5

SUMMARY AND CONCLUSION

Cancer is a dreadful disease associated with the abnormal growth, development and division of the cells. It can develop at one part of the body and spread to the other parts. It is the second most leading cause of death worldwide. Over hundred different types of cancer have been identified so far, of which leukaemia, a cancer that arises from the blood is forming tissues. It is a cancer that affects the white blood cells. Based on its origin, the leukaemia is characterized into two types they are myeloid and lymphoid leukaemia and also based on the development time, they are categorized as acute and chronic leukaemia. So there are four predominant types of leukaemia and are affecting both the children and adult, it includes, "Acute Lymphoblastic Leukaemia (ALL), Chronic Lymphocytic Leukaemia (CLL), Acute Myeloid Leukaemia (AML) and Chronic Myeloid Leukaemia (CML)". The average survival range of leukaemia is only about 63%.

There are various treatment strategies that are adopted for the leukaemia which includes chemotherapy, targeted therapy, radiation therapy, transplantation of bone marrow and immune therapy. But the major disadvantage of these treatment options is their side effects. Especially when chemotherapy and radiation therapy are opted for the treatment, it leads to a lot of side effects including hair loss, pain, swelling, redness in the skin, nausea, fatigue. These side effects are mainly due to the fact that the treatment methods opted will not only kills the cancer cells but also affects the normal cells. So a treatment strategy with minimal or no side effect is the need of the hour.

Recently silver nanoparticles gained importance among researchers as an alternative to chemotherapy as they possess some novel properties. Silver nanoparticles possess apoptosis inducing and antiproliferative properties which makes them a choice for treating cancer. Plant mediated green synthesized AgNPs are found to be potent against cancer of various types. The phytochemicals present in the plant extracts acts as reducing agents and also involve in the process of capping. Thus the phytochemicals present in the surface of the AgNPs are responsible for the anticancer potential. The major advantage of these nanoparticles is their size. Since they are nano in size, they can easily enter into the cancerous cell and can exert a targeted action.

Nanocarriers are found to contain large surface area when compared to the larger particles. The nanocarriers can be modified in such a way to encapsulate large amount of drug into it with increased circulation in blood. They can exert targeted drug delivery into the tumor site without harming the normal cells. Liposomes are lipid vesicles made up of lipid bilayer, which can act as effective drug delivery carrier. They are less toxic and permits controlled drug release and targeting the tumor site. Since they contain both aqueous and hydrophobic membrane the encapsulation of both lipophilic and hydrophilic drugs is possible.

The present study aimed to synthesize silver nanoparticles loaded Liposomes and to analyze their drug release profile, antioxidant and anticancer potential using acute lymphoblastic leukaemia cells (MOLT-3) and peripheral blood lymphocytes (Normal cells). The study was carried out in four different phases. In phase I, the silver nanoparticles and silver nanoparticles loaded liposomes of *Tabebuia pallida* was synthesized and characterized using various characterization techniques. In phase II, the drug release profile of silver nanoparticles loaded liposomes were analyzed and it was validated through various mathematical models. In the third phase the antioxidant and free radical scavenging potential of silver nanoparticles and silver nanoparticles loaded liposomes of *Tabebuia pallida* was evaluated. In the final phase, the anticancer potential of Silver nanoparticles and silver nanoparticles loaded liposomes of *Tabebuia pallida* was evaluated against Molt-3 cells.

The salient findings of the study are summarised as follows: In phase I , as a preliminary study, we have carried out the Soxhlet Extraction of *Tabebuia pallida* leaves using 7 different solvents (Water, Methanol, Ethanol, Chloroform, Acetone, Ethyl Acetate, Petroleum Ether and Benzene) and conducted Free radical Scavenging assays including, "DPPH radical scavenging assay, ABTS radical scavenging assay, Hydroxyl radical scavenging assay, Hydrogen Peroxide Radical Scavenging Assay, Reducing power assay and Nitric oxide radical scavenging assay". From the results of free radical scavenging assays, it was found that the ethanol and Water extract was found to be the best radical scavengers. So we have carried out the Soxhlet extraction using various proportion of these solvents (water, ethanol, ethanol 20:

water 80, ethanol 80: water 20, ethanol 40: water 60, ethanol 60: water 40, and ethanol 50: water 50) and carried out Sunlight mediated Green Synthesis of Silver nanoparticles.

The synthesized silver nanoparticles were analysed using UV-Visible spectrophotometer to identify the best extract for the synthesis of AgNPs. Usually the silver nanoparticles have maximum UV–Visible absorption in the range of 400–500 nm. A good and little narrowed peak at 450 nm was obtained for Ethanol 60: Water 40 extract, noise and broadened peaks were observed for other extracts which indicates that the Ethanol 60: Water 40 extract was the best for AgNP synthesis. Therefore, Ethanol 60: Water 40 extract was used for the further analysis of the study. The elemental mapping of the synthesized AgNPs was done using EDAX APEX. Various elements like Ag, Cl, C, and O are present in the synthesized nanoparticles and their percentage weight is 65.94, 3.52, 15.46, and 15.08 respectively. The XRD analysis was carried out to identify the structure and nature of the synthesized AgNPs. The XRD pattern of green synthesized silver nanoparticles reveals various diffraction peaks at 2θ values: 21.60°, 27.82°, 32.20°, 38.08°, 46.18°, 54.80°and 57.47° which corresponds the (220), (111), (200), (120), (202) planes respectively. These diffraction patterns indicate the presence of face centered cubic crystalline structure of the synthesized nanoparticles. The average crystalline size was found to be 32.18 nm.

The silver nanoparticles loaded liposomes were synthesized using thin film hydration method coupled with sonication with the use of triglyceride lipid lecithin and cholesterol in the molar ratio 2:1. The encapsulation efficiency of the AgNPs loaded liposomes was found to be 82.25%. FTIR analysis was carried out to analyze the possible functional groups that are responsible for the synthesis of AgNPs and AgNPs loaded liposomes. The involvement of phytochemicals of the plant extract that acted as a capping agent during the formation of silver nanoparticles proved by the various functional groups that are identified in FTIR. The size of the synthesized AgNPs and AgNPs loaded liposomes were analysed using Field Emission Scanning Electron Microscopy. Usually the size of the nanoparticles ranges between 1 to 100nm. The size of the synthesized nanoparticles was found to be ranging between 31.76 nm – 50.36 nm and are spherical in shape and the size of the AgNP loaded liposomes are found to be ranging between 80.69-99.71 nm and are also spherical in shape. From the results of Dynamic Light Scattering analysis, it is observed that the mean particle size of the synthesized AgNPs and AgNPs loaded liposomes was 55.4 nm and 172.1 nm with a PDI value of 0.33 and

0.381 respectively. This Polydispersity index value indicates that the synthesized AgNPs and AgNPs loaded liposomes were stable and will not undergo agglomeration for a longer period. The Zeta potential of the green synthesized silver nanoparticles was found to be -15.6 mV. The zeta potential of the AgNP loaded liposomes was found to be -21.5 mV. From this negative zeta potential, it could be confirmed that the synthesized silver nanoparticles and silver nanoparticles loaded liposomes will be stable for a longer duration since there will be a strong repulsion among the particles that prevents them from agglomeration.

In phase II, the *in vitro* evaluation of drug release profile of silver nanoparticles loaded liposomes was done and was validated through various mathematical models. The *in vitro* drug release study was carried out in three different pH like pH 5.5 which is similar to the pH of mature endosomes of cancer cells, pH 6.8 which is similar to the pH of cancer cells and pH 7.4 which is similar to the pH blood. A high AgNP release was found at a pH of 5.5 when compared to the others which clearly indicates the targeted delivery of the AgNPs by the liposomes.

This drug release profile was validated using different mathematical models including, Zero order model, First order model, Higuchi model, Korsmeyer and Peppas model and Hixson Crowel model. Among the models analysed Higuchi model was the best fitted model for the release of AgNPs from liposomes as the correlation coeffecient is higher which indicates that the drug release is by diffusion. From the Korsmeyar peppas model it could be concluded that the drug release follows Non-Fickian Diffusion and also time dependent.

In phase III, the antioxidant potential of hydroethanolic extract, AgNPs and AgNP loaded Liposomes was evaluated. Free radical scavenging and antioxidant potential of the hydro ethanolic extract, AgNPs and AgNP loaded Liposomes were evaluated using, DPPH radical scavenging assay, ABTS radical scavenging assay, Hydroxyl radical scavenging assay, Reducing Power assay, Hydrogen peroxide radical scavenging assay and Nitric oxide radical scavenging assay compared with the standard antioxidant quercetin. From these assays, it was found that the hydro ethanolic extract, AgNPs, AgNP loaded Liposomes and standard quercetin exhibited a dose-dependent scavenging potential. When the concentration of both standard and the samples increased, the radical scavenging ability was found to be increased to a greater extent. Among the various samples tested, the Silver nanoparticles loaded liposomes possess high radical scavenging potential.

In phase IV, the cell viability and anticancer activity against Molt-3 cells of the hydro ethanolic extract, AgNPs and AgNP loaded Liposomes were analyzed. Peripheral Blood Lymphocytes (PBL) was used as the normal counterpart for the Molt-3 cells. From MTT dye reduction assay and Sulphorhodamine B assay, it was found that the liposomes are very effective against Molt-3 cells and does not affect the normal PBL cells much, which indicates that the various treatment groups are not cytotoxic to the normal cells. Measurement of Apoptosis was carried out using Annexin V/FITC staining. In the control group, all the cells are in the live phase and in the treated cells, the cells are in both early and late apoptotic phase which clearly indicates the cytotoxicity of the treatment groups. In the case of silver nanoparticles loaded liposomes, the cells are in late apoptosis which confirms the high cytotoxic effect of the liposomes against MOLT-3 cells. In the case of normal PBL, most of the cells are in live phase in control and in the treated cells some of the cells are in early and late apoptosis which is considerably less when compared to MOLT-3 cells of leukaemic origin. In the case of liposomes 94% of the cells are in live phase which clearly depicts the targeted drug delivery by the liposomes to the leukemic cells.

Mitochondrial Membrane Potential of the control and treated cells were analysed using JC1 Staining. If the mitochondria are intact with normal membrane potential, the JC1 dye enters into the mitochondria and exhibit red coloured fluorescence. If there is a loss of mitochondrial membrane potential, disappearance of red fluorescence will be observed. After treating with Molt-3 cells with hydroethanolic extract, AgNPs and AgNP loaded liposomes, there was a loss of mitochondrial membrane potential to a great extent, which was confirmed by the formation of more monomers. In the case of normal peripheral blood lymphocytes, minimal loss of mitochondrial membrane potential was observed, which was confirmed by the presence of more J-aggregates.

Changes in the phases of cell cycle were analysed using flow cytometer. For Molt-3, In the case of untreated control, the cells are disbursed in all the phases of cell cycle. But in the treated cells with hydroethanolic extract, AgNPs and AgNP loaded liposomes; the cells were blocked in G0-G1 phase. In the untreated PBL control, cells were distributed in all the phases of cell cycle. Similar results were observed in the treated PBL cells which clearly indicate that the treatment groups do not produce any considerable cytotoxic effect on the normal counterpart.

To conclude, the green synthesized *Tabebuia pallida* silver nanoparticles loaded liposomes were found to possess both antioxidant and anticancer potential. The targeted drug delivery of silver nanoparticles by liposomes to the Molt-3 cells was clearly identified from the drug release assay. Therefore, *Tabebuia pallida* silver nanoparticles loaded liposomes can be used as a candidate plant for the development of new anticancer drug with lesser or minimal side effects.

Suggestions for the further research

The outcome of the present study paved a way for number of future research. Some of them are given below

- *in vivo* studies can be carried out to analyze the anticancer efficiency of silver nanoparticles and silver nanoparticles loaded liposomes in animal models
- Gene expression studies can be carried out to analyze the effect of the drugs at molecular level
- Mechanism of action of drugs on causing cancer cell death can be studied
- Clinical trial can be conducted using human volunteers for the pharmacological validation of the drug