) of PTX, Gen and their combination and PLGA based polymeric nanoparticles of PTX, Gen and their combination. NLCs were formulated by Emulsification solvent evaporation technique and Polymeric nanoparticles were formulated by nanoprecipitation technique. Response surface methodology based Box Behnken design followed by desirability based numerical optimization technique was utilized for the optimization studies. The

optimized nanoformulations were subjected to different characterization for parameters like particle size, surface charge, morphology, entrapment efficiency, drug-excipient interaction, drug release studies, stability studies, cytotoxicity, evaluation of haemolysis, platelets aggregation, pharmacokinetic studies, *in vivo* anticancer activity & biochemical estimations.