Chapter I
CHAPTER I

INTRODUCTION

Current dogma states that cancer is a multi-gene, multi-step disease originating from a single abnormal cell (clonal origin) with an altered DNA sequence (mutation). Uncontrolled proliferation of the abnormal cell is followed by a second mutation leading to the mildly aberrant stage. Successive rounds of mutation and selective expansion of the cell proliferation results in the formation of a tumor mass (Hanahan and Weinberg, 2000).

Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. Twenty five to thirty percentages of women with invasive breast cancer will die because of the severness of the disease. The vast majority of breast cancers begin in the parts of the breast tissue that are made up of glands for milk production, called lobules, and ducts that connect the lobules to the nipple. The remainder part of the breast tissue is made up of fatty, connective, and lymphatic tissues (Harris et al., 1992). Breast cancer is typically detected either during a screening examination, before symptoms have developed, or after a woman notices a lump. Most masses seen on a mammogram and most breast lumps turn out to be benign; that is, they are not cancerous, do not grow uncontrollably or spread, and are not life-threatening. When cancer is suspected, microscopic analysis of breast tissue is necessary for a definitive diagnosis and to determine the extent of spread (in situ or invasive) and characterize the type of the disease.

Most women with early stage breast cancer will have some type of surgery, which is often combined with other treatments to reduce the risk of recurrence, such as radiation therapy, chemotherapy, hormonal (endocrine) therapy, and/or targeted therapy. Patients with metastatic disease are primarily treated with systemic therapies, which can include chemotherapy, targeted therapy, and hormonal therapy. The use of chemotherapy began in the 1940s with nitrogen mustards, which are extremely powerful alkylating agents, and anti metabolites. Since the early success of these initial treatments, a large number of additional anticancer drugs have been developed (Shewach and Kuchta, 2009). Platinum complexes (e.g., cisplatin, carboplatin, oxaliplatin) and nitrogen mustards (e.g., cyclophosphamide, ifosfamide) are the two main groups of this anticancer drug sub-family.
Chemotherapy involves the use of low-molecular-weight drugs to selectively destroy tumor cells or at least limit their proliferation. Disadvantages of many cytotoxic agents include bone marrow suppression, gastrointestinal tract lesions, hair loss, nausea, and development of clinical resistance. These side effects occur because a cytotoxic agent acts on both tumor cells and healthy cells (Thurston, 2006). Anticancer drugs can be classified according to their mechanism of action, such as anti metabolites, DNA-interactive agents, anti tubulin agents, molecular targeting agents, hormones, monoclonal antibodies and other biological agents (Tovmasyan et al., 2013). Anti-metabolites are one of the oldest families of anticancer drugs whose mechanism of action is based on the interaction with essential biosynthesis pathways.

Structural analogues of pyrimidine or purine are incorporated into cell components to disrupt the synthesis of nucleic acids. 5-Fluorouracil and mercapto purine are typical pyrimidine and purine analogues, respectively. Other anti-metabolites, such as methotrexate, interfere with essential enzymatic processes of metabolism. DNA interactive agents constitute one of the largest and most important anticancer drug families, acting through a variety of mechanisms. Intercalating agents act by binding between DNA base pairs. The family includes anthracyclines (e.g., doxorubicin, epirubicin), mitoxantrone and actinomycin-D. Topoisomerase inhibitors include irinotecan and etoposide compounds. These drugs inhibit the responsible enzymes for the cleavage, annealing, and topological state of DNA. DNA-cleaving agents such as bleomycin interact with DNA and cause strand scission at the binding site. Anti-tubulin agents interfere with microtubule dynamics (i.e., spindle formation or disassembly), block division of the nucleus and lead to cell death. The main members of this family include taxanes and vinca alkaloids (Nussbaumer et al., 2011). There is an increased risk of thromboembolism immediately after major surgery (Decensi et al., 2005).

Tamoxifen is a commonly used anti-cancer drug. It is most often used against breast cancer, carcinomas, osteosarcoma and soft-tissue sarcomas. The effectiveness of tamoxifen in treating various types of cancer is greatly limited by the serious side effects caused by the drug. When tamoxifen was used as a drug, it would act as an ER antagonist in all tissue and contributes to osteoporosis (Nakamura et al., 2007). Tamoxifen appears to be associated with bone loss in premenopausal women who continue to menstruate.
after adjuvant chemotherapy (Vehmanen et al., 2006). Tamoxifen can cause rapid increase in triglyceride concentration and also steatorrhoeic hepatosis or steatosis hepatitis (Osman et al., 2007). According to Paganini-Hill et al., (2000), the drug Tamoxifen shows reduced cognition and semantic memory scores, as the major side effect. A widely researched approach of increasing the efficacy, while lowering the deleterious side effects caused by anti-cancer agents such as tamoxifen, is of developing nanoparticles-based drug delivery systems (Dreis et al., 2007).

A major problem in treatment of many diseases is delivering therapeutic compound to the target site. A conventional application of drugs is characterized by limited effectiveness, lack of bio distribution and selectivity (Nevozhay et al., 2007). Nanoparticles have been used for drug delivery to overcome the limitations in the conventional chemotherapy. Recent developments in nanotechnology have shown that nanoparticles have a great potential as drug carriers (Bhatia, 2016). Due to smaller in size (> 100 nm) the nanostructures exhibit unique physiochemical and biological properties which makes them a favorable material for biomedical applications. Nanoparticles possess enhanced reactive area and they have an ability to cross cell wall and tissue barriers (Wilczewska et al., 2012). Cell specific targeting can be achieved by attaching the drugs to individually designed carriers. The way of conjugating the drug to the nanocarrier and the strategy of its targeting is highly important for targeted therapy (Grumezescu and Ficai, 2017). The nanoscale drug delivery system ensures that the conjugated drug carrier complex acts preferentially at the selected target. Targeting of the drug nanocarrier complex can be active, whereby the complex incorporates a ligand specific for the receptor or epitope of the targeted tissue. In passive targeting, complexes diffuse and accumulate at sites with excessively leaky microvasculature, such as tumors and inflammed tissues, with normal endothelium being mush less permeable. The extravasation of complexes takes place either via transcytosis, whereby macromolecules are internalized from the blood at points of invagination of cell membrane or via diffusion through the tight junctions of endothelial cells. Particularly in cancer, an imbalance in factors that regulate angiogenesis, such as over expression of vascular endothelial growth factor (VEGF), results in both increased vascular
permeability and chaotic tumor-vessel architecture. In combination, these effects cause enhanced permeation and retention (EPR), resulting in high local drug concentrations (Malam et al., 2009).

Silver nanoparticles (AgNps) are the most stable metal nanoparticles which have received significant research attention, both due to their unique physical and chemical properties and promising applications compared to other types of nanoparticles (Tsai et al., 2010). The recent interest of AgNps is propelled by the advancement in their physical properties and their potential applications in the field of chemical and biological sensing (Sobczak-Kupiec et al., 2011), cancer treatment (Narayanan, 2010), catalysts (Kisailus et al., 2005) drug delivery (Fu et al., 2004), electronics and optoelectronic devices (Huang et al., 2003) and antimicrobial activity (Bhakat et al., 2012). The colloidal AgNps are synthesized by the reduction of silver salt (AgNO₃) in an appropriate solvent. The synthesis of AgNps has been carried out using three different approaches, including physical, chemical and biological methods. The advantages of physical methods are speed, radiation that can be used as reducing agents, and no hazardous chemicals involved, but the disadvantages are low yield and high energy consumption, solvent contamination and lack of uniform distribution (Elsupikhe et al., 2015). The synthesis by chemical method is extremely expensive and it involves the usage of toxic and hazardous chemicals such as citrate, borohydride, thio-glycerol and 2-mercaptoethanol. Nanoparticles synthesized by chemical method are not eco-friendly (Devi et al., 2012). Apart from this, the chemically synthesized particles are not of expected purity and they have surfaces which are sedimented by chemicals. To overcome the disadvantages of physical and chemical methods, biological methods have emerged as viable options. The biologically mediated synthesis of AgNps is simple, cost effective, dependable and environmental friendly and they have been shown much interest because of their therapeutic applications in cancer as anticancer agents, in diagnostics and in probing (Zhang et al., 2016). In recent years, plant-mediated synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Synthesis of nanoparticles from plant / microbial source is an easy, efficient and eco friendly approach, where most researchers are looking at the eco-friendly and to explore the ability of various herbs in
synthesizing nanoparticles. Some of the plant materials such as leaves (Satyavani et al., 2011; Cruz et al., 2010; Elumalai et al., 2010; Huang et al., 2007), seeds (Bar et al., 2009), fruits (Dubey et al., 2010; Jain et al., 2009), latex (Bar et al., 2009) tuber (Shameli et al., 2012) and barks (Sathishkumar et al., 2009) have been used in the synthesis of AgNps.

“Adenia” is a genus of flowering plants in the passion flower family, Passifloraceae. This plant is found in the hills of Karnataka, Western Ghats and Western coastal region. The plant is large woody tendrillar climber with tuberous roots. Leaves are alternate, palmately lobe, elliptic and flowers are unisexual, in axillary, panicle or cymes, fruit looks like globose or ellipsoid capsule, orange red when it ripe, seeds are numerous covered with aril. The close resemblance of the fruit to the passion fruit is partly responsible for the accidental poisoning among children. Hondala is found in South India and Srilanka. The tubers are used to treat the ailment of hernia and also for body stimulant and lactation (Udayan et al., 2005). Because of its bitter nature, it is also used for snake bite therapy (Prasad and Shyma, 2013).

Currently, natural, synthetic polymers and lipids are typically used as drug delivery vectors; the family of nanocarriers includes polymer conjugates, polymeric nanoparticles, lipid-based carriers such as liposomes, micelles, dendrimers, carbon nanotubes, and gold nanoparticles, including nanoshells and nanocages. These nanocarriers have been explored for a variety of applications such as drug delivery, imaging, photothermal ablation of tumors, radiation sensitizers, detection of apoptosis, and sentinel lymphnode mapping (Peer et al., 2007).

Polymers have become an integral part of drug delivery systems due to their improved pharmacokinetic properties. They have better circulation than conventional small drug molecules thus target tissues more specifically. Biocompatible polymers offer a safe passage for drug delivery due to their well engineered molecular architecture according to the transitions in the underlying mechanisms of the biological process. Biodegradable polymers break due to cleavage of covalent bonds between them and bi eroisible polymers bring about erosion of the polymer due to dissolution of linking chains without bringing about any change in chemical structure of the molecule. Polymers
serving as drug carrier should be water soluble, nontoxic and non-immunogenic. They work passively in minimizing drug degradation and improving circulation time (Srivastava et al., 2015).

Biopolymers such as chitosan, collagen, poly (lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), Poly (α-capro lactone) (PCL), poly (L-Lysine) (PLL) are used to prepare polymer nanocomposite for biomedical applications such as hydroxyapatite-polymer composite for bone reconstruction, aliphatic polyester nanocomposites in degradable drug delivery system and polypeptide and polysaccharide based nanocomposites in drug delivery. (Hule and Pochan, 2007).

Silver polymer nanostructures have been extensively studied because of their potential applications, ranging from electronic and optical devices to bio sensing and antimicrobial agents. Silver- biopolymer conjugates have been applied to various research fields such as biomaterials, medical devices and electronics (Kong and Jang, 2006). Chitosan is a polysaccharide composed of glucosamine and N-acetyl glucosamine linked with a β-1-4- glycosidic linkage (Crini and Badot, 2008). Chitosan is a biopolymer which is biocompatible and can be degraded by enzymes in human body, the degradation products are nontoxic (Trung et al., 2006). Chitosan coated silver nanoparticles (Ch-AgNps) is one of the nanostructure that has a capability of being used as a biosensor as well as in drug delivery. According to Govindan et al., (2012), chitosan prolongs the action of silver. However, this chitosan based nanostructure is biodegradable, and also possesses good antimicrobial and biosensing activity.

In view of these developments, the main aim of this research work was to explore a novel drug delivery for Tamoxifen based on Chitosan coated Silver Nanoparticles synthesized from the tuber extract of the medicinal plant Adenia hondala. This attempt will improve the delivery efficacy of the drug Tamoxifen in breast cancer treatment.
To achieve this, the following objectives were intended:

1. To explore the biosynthesis and characterization of silver nanoparticles from *Adenia hondala* (Gaertn.) tuber extract.

2. To analyze the *in vitro* cytotoxic and apoptotic effect of above biosynthesized silver nanoparticles in the breast cancer cell line.

3. To study the effect of chitosan mediated biosynthesized silver nanoparticles loaded with Tamoxifen drug in targeted drug delivery system for breast cancer therapy.