Cancer is recognized as a genetic disease in which cells display a diverse array of genetic alterations including gene rearrangements, point mutations, and gene amplification (Feinberg et al., 2006). Cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008. There are over 200 different types of cancer reported all over the globe. Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year (Madani et al., 2011). In 2008, approximately 12.7 million cancer cases were reported, causing approximately 7.6 million cancer deaths, out of which 64% of the deaths were reported from economically developing countries (Ferlay et al., 2010).

There are several modes of conventional treatment options available like surgical excision to remove cancerous parts, radiation therapy, and chemotherapy, but all these have their own limitations. Surgery cannot be applied for all types of cancers and might result in loss of organ, coupled with the risk of cancer recurrence. The radiation approach kills cancerous cells, but it also damages the surrounding healthy cells (Singh & Nehru, 2008). Approximately 85% human cancers are solid tumors and can be surgically removed as a first method in anticancer therapy. After surgical removal, the remaining cancer cells and tissue are treated with radiotherapy, chemotherapy, immunotherapy, gene therapy, and hyperthermia. The most effective treatment for cancer is cytotoxic chemotherapy. The objective of chemotherapy is total cell kill resulting in a total cure. However, achieving total cell kill is a difficult task especially once the cancer has metastasized. Reasons for anticancer therapy failure have been attributed to poor drug selectivity to the diseased tissue, acquired multidrug resistance, heterogeneous biology of growing tumors, low solubility of drug at physiological pH, and suppressed host immune system (Chidambaram et al., 2011). Achieving a dose that would completely eradicate malignant cells is problematic because such a dose would result in elevated toxic levels contributing to patient mortality (Sinha et al., 2006).

The destruction of cancer cells with a minimum harm to healthy tissues and delivery of high doses of drug molecules to tumor sites for maximum treatment efficacy is the need of the hour.

Cancer nanotechnology is a rapidly growing field and has made a remarkable contribution to treatment strategies by enabling a site-specific release of chemotherapeutic agents, based on their physicochemical characteristics and biological
attributes (Cho et al., 2008; Ranganathan et al., 2012). Several stability and drug-payload studies on nanoparticulate formulations have shown that they are quite stable with high carrier capacity, and are suitable for administration of both hydrophilic and hydrophobic substances by various routes (Gelperina et al., 2005). There are a variety of nanoparticulate systems currently being explored for cancer therapeutics (Haley & Frenkel, 2008).

Looking for new compounds, doctors and scientists are increasingly focusing on substances from plants used in traditional medicine. About three quarters of the natural pharmaceutical compounds commonly used today are derived from plants of the traditional medicine of the people in various parts of the world. The science of traditional medicine is supposed to add a step on to the curative aspects of cancers. In the present study, such a compound i.e. andrographolide (AD) was selected for the study, which is from natural origin having anticancer activity.

AD, one of the major constituents of *Andrographis paniculata* Nees., is a diterpene lactone, which has anticancer activity *in vitro* in many tumor cell lines including leukemia, myeloma, HeLa, colon (HT-29), human peripheral blood lymphocytes (HPBLs), human breast cancer MCF-7 and many more. It is responsible for most of the pharmacological properties of the herb including the anti-cancer activity (Jarukamjorn & Nemoto, 2008; Ojha and Ahmed, 2010). The compound is able to induce a G0/G1 cell-cycle arrest in various kinds of cancer cell, activates the death receptor pathways, induces TRAIL (TNF-related apoptosis-inducing ligand)-mediated apoptosis and causes inhibition of NF-kB transcriptional factors and various angiogenic factors (Varma et al., 2011). The major problem associated with drug is its low aqueous solubility (3.29 ± 0.73 µg/ml), high lipophilicity having log P value = 2.632 ± 0.135 and low bioavailability (Chellampillai & Pawar, 2011). Therefore, an attempt has been made to incorporate the drug into solid lipid nanoparticles (SLNs) to improve its solubility and oral bioavailability.
potential for acute and chronic toxicity (Cavalli et al., 1993; Estella-Hermoso de Mendoza et al., 2009).

The present study was an attempt to design and characterize a stable nanoparticulate formulation of AD (AD-SLNs), along with in vitro and pharmacokinetic studies, in order to increase its aqueous solubility, stability and thereby increasing the oral bioavailability.

Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) using water and acetonitrile in a ratio of 10:90 (v/v), and High Performance Thin Layer Chromatography (HPTLC) using toluene: ethyl acetate: formic acid in the ratio of 5: 4: 1, v/v/v as mobile phases, were developed and validated as per the International Conference on Harmonization (ICH) guidelines, and applied for the quantification of AD in SLN-formulation. The retention time by RP-HPLC and retardation factor by HPTLC were found to be 2.2 min and 0.24 ± 0.01, respectively. The calibration curves of AD showed good linearity with regression coefficients 0.9932 by RP-HPLC and 0.9904 by HPTLC, respectively. Both the methods were validated for accuracy, precision, repeatability, robustness, Limit of detection (LOD) and Limit of quantitation (LOQ).

A rapid, sensitive, fast and high throughput ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC/Q-TOF-MS) method was developed to study the pharmacokinetics of AD-SLNs in rat plasma using, carbamazepine as an internal standard. The recovery of analyte from plasma samples was optimized using liquid-liquid extraction. Chromatography was performed on a Waters ACQUITY UPLC™ BEH C18 (100.0 mm x 2.1 mm; 1.7 µm) column, using isocratic mobile phase consisting of acetonitrile: formic acid (10: 90, v/v). The method was validated over the linearity range of 0.051-50 ng mL^{-1}. The intra-day and inter-day precision and accuracy fulfilled the international acceptance criteria.

The drug was identified on the basis of DSC, UV, FTIR, NMR and mass spectral analysis. The drug exhibited absorption maxima at 227 nm in UV and absorption bands of C=O, C=C, C-O-C of lactone ring and O-H group of alcohol by FTIR. The proton and $^{13}$C NMR showed peaks for different types of proton and $^{13}$C, as reported in literature. The $m/z$ ratio for AD was 331.26 in negative ion mode, as its molecular mass
is 350.45 g mol\(^{-1}\) also confirmed the purity. On the basis of these studies, it was proved that the drug sample was authentic and pure. Drug-excipient compatibility studies showed no interaction between the excipients and drug.

Phase solubility studies in different organic solvents revealed that the cetyl alcohol and gelucire 50/13 showed good solubility amounting to 300 mg dissolved in ethanol. These two lipids were selected for the formulation of AD-SLNs. AD-SLNs were prepared by three different methods (Melting dispersion, solvent-emulsification evaporation and injection methods). The solvent injection method was optimized on the basis of smaller particle size and high entrapment efficiency. The solvent injection method offers several advantages like the use of pharmaceutically acceptable organic solvents, easy handling, avoidance of high pressure homogenization, less time-consumption and no involvement sophisticated equipments/ instruments (Schubrt and Muller-Goymann, 2003). This method is based on lipid precipitation from a dissolved lipid in solution. The results demonstrated successful preparation of AD-SLNs, using two lipids (cetyl alcohol and gelucire 50/13, seperately), tween 80 as an emulsifier (0.2% and 0.5% v/v), polyvinyl alcohol as a stabilizer (0.2% and 0.5% w/w), and ethyl alcohol as an organic solvent.

Optimization of formulation and process variables was performed to evaluate how the physicochemical properties of the SLN were influenced by systematically varying formulation and process parameters. The formulation variables were optimized using the surface-response method, the results showed that 0.2% concentration of stabilizer and surfactant in the ratios 1:10, v/v, aqueous: lipid phase as 25:1, v/v and drug: lipid ratio as 1:10 w/w gave the smallest particle size (152.4 nm) and uniform size distribution (PDI-0.197). The process variables were optimized as rate of stirring 2000 rpm, time of stirring 60 min at 25°C temperature. SLN dispersions were lyophilized using 10% sucrose as cryoprotectant to stabilize the SLN and the lyophilizates exhibited good re-dispersibility.

The SLNs were characterized for entrapment efficiency, drug loading, particle size, zeta potential, surface morphology and shape by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) and for crystallinity by differential scanning calorimetry (DSC) and X-ray diffraction studies (XRD). Average entrapment efficiency and average drug loading of AD-SLNs prepared by cetyl alcohol were 91.4 ± 0.4% and
18.60 ± 0.15%, respectively. For gelucire 50/13, the average entrapment efficiency and average drug loading of AD-SLNs were 87.5 ± 2.19% and 17.50 ± 0.43% and 91.4 ± 0.4% and 18.60 ± 0.15% , respectively. The yield, which refers to the quantity of SLNs recovered from the preparation process and calculated as the percentage ratio of the lipid amount in the suspension to the theoretical amount, was found to be 95.20 ± 2.3% for gelucire 50/13 SLNs and 95.72 ± 1.83% for cetyl alcohol SLNs. The developed and validated RP-HPLC and HPTLC methods were applied for quantitative estimation of AD in SLN formulation developed. The content of AD from SLNs prepared by gelucire 50/13 was 95.3 % by RP-HPLC method and 96.4% by HPTLC methods. The content of AD from SLNs prepared by cetyl alcohol was 96.0% by RP-HPLC method and 96.59% by HPTLC methods. The average particle size of an optimized AD-SLN formulation using cetyl alcohol and gelucire 50/13 were 154.1 ± 10.7 nm with PDI 0.172 ± 0.07 and 183.9 ± 8.2 nm with PDI 0.114 ± 0.05. The TEM and SEM images showed spherical to oval particles that were less dense in the core with a well-defined shell and the particle size was in agreement with the particle-size-analysis data obtained by DLS. The zeta potential was -40.3 ± 0.8 for cetyl alcohol-derived AD-SLNs and -32.3 ± 1.2 for gelucire 50/13-derived AD-SLNs. The thermogram of the drug showed a sharp melting peak at 230.38°C. The melting endotherm of the drug was almost absent in the thermogram of AD-loaded SLN, which indicates that AD was completely solubilized inside the lipid matrix of the SLN and did not occur in crystalline state in the formulation. The sharp peak of AD in the XRD pattern was indicative of the crystalline nature of drug. The characteristic peak for AD was absent in the AD-SLN. The crystalline peaks of AD were overlapped with the noise of coated lipid.

Dissolution studies were performed to compare the release of AD from SLNs with the standard AD suspension. The highest release (98.23%) was obtained for standard AD in 4 h, whereas release of AD from cetyl alcohol SLNs was 33.603% and that from gelucire 50/13 SLNs was 54.71% in 4 h, indicating the initial burst release of drug that was present at the surface of the SLNs. Subsequently, AD-SLNs showed sustained-release property and the accumulated drug release percentage was approximately 77.89% for cetyl alcohol and 73.0% for gelucire 50/13 in 36 h. The results endorsed the earlier studies showing a sustained/controlled release pattern of drug loaded SLNs (Li et al., 2009).
No dramatic increase in the size of the AD-SLNs was observed after three months of storage at 4°C. The entrapment efficiency of AD-SLNs decreased by about 4% and particle size increased by approximately 13% in case of cetyl alcohol, whereas with gelucire 50/13, the entrapment efficiency decreased by 1.5% and particle size increased by 17% at 25°C.

The drug concentration in plasma after oral administration of AD and AD-SLNs to albino wistar rats, was determined by the in-house developed and validated UPLC/QTOF-MS/MS method. The AUC of cetyl alcohol- and gelucire 50/13-derived SLNs after oral administration was 3.41-fold and 3.25-fold higher respectively, than the AUC of drug suspension of AD. The C_{max} of cetyl alcohol- and gelucire 50/13-derived SLNs was 3.92-fold and 3.56-fold greater respectively, than the C_{max} of drug suspension of AD. The t_{max} was higher than that of AD suspension, indicating the sustained/controlled release pattern of AD-SLNs. The AUC and C_{max} of cetyl alcohol-derived SLNs showed a 1.04-fold and 1.10-fold increase respectively, over the AUC and C_{max} of gelucire 50/13-derived SLNs. However, the t_{max} was the almost similar for both types of SLN.

Although the entire characters of both AD-SLN of cetyl alcohol and gelucire 50/13 were comparable, however the AD-SLNs prepared using cetyl alcohol were better in terms of size (154.1 nm), PDI (0.172), entrapment efficiency (91.4%), release rate (77.89% release in 36 h), pharmacokinetic analysis (AUC: 37.044 ± 9.15 µg h mL^{-1}; C_{max}: 3.272 ± 0.7 µg mL^{-1} and % relative bioavailability: 340.99%). Therefore, AD-SLNs-CA were considered to be final optimized formulation and subjected to antitumor activity.

The antitumor activity was carried out in vitro on different cell line (MCF-7: Human breast cancer cell line; HT-29: Human colon cancer cell line; Hep G2: Human Liver cancer cell line; SiHa: Human cervical cancer cell line; colo 320: Human colon cancer cell line; A549: Human lung cancer cell line; Hela: Human cervical cancer cell line) as well as in vivo on Balb/c mice, using the cetyl alcohol-derived AD-SLNs.

The AD and AD-SLNs showed a relatively dose-dependent inhibitory effect on the proliferation of all tested cell lines in the concentration range of 200-1.5 µg mL^{-1}. The percent cell inhibition was the maximum at a concentration of 200 µg mL^{-1} of AD and
AD-SLNs at 72 h, and the same pattern was observed in all the cell lines. According to 50% inhibition of cell proliferation, the order of sensitivity of the cell lines to this drug was MCF-7 > Hep G2 > A549 > colo 320 > HeLa > SiHa > HT-29 (Table 26). The IC$_{50}$ value of AD and AD-SLNs in MCF-7 was the least, when compared with all other cell lines. This means only 20.03 ± 1.204 µg mL$^{-1}$ and 18.25 ± 3.427 µg mL$^{-1}$ of AD and AD-LNs was sufficient to decrease the 50% population of cells. The highest IC$_{50}$ value was obtained with HT29 cells i.e. 39.01 ± 2.345 µg mL$^{-1}$ for AD and 35.97 ± 7.520 µg mL$^{-1}$ for AD-SLNs. The IC$_{50}$ values of AD-SLNs were lesser than the AD against all the tested human cancer cell lines. The results showed that AD-SLNs possessed more antitumor effect than AD. Wang et al (2012) suggested that the cellular uptake of SLNs could be accelerated because of non-specific internalization of SLNs into cells via endocytosis or phagocytosis. Because of increased solubility and dissolution rate of the drug-loaded SLNs, molecular concentration will be high around the cells.

The in vivo antitumor study revealed that the developed AD and AD-SLNs have a remarkable anti-tumor activity against Ehrlich ascites tumor cells treated mice. In EAT tumor bearing mice, a regular rapid increase in the ascetic tumor volume was observed. Treatment with AD and AD-SLNs decreased the tumor volume, tumor weight, viable tumor cells and increased life span of the tumor-bearing mice by decreasing the nutritional fluid volume and arresting the tumor growth. In EAC control group, the MST was 18.5 ± 1.8 days, which was significantly increased up to 27.6 ± 2.58 days in AD-treated group (group IV) and 33.1 ± 2.1 days in AD-SLNs treated group (group V) and 41.33 ± 2.338 days in 5-FU. The MST of AD-SLNs was significantly higher than that of AD and comparable to 5-FU. The Kaplan Meier curve showed that the survival rate was significantly increased in treated groups (Group III, IV and V) when compared with EAC control (Group II). The weight of animals in EAC group was increased from 26.5 ± 2.88 to 47.3 ± 3.38 and a minimal change was observed in treated groups (AD, AD-SLNs and 5-FU). Comparative analysis of various hematologic parameters in all the treated and control animals clearly showed that there was no significant alteration except for marginal variation in some parameters that was still within the normal range. In conclusion, the AD-loaded SLNs would potentially be useful for delivering poorly water-soluble AD with an enhanced bioavailability and improved antitumor activity.