ABSTRACT
Cancer is one of the most widespread and feared diseases in the world today—feared largely because it is known to be difficult to cure. The main reason for this difficulty is that cancer results from the uncontrolled multiplication of subtly modified normal human cells. The main method of modern cancer treatment is chemotherapy. The majority of drugs used for the treatment of cancer today are cytotoxic in nature that works by interfering in some way with the operation of the cell's DNA. A major challenge is to design new drugs that will be more selective for cancer cells, and thus have lesser side effects. Initially the specificity of drugs was worked out simply by testing on animals. But now it is possible to use the knowledge of cancer cell biology to actively design such drugs that are more specific. Animal tests nevertheless are still needed to know the efficiency of the drugs. Currently cancer treatment is carried out by radiation therapy, surgery and cytotoxic drugs. All of these methods have significant limitations. Drugs however offer the only approach to treat cases where the cancer has spread (metastasised) through the body. Recent developments have led to drugs with novel actions that are highly specific to cancer cells.

The use of dietary constituents as an alternative to the conventional medicines for cancer chemoprevention in the form of conjugated anticancer drug has become an effective approach for the treatment of cancer. Among dietary constituents unsaturated fatty acids (FA), while originally considered to be used by the body to store energy are now known to play important structural and signaling roles in altering the expression pattern of proteins. Evidences have shown that ω-3, ω-6 and ω-9 unsaturated FAs present in natural sources like castor oil, olive oil, almonds, peanuts, red meat, fish oil etc. can control several types of cancer. The inhibitory effects of ω-9 monounsaturated oleic acid on cancer development and progression are shown in cultured cells and animal models. Moreover, preclinical studies on ω-3 and ω-6 polyunsaturated fatty acids (PUFA) like docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid and linoleic acid are also observed to enhance the cytotoxicity of several antineoplastic agents. Therefore, the inhibitory effects of unsaturated FAs on cancer development and progression by
inhibition of tumor cell proliferation, induction of apoptosis and improved sensitivity to chemotherapy has led to the development of drugs containing FA conjugates.

Administration of antioxidants in synthesized drugs may further enhance the impact of chemotherapy. Propofol is one such agent that possesses antioxidant properties. Although it is an intravenous sedative-hypnotic agent, it also shows preferential scavenging of organo-radical species. It inhibits lipid peroxidation in various experimental models, protects cells against oxidative stress and increases the antioxidant capacity of plasma in humans. Propofol can therefore be used to prevent or ameliorate the effects of chemotherapeutic agents that have oxidative damage as a significant side effect, e.g., bleomycin, doxorubicin and cisplatin. Moreover, it can be used as an adjuvant with unsaturated FAs, functioning as an antioxidant, in the treatment of cancer. Their co-administration with chemotherapeutic unsaturated FAs, will enhance the cytotoxicity and inhibit the oxidative damage, thereby inhibiting the growth of tumors.

It can, therefore, be speculated that targeted delivery of chemotherapeutic unsaturated FAs in combination with propofol may offer a promising strategy to eliminate cancer. Among various FAs, oleic acid (OA), arachidonic acid (AA) and linoleic acid (LA) have been found to be effective against cancer. Anticancer activity of Ricinoleic acid (RA) which is a possible intermediate in the conversion of OA into LA has not been explored till date although it has been used for development of biodegradable polymers utilized in drug delivery. In the present work, considering the properties of above mentioned unsaturated FAs to be specific against cancer cell differentiation and progression, the synthesis of novel FA based anticancer compounds has been carried out. OA, AA, RA and LA have been covalently coupled either with the C₄ or C₆-α-hydroxy function of propofol. After synthesis of eight novel compounds their formation was established by TLC, UV absorbance and FT-IR spectroscopy. ¹H and ¹³C NMR spectral analysis was employed for characterization of the synthesized compounds. Coupling of hydroxyl function present in propofol isomers (2,4-diisopropylphenol or 2,6-diisopropylphenol) with terminal carboxylic group of FA in the presence of coupling reagent and a catalyst resulted in the formation of eight propofol-FA analogues viz., 2,4P-OA, 2,6P-OA, 2,4P-AA, 2,6P-AA, 2,4P-RA, 2,6P-RA, 2,4P-LA and 2,6P-LA. All
the proton and carbon signals from both moiety of FA and aromatic ring in the propofol come up in the $^1\text{H}$ and $^{13}\text{C}$ NMR spectrum. Results of mass spectra of all novel compounds identified a product with a parent molecular mass, which is very close to the calculated molecular mass. The results of all the spectrum data showed that target compounds were formed.

In the next phase of the study, the \textit{in vitro} efficacy of synthesized compounds was evaluated on the basis of cancer cell cytotoxicity and induction of apoptosis. Cytotoxic selectivity of synthesized compounds towards cancer cells was determined by MTT test. Panel of cancer cell lines SK-MEL-1, MDA-MB-361, HepG2, A549 and HL-60 revealed significant growth inhibition in a dose-dependent manner whereas normal HFL1 cells showed no effect except by propofol-AA analogues. Moreover, difference in IC$_{50}$ values showing 50% growth inhibition revealed the variation in activity of the compounds. To understand the signaling pathway involved in growth inhibition of cancer cells by propofol-FA analogues, two apoptotic factors; cytochrome \textit{c} and caspase-3 were screened. Cancer cells showing highest growth inhibition by propofol analogues of same FA were examined for induction of apoptosis. Hence, the effect of propofol-AA/LA analogues on HepG2, propofol-OA analogues on MDA-MB-361 and propofol-RA analogues on SK-MEL-1 was determined for induction of apoptosis. The down-regulation of cytochrome \textit{c} and caspase-3 in cancer cell was slightly up-regulated on treatment with parent controls. These reduced expression levels were restored by propofol-FA analogues. The compounds at 15µM concentration increased the incidence of apoptotic cell death by enhancing the release of cytochrome \textit{c} and expression of caspase-3.

For interpreting the suitability of propofol-FA analogues as potential anticancer agents in clinical setting, it is desirable to design drug delivery system that can modulate pharmacokinetics as well as pharmacodynamic properties of these compounds thereby making them more efficacious. The best way to increase their efficacy is to direct these drugs to their target and maintain their concentration at the site for a sufficient time for therapeutic action to take effect. The efficiency of drug delivery to various parts of the body is directly affected by particle size. Nanoparticle-mediated drug delivery enhances
drug bioavailability, improve the timed release of drug molecules, and enable precision drug targeting. Nanoparticles are able to penetrate tumors due to the discontinuous, or “leaky,” nature of the tumor microvasculature, which typically contains pores ranging from 100 to 1000 nm in diameter in contrast to the tight intercellular junctions of less than 10 nm in healthy tissues. Therefore, tumors within these tissue types can be selectively targeted. Various drug delivery systems, such as micro-emulsion, nano-emulsion, liposomes, niosomes, poly-lactic glycolic acid (PLGA) and others have been reported to improve the delivery of drugs. Interestingly, PC liposomes, escheriosomes, niosomes and PLGA microspheres show characteristics such as biocompatibility, biodegradability and low toxicity. Due to their advantages, nanoparticle formulations provide a substantial increase in anti-tumor efficacy comparing with the free drug or standard chemotherapy schedule. In this regard, considerable attention has been focused on the use of natural as well as tailor-made nanoparticles to enhance the therapeutic potential of anticancer agents.

In the present study, chemopreventive effects of propofol-FA analogues entrapped in various nanoparticle formulations (PC liposomes, escheriosomes, niosomes and PLGA microspheres) were evaluated. The ideal formulations were selected on the basis of parameters such as favorable entrapment efficiency for the compounds, acceptable release kinetics and zeta (ζ)-potential of the nanoparticles as well as negligible toxicity issues upon its administration in host. Various nanoparticle formulations loaded with propofol-FA analogues separately were used for treatment of skin, breast and liver cancer in animal models. Initially, breast carcinoma and skin papilloma were induced using Dimethyl benzoanthracine (DMBA) whereas liver cancer was induced by di-ethyl nitrosamine (DEN). Compounds which showed highest anticancer activity on respective cell line in in vitro studies were used for in vivo studies. Therefore, the effect of 2,6P-OA, 2,6P-AA, 2,6P-RA and 2,6P-LA nanoparticle formulations on breast, liver, skin and liver cancer, respectively was evaluated in Swiss albino mice. Various nanoparticle formulations of 2,6P-RA were applied topically to the animals, 2,6P-AA and 2,6P-LA formulations were administered intraperitoneally and 2,6P-OA formulations were given orally. Dose of 500µg (for 2,6P-AA and 2,6P-LA) and 1000µg (for 2,6P-RA and 2,6P-OA) was used for each category of treatment. Further, their in vivo efficacy was assessed
on the basis of regression in size of skin tumor, histology and survival. The results of the present study showed that nanoparticle formulations of 2,6P-RA could effectively reduce the cumulative numbers and size of skin papillomas in treated mice. Histopathological sections of all three types of cancers distinctly revealed the effect of nanoparticle formulations whereby apoptosis and necrosis resulted in killing of cancerous cells. The nanoparticle formulations were also assessed for their effect on expression profile of p53wt, p53mut, p21/Waf1, bax, cytochrome c and caspase-3. The treatment by 2,6P-RA bearing nanoparticle formulations resulted in up-regulation of cytochrome c and caspase-3. No other factor was visualized with 2,6P-RA bearing formulations. Results of 2,6P-OA bearing nanoparticle formulations revealed expression of cytochrome c, caspase-3 along with p53mut and p21/Waf1. 2,6P-AA bearing nanoparticle formulations enhanced levels of bax, p53wt and p21/Waf1 whereas p53wt, p53mut, bax and caspase-3 were recorded upon treatment with 2,6P-LA bearing nanoparticle formulations. Overall, escheriosome formulations showed highest efficiency in delivering the encapsulated compound.

Tendency of propofol-FA analogues bearing various nanoparticle formulations in anticancer action provides an opportunity to effectively deliver these therapeutic agents to cancer cells. The formulations are efficient vehicles for the in vivo delivery of the drugs to cytosol of the target cells resulting in the destruction of tumor cells more efficiently. This bio-distribution can be of benefit for the chemotherapeutic treatment of cancers. Interestingly, histopathological examination shows a considerable effect of nanoparticles on the cancer cells where they are able to induce the release of compound, leading to a gradient of drug concentration, favorable for a prolonged diffusion of the free and still active drug towards the neighboring metastatic cells. These results imply usage of drug bearing nanoparticle formulations in chemotherapy against various forms of cancer.
The last part of the study is designed to understand the role of siRNA nanoparticles in cancer therapy. Polo like kinase family enzyme \textit{viz.} PLK1 plays a critical role in the development of skin, prostate, liver and breast cancer \textit{etc}. The silencing of PLK1 is anticipated to result in killing of human cancer cells via inactivation of cyclin dependent kinase1 (Cdc2)/cyclin B1 mediated mitotic arrest followed by apoptosis. Thus, if the proposed concept is tested to be true, it is conceivable that therapeutic approaches aimed at PLK1 or the pharmacological inhibitors of PLK1 may be developed as potential measure to treat skin and liver cancer. Furthermore, in spite of being a promising approach, siRNA mediated gene silencing has not yet entered clinical trials mostly because of its degradation in plasma and also because of poor delivery to the target cells. Establishment of a functional drug delivery system implying nanoparticles will be crucial for development of therapeutically viable nucleic acid–related medicines. Administration of nanoparticle encapsulated siRNA will provide a high concentration of siRNA to the target cancer cells during a limited time.

For this, chapter 5 is focused on development of nanoparticle based gene delivery system for targeted and safe delivery of the encapsulated siRNA considering the fact that these nanoparticles will selectively bind to cancerous hepatocytes \textit{in vivo} which then allows for the selective treatment of the target liver cancer. To achieve the delivery of PLK1 siRNA to the cancer cells we prepared fusogenic liposome (escheriosome) from membrane lipid of \textit{Escherichia coli}. The incorporation of siRNA in escheriosome nanoparticles was successfully achieved. siRNA bearing escheriosome (EC-siRNA) nanoparticles revealed ideal fusogenic potential as established by their entrapment efficiency, $\zeta$-potential and release kinetics. Moreover, EC-siRNA nanoparticles were thoroughly characterized by transmission electron microscopy (TEM), atomic force microscopy (AFM) and nanophox particle analyzer. The studies revealed the EC-siRNA nanoparticles to be monodispersed spherical particles of 150 nm size.

To determine the \textit{in vivo} anticancer efficacy of EC-siRNA nanoparticles, a dose of EC-siRNA nanoparticles was administered in different groups of DEN induced liver
cancer in Swiss albino mice. Higher efficacy of EC-siRNA nanoparticles in comparison with free siRNA as well as sham escheriosome was observed. Histopathological tissue sections of treated mice revealed positive recovery in general architecture. Moreover, induction of various apoptotic factors was also visualized where EC-siRNA nanoparticles were able to mediate up-regulation of p53wt and caspase-9 and down-regulation of p53mut and PLK1 in treated cancerous mice. Full blown tumorogenesis in terms of survival of tumor free animals at different time intervals established the efficacy of EC-siRNA nanoparticles. Interestingly, EC-siRNA nanoparticles treated animals showed tremendous increase in survival rate up to 75% till 12 weeks when compared to control. In further study, we evaluated the appearance of caspase-9 upon treatment with EC-siRNA nanoparticles with the help of confocal microscopy. Here also, positive results were observed. Induction of apoptosis was evident in confocal images where enhanced expression of caspase-9 was visualized.

In conclusion, nanocarriers hold great potential for successful and safe in vivo delivery of siRNA-based therapeutics. On the basis of the data obtained in the present study it can be inferred that nanoparticle based formulations of siRNA can successfully regulate mouse liver cancer in model animals. It can be speculated that the strategy will be effective against other forms of cancer as well. Escheriosomal siRNA-based therapeutics or other safe nanocarriers offer great hope for targeted therapies for cancer and other diseases.