# 6. Summary and Conclusion

The current anticancer drugs do not greatly distinguish normal and cancerous cells, thereby results in systemic toxicity and adverse effects (Nurgali et al., 2018). Drug delivery via nanoparticles makes it easy to acquire the desired drug concentration at a particular site, thereby reducing the adverse effects and minimizing the toxicity, dose dumping, etc. (Wen et al., 2015).

6.1. Chapter 1 discusses about the nanoparticles in cancer, tamoxifen citrate, nanosuspension on drug delivery, pathological changes in Breast Cancer, cancer biology behind Breast Cancer, and altered gene in Breast Cancer. Tamoxifen citrate can greatly reduce the risk of cancer recurrence and invasive cancer. The daily oral treatment stops cancer cells from using estrogen and progesterone to grow and spread. Nanodrug formulation are the new trends in medical world for easy drug delivery system. The study aims in designing, developing, and evaluating tamoxifen citrate modified nano-suspensions for the treatment of Breast Cancer. Tamoxifen exhibits numerous biopharmaceutical and toxicological issues such as high susceptibility to liver metabolism and precipitation as it is free in acidic environment of stomach which contributes to low tamoxifen bioavailability. Owing to its hydrophobic nature, it was encapsulated in solid lipid nanocarrier and further modified to accumulate at cancer cell site by active targeting.

6.2 Chapter 2 explains the aim and objectives of the study. The aim of the present study was to design, develop and evaluate tamoxifen citrate modified nano-suspensions for treatment of Breast Cancer. The present study formulated the newly developed nanoformulation for the delivery of tamoxifen citrate and assessed the characterization of the nanosuspension. Compatibility studies of tamoxifen citrate nano-suspension was studied using X-ray diffraction, Differential scanning colourimetry, Forced degradation study, Infrared spectroscopy and HPLC. In vitro studies of nano-suspension of tamoxifen citrate against Breast Cancer MCF-7 cell lines were performed. Cytotoxicity study of tamoxifen citrate nano-suspension was done by MTT assay and percentage of cell viability was calculated. The cell cycle arrest and antiproliferation efficacy of the nanosuspension was analyzed by flow cytometry. The apoptosis action of tamoxifen citrate nanosuspension on breast cancer was demonstrated by DAPI staining and methylene blue assay.

6.3 Chapter 3 describes the available literature on the topic and updates the knowledge on the different concept of the present study. This chapter explains the national and international status of breast cancer occurrence. Adverse effects of pharmacological drugs for Breast Cancer have been elaborated in this chapter. Various techniques and protocol used for preparing nanosuspension was discussed. Types of nanosuspension preparation of nanosuspension and its easy drug delivery system were also described and anticancer drug tamoxifen citrate target effect on breast cancer was also elaborated.

6.4 Chapter 4 describes about the formulation and evaluation of tamoxifen citrate nanosuspension in detail. The outcome of the present investigation proposes a novel formulation of Tamoxifen citrate nanoparticles which is prepared by a multiple emulsion solvent evaporation technique. The nanosize particles with a desired drug polymer ratio can also be produced. The size, drug loading and the drug release kinetics were studied with various ratios of formulation. The characterization of the tamoxifen citrate nanosuspension was discussed in detail in this chapter.

6.5 Chapter 5 explains about the in vitro study of tamoxifen citrate nanosuspension against Breast Cancer (MCF-7) cell line in detail. Tamoxifen citrate nanosuspension was internalized well in Breast Cancer cells in vitro, suggesting that they are very well suitable in treating Breast Cancer. The cytotoxic effect of Tamoxifen citrate nanosuspension reduced the cell viability from 100% to 20.3% remarkably, whereas nanosuspension without the drug showed the cell viability of 99.5%. The significant decrease in cell viability was found to be from 100% to 30.9% in Tamoxifen citrate free drug. We analyzed that Tamoxifen citrate nanosuspension could inhibit the growth of MCF-7 cells by induction of apoptosis and arresting cell-cycle progression. Tamoxifen citrate induces anticancer activity against MCF-7 cells by modulating tumor genes such as BRCA1 in formulation manner than in free form.

The breast cancer type 1 susceptibility protein (BRCA1) gene alteration and loss of BRCA1 expression are described to have more risk of triple-negative breast cancer (TNBC). Moreover, TC nanosuspension was more significant than tamoxifen citrate.

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in Breast Cancer cells in vitro, suggesting that they are suitable in Breast Cancer treatment. In the present study, our data show an increase in apoptosis in the TC nanosuspension treatment group as compared with that of Tamoxifen citrate group. In summary, TC nanosuspension suppressed the MCF-7 proliferation and enhanced the apoptosis of cancer MCF-7 cells. These data suggest that nanosuspension may be an optimized Tamoxifen citrate delivery formulation for breast cancer therapy.

It is reasonable to conclude that research efforts are increasing into new nanoformulated TAM. The recent investigations reported successful preparation of TC nanosuspension to target the tumour point and exhibited enhanced efficacy in breast cancer therapy. Further research and clinical trials are required, especially when their benefits clearly outweigh the risks, to establish the most effective strategies to utilize the targetability of the versatile TAM nanosuspension. Through these nano formulation technologies, TAM can be a potent lifesaving drug to fight Breast Cancer.

Most of the present-day anticancer drugs mediate their tumor arrest via induction of apoptosis in cancer cells, and it is recommended as one of the key mechanisms for the targeted therapy of breast cancer (Pistritto et al., 2016). TAM may induce apoptosis in Breast Cancer cells by membrane receptor pathway, the release of cytochrome c (Cyt C) and several biochemical pathways that are activated by caspase family of proteins (Rouhimoghadam et al., 2018).