Evaluation of the anti-cancerous effect of ciprofloxacin loaded liposomes against leukemia in rat model.

Author

Sahar Fatima

Regn Number

00000328959

Supervisor

Dr. Nosheen Fatima Rana

DEPARTMENT OF BIOMEDICAL ENGINEERING & SCIENCES

SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY ISLAMABAD

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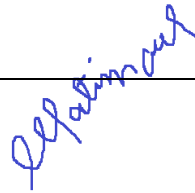
Author

Sahar Fatima

Regn Number

00000328959

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Thesis Supervisor:

Dr. Nosheen Fatima Rana

Thesis Supervisor's Signature: _____

DEPARTMENT OF BIOMEDICAL ENGINEERING & SCIENCES SCHOOL
OF MECHANICAL & MANUFACTURING ENGINEERING
NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY.

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Signature: _____

Name (Supervisor): Nosheen Fatima Rana

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Signature (HOD): _____

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Declaration

I certify that this research work titled "**Evaluation of the anti-cancerous effect of ciprofloxacin loaded liposomes against leukemia in rat model.**" is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources it has been properly acknowledged / referred.

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Copyright Statement

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I am thankful to my Creator Allah Subhana-Watala to have guided me throughout this work at every step and for every new thought which You setup in my mind to improve it. Indeed, I could have done nothing without Your priceless help and guidance. Whosoever helped me throughout the course of my thesis, whether my parents or any other individual was Your will, so indeed none be worthy of praise but You.

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Table of Abbreviations

AML	Acute Myeloid Leukemia
IARC	International Agency for Research on Cancer
DOX	Doxorubicin
FITU	Ferrocene-Incorporated Thiourea
ALL	Acute Lymphoblastic Leukemia
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myelogenous Leukemia
Np	Nanoparticles
UV-Vis spectroscopy	Ultraviolet–visible spectroscopy
XRD	X-ray Diffraction
FTIR	Fourier Transform Infrared
EDX	Energy Dispersive X-Ray Analysis
SEM	Scanning Electron Microscopy
ER	Estrogen Receptor
ROS	Reactive Oxygen Species
DOPC	1,2-Dioleoyl-sn-glycero-3-phosphocholine
DPPC	Dipalmitoyl phosphatidyl choline
PEG	Polyethylene glycol

Abstract

Acute myeloid leukemia is an aggressive type of leukemia with a high mortality rate. It involves the anomalous proliferation and differentiation of the hematopoietic cells. The biological and clinical disparity of this disorder makes treatment complex and emphasizes the need to explore novel therapeutic drug delivery systems. These carriers must exhibit less toxicity and more efficacy than the conventional chemotherapeutic drugs presently available against leukemia. In this study, the anti-cancer potential of Ciprofloxacin, which is a potent antibiotic as a free drug and as a liposomal nanoparticle, was investigated in benzene-induced leukemic rat model. The result of free ciprofloxacin and nanoparticles of ciprofloxacin was compared to free doxorubicin and nanoparticles-based doxorubicin respectively as doxorubicin is well known anti-leukemia drug and is considered a positive control in this study. The liposomes are prepared by thin film hydration method and are pegylated to improve cell internalization of drug. Characterization of nanoparticles was done by SEM, UV, Zeta potential and FTIR analysis. The biological activity of doxorubicin, ciprofloxacin and nanoparticles of ciprofloxacin and doxorubicin was tested by different cytotoxicity and antioxidant assays. The free drug and nanoparticles were then administered by intravenous route to benzene induced leukemic rat model, to investigate its anti- leukemic potential. For this purpose, different parameters were analyzed including hematological profiles, morphology of blood cells, hepatic & renal enzyme activity in samples obtained from the experimental groups. It was observed in biological assays that free drugs have higher cytotoxicity as compared to nanoparticles of drugs. It was observed in rat model that ciprofloxacin has remarkable recovery of cellular indicators as compared to leukemic conditions. The result of liposomal ciprofloxacin is as significant as that of doxorubicin liposomes. The blood cell counts of Ciprofloxacin-treated leukemic rats improved, as did hepatic and renal biomarker levels. whereas additional research is required to assess the efficacy of ciprofloxacin liposomal chemotherapy against leukemia.

CHAPTER 1: INTRODUCTION

1.1 Cancer

Cancer is a primary cause of mortality and a significant impediment to extending man's average lifespan. In 2020, approximately 19.3 million new cancer cases were diagnosed globally, with over 10 million cancer fatalities (World Health Organization, 2020). Cancer kills three people for every ten who die prematurely from non- communicable diseases (Bray et al., 2021). Cancer may begin practically anywhere in the body. Normal cells grow and multiply by cell division, consequently old and damaged cells are replaced by new cells. When this well-organized mechanism fails, aberrant and damaged cells grow and proliferate when they shouldn't (Williams & Stoeber, 2012). These cells can clump together to form tumors, which may be benign or malignant. Malignant tumors invade other body tissues to produce new tumors by metastasis (Fares *et al*., 2020). While most cancers produce tumors that are solid, blood cancers like leukemia do not (Suhail et al., 2019). The Hallmarks of Cancer were postulated by Hanahan and Weinberg in 2000, and then updated in 2011 (Hanahan & Weinberg, 2011) as a set of functional attributes critical for the formation of malignant tumors. These characteristics are acquired by cells as they shift from normal to neoplasm development.
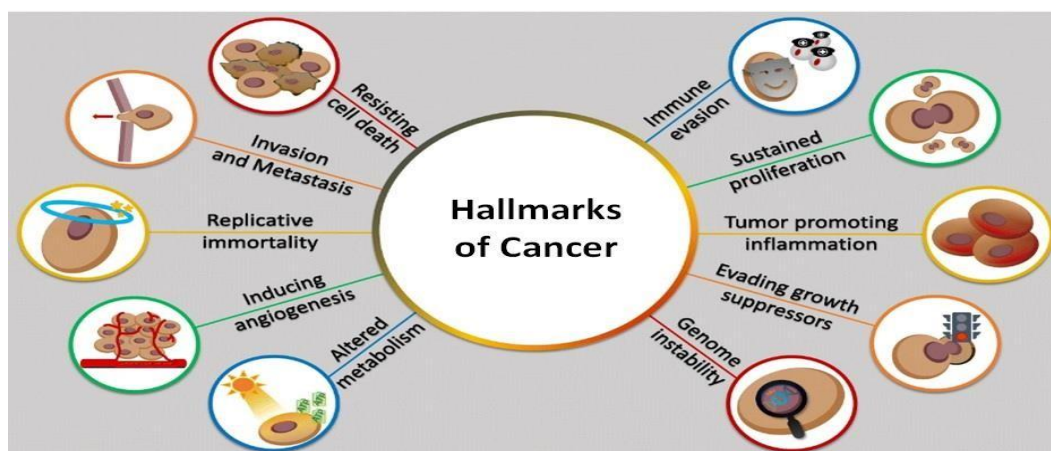
Figure 1.1 Hallmarks of Cancer (Zhong et al., 2020).

1.2 Causes of Cancer

Cancer is a hereditary disease caused by mutations in the genes that control how our cells function, especially how they divide and proliferate. Cancer-causing genetic mutations can originate from (a) cell division errors, (b) DNA damage caused by environmental toxins like some chemicals and radiations, or (iii) genetically inherited mutations (Wu *et al.*, 2016).

Normally, the body kills cells with damaged DNA before they become cancerous. However, this ability of the body diminishes with age as observed why older people are more prone to developing cancer (Fouad & Aanei, 2017). DNA repair genes, as is obvious from the name, are responsible for fixing errors during DNA replication. Mutations in these genes impart errors to other genes on the cell generating chromosomal alterations like deletions and duplications which may contribute to malignancy. Tumor suppressor genes are involved in the regulation of cell division and proliferation. Their mutations can promote uncontrollable cell proliferation. Proto-oncogenes promote cell to divide and grow in an appropriate manner. These genes can transform into oncogenes, i.e. cancer causing genes, upon mutation and lead to abnormal cell growth. This can instigate neoplastic activity (Vogelstein & Kinzler, 2004).

1.3 Stages of Cancer Progression

Cells undergo aberrant alterations known as hyperplasia and dysplasia before becoming malignant. With hyperplasia, the number of cells increases more rapidly than normal. Dysplastic cells appear abnormal under a microscope, yet they are not malignant. Hyperplasia and dysplasia can both lead to cancer. Carcinoma in situ is a stage of cancer in which the tumor comprises mostly abnormal cells and is increasing in size but has not spread beyond the site of origin. When a tumor begins to infect surrounding or distant tissues, it has turned malignant and poses a significant risk of death (Kanwal, 2013).
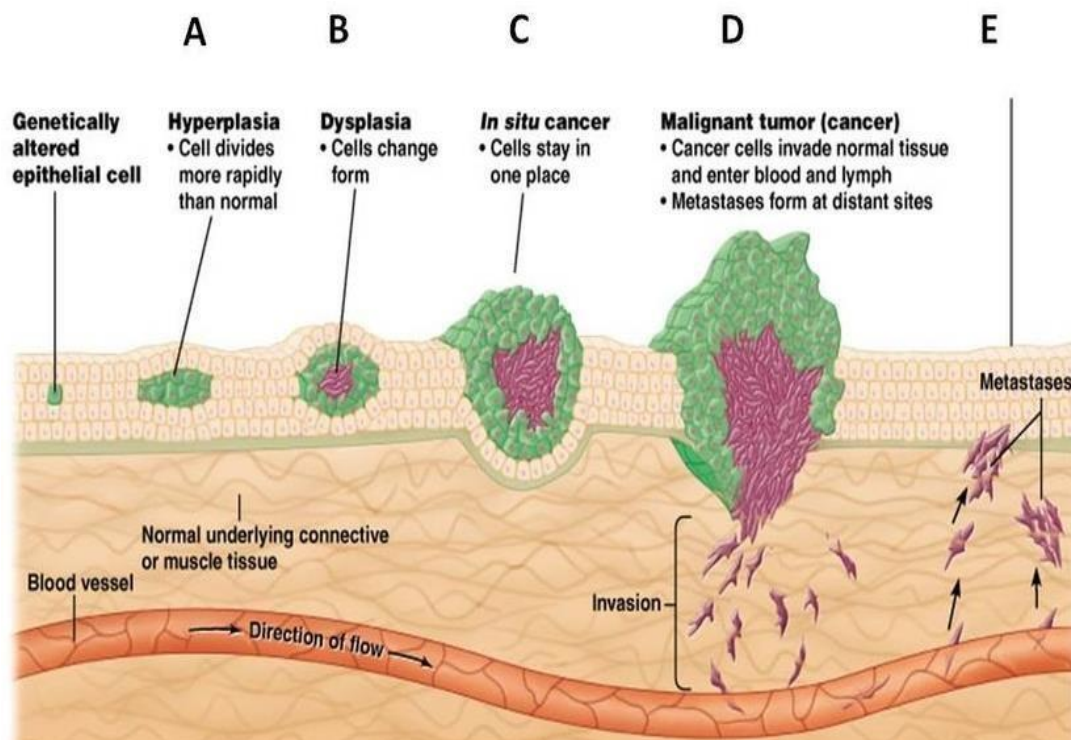
Figure 1.3 Stages of Cancer Progression (Kanwal, 2013)

1.4 Types of Cancer

There are numerous distinct forms of cancer. They are usually named after the anatomical tissue in which they originate. Moreover, cancers are also categorized according to the type of cell that generated them, for instance, squamous or epithelial cells. (Hoadley et al., 2018).

These types of cancers are classified into five categories: carcinoma, sarcoma, melanoma, lymphoma, and leukemia (Mitra *et al*., 2018). Carcinomas are the most common type of cancer and develop in the lungs, pancreas, skin, breasts, and other organs and glands. Sarcomas are less common and occur in muscle, cartilage, bone, blood vessels, or other soft or connective tissues. Melanomas form in the pigment- producing cells of the skin. Lymphomas are the cancer of the lymphocytes while leukemia is a blood cancer (*The Development and Causes of Cancer - The Cell - NCBI Bookshelf*, n.d.).

1.5 Leukemia

For generations after detecting solid tumors, doctors were ignorant of a comparable disease in the blood. Even after the invention of the microscope and the identification of white blood cells, hematological diseases were mostly seen as pus and inflammation. Only in the early nineteenth century did European physicians identify individuals with unusual blood changes, which were initially thought to be caused by inflammation. However, a young pathologist named John Hughes Bennett performed autopsies on these patients and published the results, concluding that the blood changewas not caused by inflammation and that the disease affected the entire blood system (Tallman & Hughes Bennett, 2008). Soon after, Rudolf Virchow described the case of a woman who had an increase in circulating white blood cells, which may be classified as chronic leukemia. It was first termed as 'Weisses Blut' (white blood) by Virchow, and then as Leukemia by succeeding researchers (Mehranfar et al., 2017).

1.5.1 Hematopoietic Discrepancies Leading to Leukemia

All types of blood cells are generated in the bone marrow through a complicated biological mechanism of cell replication and differentiation from the hematopoietic stem cells. Myeloid and lymphoid differentiations are the two main branches of hematopoietic development, and each includes a succession of differentiation phases (Paschka, 2008).

Each step of each route results in a unique progenitor cell capable of further differentiation. Leukemia can arise if oncogenic mutations are identified in any of these progenitors. It is defined by the interruption of systems that control self-renewal, differentiation, and hematopoietic cell growth, resulting in an increase of immature, non-functioning neoplastic cells (Sive *et al*., 2014).

1.5.2 Types of Leukemia

Acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphocytic leukemia (CLL), are the four primary kinds of leukemia (Rousseau et al., 2014). Generally, acute leukemia refers to cells that resemble the hematopoietic stem cells, whereas the word "chronic" specifies highly differentiated cells(Szczepański et al., 2003). Despite accounting for only 30% of newly diagnosed leukemia, AML is responsible for 48% of leukemia fatalities (*Key Statistics for Acute Myeloid Leukemia (AML)*, n.d.). The high death rate reflects the aggressiveness of AML as well as its diverse character (Shallis *et al*., 2019).

Table 1.1 Types of Leukemia and their potential causes

Type of Leukemia	Causes and Symptoms	Reference
Acute lymphocytic leukemia (ALL)	1. Hematopoietic stem cell genetic abnormalities (HSCs) 2. Chromosomal translocation activates certain genes	(Terwilliger & Abdul-Hay, 2017)
Chronic lymphocytic leukemia (CLL)	1. Homogenous disease of immatureB-cells 2. Malfunctioning apoptosis. 3. Inability to stimulate immune response	(Hallek *et al*, 2018)
Acute myeloid leukemia (AML)	1. Genetic alteration and malfunctioning of HSCs leading to clonal hematopoietic irregularity i.e. AML. 2. Distortion of rational differentiation. 3. Unrestrained growth of blast cells (called immature leukemiccells). 4. Fever, fatigue, hemorrhage, bone- pain and CNS malfunctioning 5. If untreated, causes severe bleeding, fatal infections and organ infiltration.	(Behrmann *etal*., 2018)

Chronic myeloid leukemia (CML)	1. Dysfunctioning of clonal HSCs. 2. Formation of Philadelphia Chromosome.	(McCafferty *et al*., 2018)

1.6 Acute Myeloid Leukemia: Pathology, Epidemiology and Etiology

The myeloid lineage is affected by differentiation block and increased proliferation in AML, resulting in a buildup of aberrant undifferentiated cells called blasts in the bone marrow and blood that are unable to differentiate into granulocytes or monocytes. Driver mutations, such as DNMT3A (Fig. 1.3), can develop in hematopoietic or myeloid stem cells in AML. Cooperative mutations, e.g. FLT3-ITD, then result in the formation of a completely converted leukemic stem cell with infinite self-renewal ability and poor maturation (Loghavi et al., 2014). Cytogenetic assessment detects chromosomal abnormalities in patients, which are then used to identify and evaluate prognostic risk.

The earliest clinical signs and symptoms of AML include exhaustion, bleeding or infections, and fever, which are caused by reductions in red blood cells and platelets. Various symptoms might result from leukemic infiltration of other tissues, such as the liver, lymph nodes, bones and central nervous system. For cancer diagnosis, blasts of the myeloid lineage must make up 20%, at the very least, of cells in a bone marrow aspirate or blood specimen (Orazi, 2007).
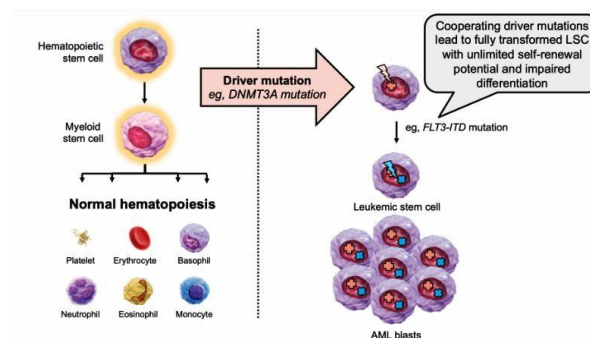
Figure 1.3 Pathogenesis of AML

CHAPTER 2: LITERATURE REVIEW

2.1 Benzene

Benzene is a well-known myelotoxin, as a matter of fact, it is often regarded as an environmental leukemogenic agent, with persistent exposure incidental to a heightened risk of AML (Snyder, 2012). It is used in the manufacturing of everyday products such as lubricants, plastics, dyes and pesticides. Ambient benzene may play a role in many cases of "de novo" AML that do not have hereditary predispositions. Exposure to benzene also causes a variety of hematological system damage, including varying degrees of pancytopenia and aplastic anemia (Mchale et al., 2012).

2.1.1 Benzene Toxicokinetics and Toxicodynamics in AML

Since the bone marrow is the most notable target of benzene carcinogenesis, benzene metabolism is an important aspect in the development of AML. Benzene can infiltrate the body by skin absorption, breathing, or oral consumption, depending on the source of exposure; nevertheless, inhalation is the most common mode of benzene exposure. Once within the body, enzymes in the lungs and liver degrade benzene into metabolites and reactive oxidative stress (ROS) that are hazardous to bone marrow (Bette Meek & Klaunig, 2010; Irons, 2000).

Benzene is first oxidized into benzene oxide in the lungs and liver by different cytochromes. In the liver, benzene oxide, immediately converted to phenol (PH) or is hydrolyzed into benzene dihydrodiol. PH is eliminated in the urine or further metabolized to hydroquinone by CYP2E1. Oxepin is also broken down in the liver, generating reactive muconaldehydes and E,E,-muconic acid (Cheng Peng & C Ng, 2016).

Toxic metabolites to bone marrow and hematopoietic stem cells (HSCs) include benzene oxide, muconaldehyde, benzene dihydrodiol, and hydroquinone, as well as semiquinone radicals and benzoquinone, which cause reactive oxidative stress (ROS) (Fig 1.4). These metabolites, together with the resulting oxidative stress, rapidly react with proteins and disrupt cellular activities, resulting in the development of AML (Bowen, 2006). Apoptosis is also influenced by the intermediates of benzene metabolism (Ross, 1996). programmed cellular death is known as apoptosis that is generally a protective process that stops damaged cells from reproducing by triggering internal signals that lead to self-destruction. In numerous ways, dysregulated apoptosis can contribute to the progression of leukemia.
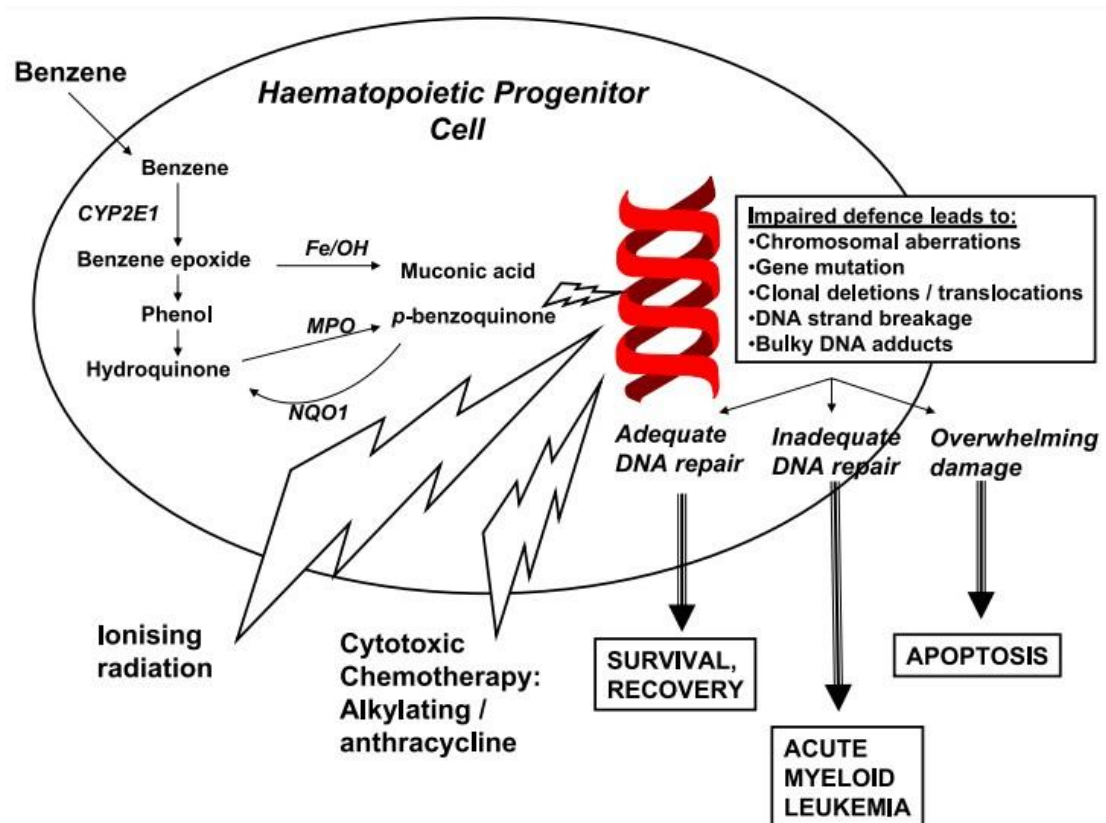
Figure 2.1 Benzene Toxicity leading to AML (Bowen, 2006)

2.1.2 Diagnosis of AML

AML can be detected in a variety of methods, but blood tests are usually the initial step in the process. Complete blood count and peripheral blood smear are two of the most popular

tests used to diagnose and characterize AML (Döhner *et al*., 2017). These tests are used to monitor changes in the quantity and form of peripheral blood cells, myoblasts, and bone marrow cells in leukemia. Increased nucleus to cytoplasm ratio, decreased cytoplasmic content, and deformation in nuclear shape are all morphological alterations seen in immature last cells. Acute Myeloid Leukemia is diagnosed when 20% or more of the cells in the blood have the characteristics of blastcells (Arber et al., 2003).

Numerous staining processes can be used for morphological research; nevertheless, Giemsa staining is the most preferred and practical. May-Grünwald/Giemsa is adifferential staining technique that uses a combination of methylene blue, eosin and azure dyes. It is selective for the phosphates of the DNA and is utilized to visualize the nuclear and cytoplasmic morphology of distinct blood cells such as RBCs, WBCs,and platelets, as well as to produce differential white blood cell counts (Blackburn *et al*., 2019).

Counting and examining cells alone isn't always enough to make a definitive diagnosis. Other tests, such as genetic study of patients, may be utilized to confirm an AML diagnosis since they have non-random chromosomal abnormalities. The therapyresponses can also be tracked on an individual basis. Changes in hepatic (ALT, ALP, and AST) and renal biomarkers in afflicted patients' serum owing to benzene-induced hepatic cytotoxicity can also be used to detect AML (Delaunay et al., 2014). In leukemic cells, the expression of apoptotic genes is decreased while that of cell proliferation genes is increased.

2.1.3   Treatment of AML

AML is an umbrella diagnosis that includes several subtypes with varying prognostic and predictive markers that can be effectively treated with selective and targeted medicines that are currently being refined. Chemotherapy, radiation therapy, surgery, hormonal, and immunotherapy are examples of these treatments. Acute myeloid leukemia is treated by gene and stem cell transplantation to enhance and extend the quality of life of patients

for a few years. One of the most difficult aspects of fighting cancer is the evolution of treatment resistance and non-specific target activity. Conventional medicines have a high level of toxicity for normal cells and can harm key organs. Chemotherapy has a number of negative impacts on normal cells due to their inability to distinguish between normal and malignant cells (Singh *et al*., 2016). As a result, current research is focusing on numerous AML subgroups and treatment combinations in order to lessen chemotherapy's side effects and increase its efficacy. These include: 1) innovative intense chemotherapy regimens for younger and fit older patients, which include high-dose nucleoside analogues; 2) lower-intensity chemotherapy regimens for older/unfit patients, which include hypomethylating drugs; and 3) novel therapeutics (Saygin & Carraway, 2017). Small molecule–targeted treatments in combination with or without conventional chemotherapy may enhance survival in AML subgroups and cure rates in previously incurable AMLs. Therefore, the quest for discovering novel, more effective compounds in the treatment of AML that have fewer side effects and target a range of cellular functions such as signaling pathways is very promising.

2.2 Doxorubicin (DOXO)

Doxorubicin (DOXO) was obtained from a cultured fluid of the actinobacterium *Streptomyces peucetius var. caesius* and later synthesized. It is a cytotoxic anti-neoplastic drug used to cure different types of cancers, including numerous childhood cancers, blood disorders including leukemia, adulthood cancer both benign and malignant e.g. hyroid and osteosarcoma (Thorn et al., 2011; Agrawal, 2007). The most common mode of administration is intravenous.

2.2.1 Mechanism of Action of DOXO

By inhibiting topoisomerase II activity and acting as a DNA incorporating agent, doxorubicin stops replication by inhibiting the DNA double-stranded structure from resealing. This sets off chemical reactions that result in cell death.
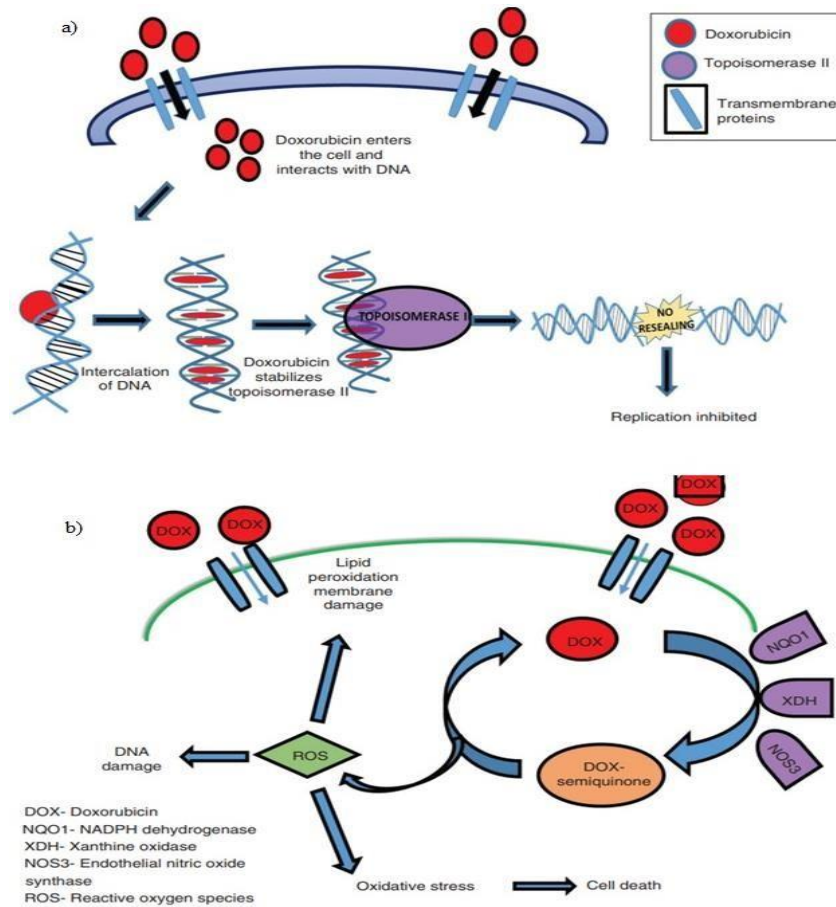
Figure 2.2 Action of (a)Intercalation of

Mechanism of Doxorubicin Doxorubicin

(Roychoudhury et al., 2020)

Another anticancer mechanism of DOXO is the formation of free radicals causing cellular membrane, DNA, and protein damage. Succinctly, DOXO is oxidized to semiquinone, an unstable metabolite that is then transformed back to its prior form, releasing ROS in the process. ROS cause lipid peroxidation and membrane disruption, along with oxidative

stress, DNA damage, and cell death through apoptosis. However, the risk of cardiotoxicity associated with increased cumulative dosages has tainted doxorubicin's efficacy as an anticancer agent, necessitating the development of other, less toxic, and more effective medicines (Roychoudhury et al., 2020).

2.3 Antibiotics as Anticancer

Surgery, radiation, chemotherapy, immunotherapy, and targeted therapy are only a few of the therapeutic options used for cancer. Since radiotherapy has little to no side effects on the body, it is the treatment of choice in about 50% of cancer patients. Tumours have long been successfully treated through surgery. But only localised malignant tumours that are restricted to particular organs can benefit from these treatments. Chemotherapy and targeted therapy have become more crucial as we come to understand cancer as a systemic illness and how to treat cancer cells (Rajaraman et al., 2006).

Antibiotics are substances that can be found in higher organisms as well as in microbes including bacteria, fungus, and actinomycetes. They have anti-pathogenic qualities as well as the capacity to slow the growth of other cells (Reuter, 2001). Recent research has shown that antibiotics can cause cancer cells to undergo apoptosis, slow the growth of cancer, and prevent metastasis. However, using antibiotics could unintentionally result in the death of healthy tissue.

The intestinal microbiota plays a significant role in the cancer therapy process (Reuter, 2001). Therefore, administering antibiotics alters not just the microbiome but also the immune system of the body and causes inflammation. These consequences have the potential to impair and reduce the efficacy of cancer treatment (Besnier & Leport, 1990).

2.4 Mechanism of Anticancer Antibiotic

Antibiotics are drugs that have the power to either kill or stop the growth of germs. Microorganisms can create substances called anticancer antibiotics that have strong anticancer characteristics (Cragg & Newman, 2001). These antibiotics, whose main ingredients are peptides and anthraquinones, successfully prevent the unchecked growth, aggressive proliferation, and spread of malignant tumours. Anticancer antibiotics include anthracyclines, endiyne (Cragg & Newman, 2001), mitomycin, bleomycin, actinomycin, and guanorycin. These antibiotics have intricate and powerful anticancer properties. Daunorubicin, Doxorubicin, Epirubicin, and Mitoxantrone are anthracycline anticancer medicines, with Doxorubicin being Particularly Notable for its Broad Clinical Spectrum of Anticancer Activity (Cheng et al., 2017; Miladiyah et al., 2020). The anthracyclic plane that daunorubicin and doxorubicin share enables them to bind securely to DNA and insert between DNA base pairs, causing disruption of DNA's spatial structure. Purine nucleosides are specifically targeted by this activity, which inhibits the production of DNA and DNA-dependent RNA. Additionally, daunorubicin and doxorubicin cause DNA double and single strand breaks by inhibiting nuclear topoisomerase activity and having cytotoxic effects on cell. Mitomycin functions as a non-specific medication in the cell cycle and exhibits anticancer effects on a variety of malignancies (Miladiyah et al., 2020). Although it has rapid curative effects and a wide range of anticancer activity, it also has a low therapeutic index and considerable toxicity. Most interactions between mitomycin and DNA take place on the single strand, and some interactions result in cross-links that prevent DNA double strands from disassembling, partially breaking the stable double helix shape of DNA (Wang et al., 2019). By separating the first glycopeptide antibiotic from Streptomyces rotundus, Metzibinoff of the Japanese Institute of Microbiology and Chemistry made the discovery of bleomycin (Aarts et al., 2020). It successfully prevents DNA synthesis and breaks both single-

stranded and double-stranded DNA (Murray et al., 2018).Actinomycin belongs to a group of antibiotics called cyclic peptide antibiotics. By attaching itself to the DNA double helix, it can form a complex with DNA that hinders RNA polymerase's ability to function and ultimately stops RNA synthesis from occurring (Prouvot et al., 2018).

Defuminomycin disrupts the DNA template by creating complexes that are stable with DNA, which notably prevents the creation of RNA. Antibiotics based on endomyne has anticancer qualities that are similar to those of actinomycin..

2.5 Application of different antibiotics as anticancer

Antibiotics that treat cancer have two distinct modes of action. They can kill cells at any stage of the cell cycle, even those in the G0 phase, which enables them to stop the growth of cancer cells by interfering with the cell cycle. Cyclinenon-specific medications (CCNSC) (Zhou et al., 2013)provide an illustration of such effect. There are two different ways in which antibiotics work to treat cancer. They can destroy cells at any stage of the cell cycle, even those in the G0 phase, which gives them the ability to disrupt the cell cycle and halt the proliferation of cancer cells. Medications that target clinenons (CCNSC) (Zhou et al., 2013)give an example of this effect. Anticancer antibiotics also regulate the epithelial-mesenchymal transition (EMT), which prevents cancer cells from metastasizing and acts as an anti-metastatic agent (Pratheeshkumar & Kuttan, 2011; Sanchez-Tillo et al., 2014). Salinomycin, for instance, suppresses proliferation and EMT during the growth of malignancies, hence helping to decrease cancer metastasis (Pratheeshkumar & Kuttan, 2011), whereas ciprofloxacin has pro-apoptotic properties.

Numerous scientists have noticed that some malignancies show increased susceptibility to recently developed anticancer antibiotic groups that they have not previously been exposed to. When compared to antibiotics that the cancer patients have previously been exposed to, these

novel medicines show more effectiveness in suppressing malignancies. From the first anticancer antibiotics, such as doxorubicin, epirubicin, and mitomycin, to more recent discoveries, like adriamycin, salinomycin, and fluoroquinolones found in the most current study, the importance of employing antibiotics for cancer treatment has grown over time. As a result, the use of antibiotics in cancer treatment has grown in significance.

2.5.1 Dactinomycin

Only the dactinomycin D and C subtypes, commonly known as actinomycin, possess clinical application value among the at least 50 distinct forms of dactinomycin that have been discovered thus far. A polypeptide antibiotic called dactinomycin D was produced from Str. Parvulus nutrition solution (Hayward et al., 2013). Two allelic cyclic peptide chains are joined by a phenoxy ring in its chemical structure. The DNA molecule's deoxyguanine can interact specifically with these peptide chains, leading dactinomycin D to bind to the groove of the DNA double helix and create a complex with DNA. This process interferes with RNA polymerase activity, preventing the generation of mRNA in particular and thereby preventing the development and spread of cancer (Van Hazel et al., 1983). Dactinomycin is primarily used to treat nephroblastoma, chorionic epithelial carcinoma, rhabdomyosarcoma, and neuroblastoma. It has a narrow anticancer scope (Bowman et al., 1991).

2.5.2 Daunorubicin

Streptomyces peucetius in culture was successfully used as the source of pilomycin by scientists at Farmitalia's laboratory in 1957. After that, in 1963, Di Marco et al. carried out a preclinical trial to show daunorubicin anticancer properties (Di Marco et al., 1977). The identical chemical, erythrobicin, was discovered from the culture solution of Streptomyces ceruleorubidus by French researchers Phome-phouleuc et al. around the same period. Later, in the same province of Hebei,

Chinese researchers discovered the same strain, isolated the same chemical, and gave it the name candimycin . Eventually, the name daunorubicin was given to all of these comparable "mycin" substances (Bloomfield et al., 1973). First-line chemotherapy drugs like daunorubicin are frequently used to treat cancers including acute leukemia, lymphocytic leukemia, and other tumours (Di Marco et al., 1977; Waters et al., 1999). Through chimerism with the DNA base pairs of cancer cells, it binds to DNA and blocks DNA spatial structure as part of its mechanism of action. This binding successfully slows the development of cancer(Waters et al., 1999).

2.5.3   Epirubicin

A brand-new anthracycline antibiotic called epirubicin has the unusual capacity to insert itself right in the middle of DNA base pairs. By doing this, it obstructs transcription, prohibiting the synthesis of mRNA, and successfully preventing the production of both DNA and RNA . The topoisomerase II enzyme is also inhibited by epirubicin (Waters et al., 1999). It works well against a variety of transplanted malignancies as a cell cycle nonspecific medication. Cancer, and other illnesses of a similar nature are among those for which epirubicin is frequently used. It's crucial to remember, though, that in addition to its medicinal advantages, epirubicin has also been linked to some negative side effects. These include high fever, mucositis, gastrointestinal system responses, hair loss, mucotoxicity, bone marrow suppression, and heart toxicity.(Waters et al., 1999)

2.5.4   Gemifloxacin

A fluoroquinolone antibiotic called gemifloxacin (GMF) works by preventing bacterial DNA gyrase and topoisomerase IV from functioning. It's interesting to note that this antibiotic has anti-proliferative and pro-apoptotic properties in addition to having anti-metastatic activity (Appelbaum et al., 2004). The first investigation demonstrating GMF's ability to reduce NF-B

activation and consequently prevent cancer necrosis factor (TNF)-induced cell migration and invasion was conducted by Tun-Chieh Chen et al. (Chen et al., 2014). In order to prevent and treat cancer metastasis, GMF exhibits promise as a potential innovative anticancer drug (Chen et al., 2014).

2.5.5 Mitomycin

As one of the most widely utilised cycle non-specific medications, mitomycin is an efficient anticancer medicine developed from actinomycin culture that is widely used for treating a variety of solid tumours. Three active groups are present in its structure: benzoquinone, ulatan, and ethylene imine (Dees et al., 1987). Mitomycin acts by depolymerizing DNA and impeding DNA replication when reductase in cells activates it. Additionally, it prevents the creation of RNA and proteins at high quantities. It mostly functions in the late G1 and early S phases, and it is still efficient in an acidic and hypoxic environment. Reduced membrane permeability and intracellular concentration are the primary contributors to drug resistance, and degradation speeds up the so-called mutation-selection pathway. Three anticancer medications make up mitomycin: mitomycin A, mitomycin B, and mitomycin C (MMC) 46. DNA replication and synthesis are inhibited by the mechanism of action, which includes the formation of double or intra-strand cross-links with DNA (Kim & Rockwell, 1995). The production of oxygen radicals caused by MMC may possibly be a factor in the anticancer effect of the compound. A side effect of this medication is bone marrow suppression, which is predominantly characterised by lower platelet counts (Kim & Rockwell, 1995). Additionally, medication exosmosis, particularly when paired with doxorubicin, might result in tissue ulcer necrosis.

2.5.6 Mitoxantone

Mitoxantrone is a member of the class of antibiotics used to treat cancer. Its anticancer properties

are comparable to those of doxorubicin. The absence of an amino sugar structure in mitoxantrone, however, makes it different in that it does not produce free radicals when lipid peroxidation is inhibited. Because of this, Mitoxantrone is less harmful to the heart than Doxorubicin. Furthermore, Mitoxantrone can penetrate cells and interact with mitochondria, effectively halting the progression of cancer. (Fox et al., 1986)

2.5.7 Plicamycin

Plicamycin, commonly referred to as brilliant-mycin, is an antibiotic made from the S. 684 strain of the culture strain of Streptomyces tanushiensis. Through its method of action, which involves DNA binding, RNA synthesis inhibition, and targeting cell proliferation at multiple phases, this antibiotic exhibit strong inhibitory effects on a variety of malignancies. Plicamycin is primarily used in clinical settings to treat testicular embryonal cancer and different malignant tumours linked to hypercalcemia. Additionally, it can be used to treat diseases including lymphoma and glioma. It is important to remember that Plicamycin may cause a number of adverse effects, including gastrointestinal problems, bleeding, as well as severe liver and kidney damage.(Thürlimann et al., 1992)

2.5.8 Salinomycin

Salinomycin was isolated from Streptomyces alba bacteria and produced through a tank fermentation technique initially used as an agricultural antimicrobial substitute for eradicating bacteria, fungi, and parasites as well as for improving feed efficiency in ruminants. This antibiotic is a monocarboxylic polyether ionophore with a molecular weight of 751 Da that also serves as an ionophore (Miyazaki et al., 1974). It has weak acidic characteristics. Specifically, salinomycin interferes with transmembrane potassium potential by acting as an ionophore with stringent selectivity for alkali ions and demonstrating a considerable affinity for potassium.

Particularly with regard to different types of cancer stem cells (CSCs), salinomycin has shown promise anticancer capabilities, and it also sensitises multidrug-resistant human cancer cells (Xipell et al., 2014).

2.6 Ciprofloxacin as anticancer

A second-generation fluoroquinolone called ciprofloxacin is very effective against Pseudomonas aeruginosa and other gram-negative bacilli (Zemelman et al., 1993). This medication has a wide distribution throughout the body and a high bioavailability (Zemelman et al., 1993). Ciprofloxacin is frequently used to treat respiratory, urinary, and gastrointestinal infections because of its favourable pharmacokinetics and capacity to reach larger concentrations in tissues compared to plasma (Peloquin et al., 1989).

Additionally, in vitro tests using human bladder cancer cell lines, human colorectal cancer cell lines, hamster ovarian cancer cell lines, and human hepatocellular carcinoma have shown that ciprofloxacin has anticancer properties (Beberok, Wrześniok, Minecka, et al., 2018) (Shipley & Butera, 2009). Pseudomonas aeruginosa and other gram-negative bacilli are effectively inhibited by ciprofloxacin, a second-generation fluoroquinolone (Zemelman et al., 1993). According to Zemelman et al. (1993), this medication has a high bioavailability and is widely distributed throughout the body. Because of its favourable pharmacokinetics and ability to reach higher concentrations ciprofloxacin is used to cure infections (Peloquin et al., 1989).

Furthermore, ciprofloxacin has been demonstrated to exhibit anticancer effects in in vitro experiments employing human different cell lines (Beberok, Wrześniok, Minecka, et al., 2018) (Shipley & Butera, 2009).

2.7 Liposomal Nanoparticle for Treatment of AML

Phospholipid bilayers make up the spherical vesicles that make up liposome-based nanomedicines. In order to facilitate effective transportation without affecting their structural integrity, these vesicles can contain hydrophilic chemicals inside their aqueous core or lipophilic compounds within the lipid bilayer (Yang et al., 2015) .Since they can improve the solubility, bioactivities, and distribution of medicinal drugs, liposomes have drawn a lot of attention and are now a commonly used class in medicine (Wang et al., 2014) .The FDA or the European Medicines Agency (EMA) have currently approved 16 liposomal medications, demonstrating their expanding significance in the pharmaceutical industry (D'Mello et al., 2017).

Cytarabine and daunorubicin are present in CPX-351 (Vyxeos), a liposomal nanomedicine that has been approved for the treatment of AML, in a set molar ratio (5:1). Its usage in newly discovered therapy-related AML and/or AML with myelodysplastic alterations received FDA and EMA approval in 2017. Initial in vitro and in vivo testing was done on five liposomal drug carriers utilizing leukaemia cell lines. In comparison to using drugs at their maximum tolerated doses (MTD) alone, the results showed that liposomal-encapsulated drugs exhibited a highly advantageous synergistic effect and minimal antagonism at the 5:1 ratio, leading to higher response rates, more robust remissions, and longer maintenance periods in bone marrow (Lim et al., 2010; Tardi et al., 2009).

A human leukaemia xenograft model was used to conduct additional analysis of the therapeutic efficacy and frequency of these liposomal drug carriers (Kim et al., 2011). The effects of liposomal drug carriers and the same ratio of free medicines in primary blood cells from AML patients and normal bone marrow donors were also investigated in a later investigation. The results demonstrated that AML cells preferentially accumulated liposomal drug carriers over

normal cells, as seen by normal peripheral blood and bone marrow demonstrating better sensitivity to free medicines (Feldman et al., 2011).

2.8 Objective of Research

- The main aim of this research is to determine the antileukemic properties of ciprofloxacin liposomes in comparison with doxorubicin liposomes.

- The objective of the research involves the formation of liposomal nanoparticles, bank as well as doxorubicin and ciprofloxacin loaded. Furthermore, more characterization of drugs and nanoparticles is performed to ensure the formation of drug-entrapped nanoparticles.

- Objectives also include measuring the cytotoxicity of drugs and their respective liposomes by different biological testing.

- The objective of the research also involved evaluating the effects of benzene, drugs, and their liposomes on histological, morphological, and blood parameters in the leukemic rat model.

- Hepatic and renal biomarkers activity is measured in benzene, drug-treated, and drug-loaded liposomal groups to find the efficiency of the drug against acute myeloid leukemia.

CHAPTER 3: METHODOLOGY

3.1 Experiment Design

3.1.1 Materials

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) lipid, dipalmitoyl phosphatidylcholine (DPPC), Cholesterol, commercially available ciprofloxacin and doxorubicin drug, Polyethylene glycol (PEG- with Molecular weight 1000) were purchased from Sigma-Aldrich USA. Balb/c female rats were purchased from Animal House of ASAB (Atta-ur-Rahman school of Applied Biosciences), National University of science & technology (NUST), Islamabad.

3.1.2 Synthesis of Ciprofloxacin and Doxorubicin loaded-liposomes Nanoparticles

In order to create liposomes, a 4:1:1 molar ratio of the components of liposomes—DMPC, DPPC, and cholesterol—was created. To make a 100 μMolar solution, the lipids were first precisely measured and dissolved in ethanol. Doxorubicin and ciprofloxacin 200-Molar solutions were also made in ethanol in separate phases. Next, the lipid solutions were mixed with 500 μL of each drug solution. For 40 minutes, the combined mixes were subjected to sonication at 80 MHz. Next, in a water bath, a lipid phase and 10 mL of water were heated separately until the temperature reached 60°C. Following the proper heating of both phases, they were combined and vigorously stirred at a speed of 90 revolutions per minute for ten minutes to create a dispersion mixture. Again, this new mixtures were sonicated at 50 MHz for 40 mins and then allowed for rotary evaporation (greater than 50 °C which is the phase transition temperature) to get rid of the excess ethanol (Chorachoo et al., 2013). Finally, unentrapped drug was removed via dialysis tube with size 2 Inf Dia 18/32 – 14.3mm and with a pore size of 12- 14000 Daltons.

3.1.3 Pegylation of Ciprofloxacin and Doxorubicin loaded-liposomes Nanoparticles

The mixture of Ciprofloxacin and Doxorubicin loaded-liposomes Nanoparticles was diluted to a volume of 50 ml, at this point 0.25% PEG (1000) was added drop by drop, stirred continuously, and allowed for rotary evaporation until only 10 ml of solution remained. A dialysis tube was used to remove any unentrapped drug.

3.2 Physical Characterization

To assess and study the particle size, shape, surface charge, drug encapsulation, release efficiency, and dispersity Index of Ciprofloxacin nanoparticles doxorubicin loaded nanoparticles, characterization was conducted through different characterization techniques were carried out.

3.2.1 U.V-Vis Absorption Spectroscopy

UV-Vis spectroscopy is a technique mostly used in chemical and clinical laboratories. It measures the extent of absorption in the sample, when light beams pass through it and the absorption is measured from reflected beam. The beam of light is split where half of the beam is focused all the way through the cuvette containing the measuring sample and the remaining half is guided to a cuvette containing only the solvent as control. Absorption can be measured at a given wavelength and a target range, and a spectrum is obtained that maps entire wavelength range versus its absorption at particular wavelengths. Maximum absorption is called as lambda max at specific wavelength. It analyses the electronic molecular transformation and obeys the Beer Lambert Law theory. The molar absorptivity, a measure of absorption that is used to compare various compound spectra, is proportional to the molar concentration in the sample cuvette. As the Beer-Lambert Laws says,

$$A=EcL$$

Molar absorptivity E= A/ cl (where A= absorbance, c= sample concentration in moles/ liter and L= length of light path through the cuvette in cm). This law enables UV-VIS spectroscopy as a useful device for quantitative analysis (Amendola & Meneghetti, 2009).

U.V-Vis spectra of Blank nanoparticles and drug loaded ciprofloxacin and doxorubicin nano particles were measured by using Shimatzu UV-Vis 2800 BMS Scientific Technical Corporation (PVT) spectrophotometer, from 200-450nm at a resolution of 1nm. For reference purpose, deionized water was used. The UV spectra of ciprofloxacin drug, PEG-Coated Ciprofloxacin nanoparticles, Blank nanoparticles, free doxorubicin, and doxorubicin loaded nanoparticles   were recorded.

3.2.2 Fourier transform infrared spectroscopy (FTIR) analysis.

It is a systematic technique used for the detection of mostly organic and a few inorganic substances. The sample material is measured by absorbing infrared radiation (IR) versus wavelength. The absorption bands in the IR describe the molecular components and structures of the sample. When an infrared radiation irradiates a substance, the molecules go to the exciting state with greater vibration due to the absorbed IR radiation. The difference in energy between the excited vibrational state and the resting state determines the wavelength of light that a single molecule absorbs. The molecular structure of the sample is defined by the wavelengths it absorbs (Zeeshan et al., 2019).

 Samples were air dried before being processed for FTIR analysis using compressed KBr discs. FTIR spectra were captured using the Bruker FTIR Spectrophotometer ALPHA II between 4000 and 350 cm^{-1}. FTIR analysis of all formulating constituents was done including DMPC, Cholesterol, Ciprofloxacin, Doxorubicin, Blank liposomes (BNPs), Pegylated-ciprofloxacin loaded liposomal nanoparticles and Pegylated- doxorubicin loaded liposomal

nanoparticles.

3.2.3 Particle size and Area Distribution

Scanning electron microscopy (SEM) was utilized to examine the practical size. Using the "image j software," the nanoparticles' area distribution is determined. An analysis of the chosen field is conducted. In binary or threshold pictures, the 'Analyze Particles' command counts and measuresitems. It operates by scanning an area or image until the edge of an object is detected. Values for particle size are provided in the 0 to 'Infinity' range. In this region, particles with circularity values beyond the defined range will likewise be disregarded. Analyzed the 8-bit binary image of the best-fitting ellipse of the observed particle (Zhou et al., n.d.).

By using a micropipette to place a little portion of the sample on a cover slip, all types of nanoparticles (BNPs, Ciprofloxacin and doxorubicin nanoparticles) were photographed.. The National University of Science and Technology, Islamabad's VEGA3 LMU scanning electron microscope was used to capture the images. Malvern Zeta Sizer Ver. 7.12 was used to measure the size distribution and dispersity of both types of NPs using Dynamic Light Scattering (DLS).

3.2.4 Zeta Potential

Zeta potential is frequently the sole value that can be used to describe the double-layer characteristics of the colloidal dispersion because it is not equivalent to the electrical surface potential of a double. layer or to the Stern potential. Zeta potential is expressed in millivolts (mV) and is also known aselectro kinetic potential. Zeta potential analyzer was known to have surface charge and zeta potential. Zeta potential explains about the nanoparticles' stability,

surface charge and average size. Zeta potential in colloids is the differential in the electrical potential via the ionic layer around a charged colloid ion. It is, in other words, the potential at the slipping plane of the double layer interface. The stability of the colloid will typically increase with the zeta-potential. Particle agglomeration often starts at zeta potentials that are less negative. If the colloid's zeta-potential is equal to zero, it will solidify (Z Zeta-Potential, n.d.).

The potential difference across phase boundaries between solids and liquids is known as the zeta potential. It is a measurement of the electrical charge of a suspended particle in liquid. The zeta potential (surface charge) of all type of LNPs (BNPs, Ciprofloxacin and Doxorubicin nanoparticles) was assessed by Dynamic Light Scattering (DLS) using Malvern Zeta Sizer Ver. 7.12.

3.2.5 Drug Encapsulation and Release Efficiency

Drug efficiency delineates the amount of drug to be entrapped with in the vesical of liposomes. A feasible linear standard curve was constructed by analyzing various drug dilutions using a UV spectrophotometer at 280 nm absorbance to determine the effectiveness of drug encapsulation.. The unentrapped drug was calculated further using this standard curve value. To find the drug fraction that was not entrapped, samples were centrifuged at 4500 rpm for 1 hour. The supernatants were then examined using UV-visible spectroscopy. (2005) Nii & Ishii Following that, the given formula was employed with the calculated data.

$$Encapsulation\ Efficiency = \frac{Total\ drug - Unentrapped\ drug \times 100}{Total\ drug}$$

3.2.6 Drug Release

 The release behavior of drug from nanoparticles vector has great importance in treating with

nanomedicines. Release of drug cargo in time dependent manner at targeted site is the main concern of nano formulation that results in controlled or sustained release. Drug release LNPS and PEG-LNPs were examined up to 48 hours along with the addition of specified volume of Phosphate Buffer Saline (PBS). From both 25 ml solutions of BNPs, PEG coated Doxorubicin liposomes and PEG coated Ciprofloxacin nanoparticles , 3ml samples wereplaced into separate centrifuge tubes and allowed for 10 min centrifugation at 4500 rpm at room temperature. While on other hand 3ml of PBS was poured to BNPs, PEG coated Doxorubicin liposomes and PEG coated Ciprofloxacin nanoparticles solutions. After this, supernatants were collected and allowed for UV spectrophotometer analysis. Same procedure was conducted after 1,2,4,6,12, and 15hr. At 280nm of wavelength, absorbance values were taken and used as cumulative drug release. Entire analysis was performed by using empty nanoparticle solution as control.

3.3 In-Vitro Assays

Multiple assays were performed to check the biological activity of free drug Ciprofloxacin, Doxorubicin, blank nanoparticles, and peg-coated doxorubicin and ciprofloxacin loaded liposomes. All assays (in-vivo and in-vitro) were performed under standard conditions, with optimized protocols. Various tools, materials and molecular biology techniques were utilized to obtain desired results.

3.3.1 Stock preparation for invitro assay

Dilutions of drug and nanoparticles are prepared to test different biological activities. The dilution of nanoparticles is 100, 80, 60 percent of original nanoparticles. Nanoparticles are diluted with Pbs. For drugs solution 500 μL from 4mM solution of drugs is added in 10ml of distilled water and further from this stock 80, 60 and 40 percent stock solutions are prepared

for testing.

3.3.2 Brine shrimp assay

The Brine shrimp assay was used to determine the optimal medication dosage for chemotherapy. Under fluorescent lights, brine shrimp eggs were allowed to incubate in a sea salt solution for a whole day. A 96-well plate was filled with different concentrations of free pharmaceuticals, specifically Doxorubicin and Ciprofloxacin, along with blank nanoparticles, peg-coated liposomes containing Doxorubicin, and Ciprofloxacin. In each dilution, ten brine shrimp specimens were added. Lethal concentration (LC50) was calculated by counting the number of prawns that survived compared to the number that died during a 24-hour incubation period.

3.3.3 Hemolysis assay

The in vitro hemolysis assay assesses haemoglobin release in the plasma (as a marker of red blood cell lysis) after test agent exposure. Human blood is combined with serial dilutions of the substance, which is then incubated for 45 minutes at 37°C. After centrifuging the cells, the absorbance of the supernatant, which contains plasma and lysed erythrocytes, is calculated. Using a standard curve of lysed erythrocytes, the percentage of lysis is computed. Percentage hemolysis was used to assess the hemolytic assay of the free drug Ciprofloxacin, Doxorubicin, blank nanoparticles, and peg-coated doxorubicin and ciprofloxacin loaded liposomes. From healthy male fresh blood sample is collected in EDTA tubes. 6 mL of PBS buffer were added to 3 mL of blood sample, and the mixture was centrifuged at 3000g for 10 mins to suspend the cells in PBS. After repeating the washing process, PBS was used to suspend the cells back into their original volume.

For the hemolytic assay, dilutions of free drug Ciprofloxacin, Doxorubicin, blank

nanoparticles, and peg-coated doxorubicin and ciprofloxacin loaded liposomes were utilised.100µl of blood sample with 100µl of each nanoparticle sample was incubated at 37°C for 4hr. PBS served as the negative control, and Triton-X100 as the positive control. They were combined after the incubation and centrifuged one more for 10 minutes. A 96-well plate was filled with 100µl of supernatant, and the Thermoscientific Multiskan Sky microplate reader was used to detect the absorbance at 540 nm. The formula was used to determine the proportion of hemolysis.

3.3.4 Antioxidant Assays:

DPPH Activity:

As an indicator of antioxidant activity, the DPPH assay was used to assess the capacity to scavenge 1,1-diphenyl-2-picrylhydrazyl, or DPPH free radicals. The positive and negative controls used were autoclaved distilled water and ascorbic acid (1 mg/ml). 3.9 mg of solid DPPH was dissolved in 100 ml of methanol to create a 0.1 mM DPPH solution. By diluting the DPPH stock solution with methanol, the optical density was fine-tuned to 0.96 ($\pm$0.03). 180 µl of the DPPH solution and 20 µl of the previously specified sample dilutions were mixed together on a 96-well plate. The mixes were incubated for 30 minutes at 37°C in the dark while this reaction was carried out in triplicate. After the samples were incubated, their absorbance was measured at 517nm.

Reducing Power Assay:

Moein et al. (2008) developed a method for determining the TRP (Total Reducing Power) value. The TRP assay is used to evaluate a compound's potential for decrease. In order to prepare the stock solutions, 1.42 g of $Na_2HPO_4.2H_2O$ and 1 g of NaH_2PO_4 were dissolved in 50 ml of double-distilled water to form a 0.2M phosphate buffer. After adjusting the pH of the solution to

6.6, it was thoroughly dissolved by being agitated using a magnetic stirrer. In a different preparation, 1g of K3Fe(CN)6 was dissolved in distilled water to yield about 1% potassium ferricyanide. 10g of trichloroacetic acid (TCA) was dissolved in 100ml of distilled water (DW) to create a 10% TCA solution, and 0.1g of FeCl3 was dissolved in 100ml of DW to create a 0.1% FeCl3 solution. 150ul of samples dilutions mentioned above was added in eppendorf along with 425ul 0.2M Phosphate buffer and 425ul potassium ferricyanide. Solution was incubated at 50 °C for 20min. 500ul of 10% Trichloroacetic acid addition was then followed by centrifugation for 10min at 3000 rpm. 500ul of supernatant was picked from Eppendorf and 100ul of 0.1% ferric chloride was added. Ascorbic acid was used in the concentration of 1mg/mL as the positive control for antioxidant assay while H_2O is used as the negative control. Absorbance was measured at 630nm.

Total antioxidant capacity (TAC) assay:

The total antioxidant capacity assay is used to quantitatively determine the antioxidant capacity of samples by a spectroscopic technique. The total antioxidant assay based on reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V). Chemicals were carefully weighed at first. 0.247g of $(NH_4)_2MoO_4$ was mixed with 1.679g NaH_2PO_4 and were then dissolved in 50mL of distilled water. Then 1.63mL of H_2SO_4 was added into the solution. Final solution prepared was the TAC reagent. Ascorbic acid was used in the concentration of 1mg/mL as the positive control for antioxidant assay while H_2O is used as the negative control. 20ul sample and 180ul of TAC reagent was added in triplicates into 96 wellplate. Ascorbic acid and H_2O were added as controls. It was then incubated at 95° for 90min, cooled and absorbance was taken at 630nm.

3.4 Experimental Strategies for animal testing

Female BALB/c rats with the age of 7 weeks and weight 100-150g were purchased from ASAB animal house. The animals were made into groups randomly and subjected to an acclimatization period for about 1 week with free access to food and water as per the requirement. Rats were keptin Standard homcages ($42 \times 26 \times 18$ cm). Home cages were filled with fresh sawdust that was replaced after every 2 days and a consistent 9:15 h light/dark cycle was kept. Temperature was maintained at 27°C ± 2°C with humidity 600% ± 5%. Rats were divided into seven groups for each type of AML model with five mice per group.Weights of Rats were recorded twice a week throughout the depression and treatment timeline. The average weight, per week, is presented in results. Mice handling and care were governed by US FDA guidelines for proper laboratory practise. (Food and Drug Administration) in 1978.

3.4.1 Grouping of rats

Group 1 is the control group. Group 2 is a diseased group. Group 3,4,5,6,7 are diseased group and are given treatment with free Ciprofloxacin (dosage 5mg/kg), free Doxorubicin (dosage 5mg/kg), Blank nanoparticles, peg-coated Ciprofloxacin, peg-coated doxorubicin administrate intravenously for 4 weeks on alternative days.

3.4.2 Induction of AML

Benzene is used to induce AML. Benzene injections were prepared by mixing benzene isopropanol and water in a (1:2:1) ratio. A fixed proportion of 0.5 mL benzene, 1 mL isopropanol, and 0.5 mL water were used to make the stock solution. For four weeks on alternate days, 100 µLof this stock was administered intravenously into each rat of groups 2, 3, 4, 5, 6, and 7.

3.4.3 Treatment

The free drugs and liposomes are administered for 4 weeks on alternative days according

to dosage mentioned above.

3.4.4 Animal Dissections

Following completion of dosage, rats were dissected according to the measures determined by the Institutional Animal Care and Use Committee (IACUC). Chloroform was used to render the rats unconscious, and the abdominal cavity was opened using surgical equipment while the rats were placed on the dissection table. Essential organs such as the liver, kidney, and heart were acquired.

3.4.5 Blood Collection and Storage

After extracting blood directly from the heart with heparinized syringes, blood was collected immediately in yellow color vacutainer serum tubes and purple color vacutainer EDTA tubes to avoid clotting. Serum was extracted by centrifuging serum tubes for 15 minutes at 3000 rpm. To prevent hemolysis, serum tubes were kept at - 40 °C and EDTA tubes at -4 °C.

3.5 Morphological Analysis

Blood smears were made from fresh blood for morphological study. A blood drop was placed on glass slides, and then a cover slip was slide at 45 degrees on a blood dot to form a smear, which was then air dried. Smears were fixed by dipping them in cooled methanol for 3 minutes and then air drying them. After that, Giemsa staining was performed by immersing smears in a 10% Giemsa staining solution for 10 minutes before washing with distilled water and air drying again. The slides were examined using a compound microscope with a 100X lens to examine cell morphology. ISCapture software was used to capture the digital pictures recorded by the camera.

3.5.1 Blood complete picture

Different factors such as total blood cell count, hemoglobin, and so on were measured in order to provide a complete picture of the blood. The total blood cell count was performed using automated equipment at the Rawalpindi Institute of Urology and Transplantation (RIUT). 10 uL of blood taken in EDTA tubes was sent to RIUT for blood CP analysis.

3.5.2 Serological testing

For ALP, AST, ALT and Uricase activity is also measured by at the Rawalpindi Institute of Urology and Transplantation (RIUT).

CHAPTER 4: RESULTS

4.1 Physical Characterization

Physical Characterization of Ciprofloxacin and Doxorubicin Loaded LNPs and Blank LNPs

Both Ciprofloxacin and Doxorubicin-loaded liposomes and blank liposome nanoparticles were successfully synthesized, as shown by physical characterization.

4.1.1 UV-VIS absorption Spectroscopy

UV-VIS absorption spectroscopy of ciprofloxacin medication exhibited the surface plasmon resonance (SPR) peak primarily at 270 nm, Doxorubicin at 240nm and 460nm, blank liposomes at 207 nm, ciprofloxacin loaded-LNPs at 212 nm, and Doxorubicin loaded-LNPs at 230 nm. The peaks of cholesterol were at 204 nm, DPPC at 202 nm, and PEG 1000 at 225nm and 250 nm. The Lipid DMPC showed a peak at 210nm.
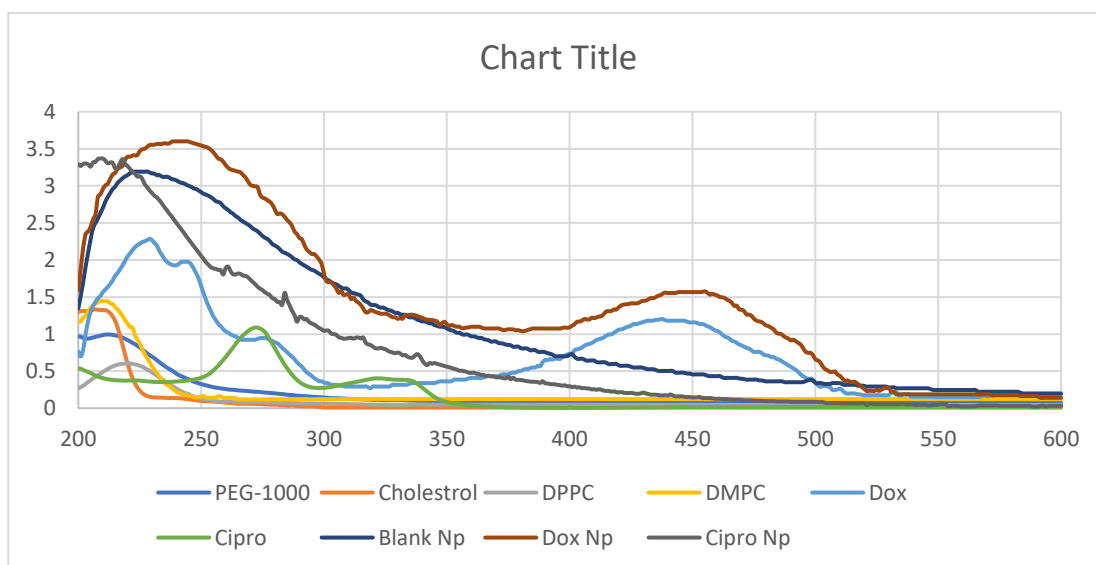

Figure 4.1 UV-Vis Spectrum absorption Spectroscopy of all drugs and constituent of nanoparticles.

4.1.2   Fourier transform infrared spectroscopy (FTIR) Analysis

The FTIR spectrum of Cholesterol indicated peaks or bands at 2850/cm (CH stretch, Alkanes), 873/cm (Tri-substituted Aromatics). DPPC spectrum indicated peaks at 2919/cm (CH stretch, Alkanes), 1632/cm (R-NH2, Amines), 1115/cm (C-O stretch, Ether), 720/cm (RCH2CH3, Bending mode). DMPC spectra indicated peaks at 1500/cm (O-H stretch, hydroxyl group) and 2830/cm (C-H stretch, indicating alkane). PEG-1000 spectrum delineated peaks at 3429/cm (O-H stretch, Alcohol), 2923/cm (CH stretch, Alkanes) and 1638/cm (C=C stretch, Alkenes). The Ciprofloxacin shows peak at 3410/cm due to (N-H bond) of imino moiety present on piperazine group. The primary amine (N-H) bend of pyridine moiety shows peak at1655/cm, and C-F functional group shows peak at1070/cm The observed changes in infrared bands proved the conformational changes in lipid biomolecules' by incorporating ciprofloxacin and Doxorubicin drug and PEG 1000, which masked the peaks due to coating.
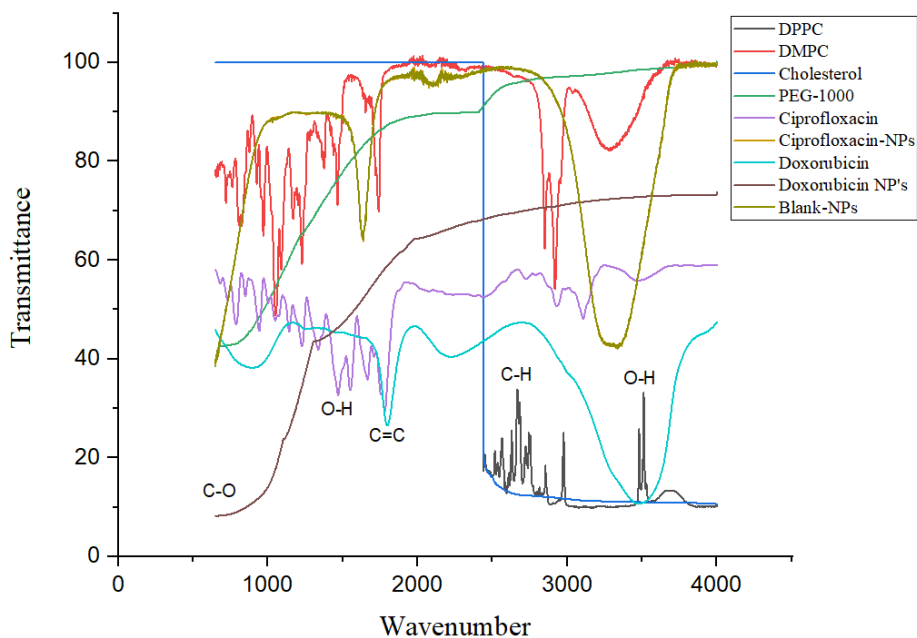
Figure 4.2 FTIR of drug, component of nanoparticles and nanoparticles.

4.1.3 Particle size

Scanning electron microscopy is used to determine the size of the doxorubicin and ciprofloxacin loaded- LNPs, and blank LNPs. A scanning picture revealed that the ciprofloxacin, doxorubicin-loaded liposome nanoparticles were spherical in form and had an average size larger than blank liposomes, while the blank nanoparticles had an average size of less than drug-encapsulated particles. This shows the encapsulation of the drug within nanoparticles. Figure A shows blank liposomes, B shows ciprofloxacin liposomes and C shows doxorubicin liposomes.

Figure 4.3 SEM of blank and drug-loaded nanoparticles.

4.1.4 Zeta Potential

Compound	Zeta Potential (mv)
Blank Nano particles	- 4.6
Doxorubicin Nano particles	- 6.07
Ciprofloxacin Nanoparticles	-7.08

Figure4.4 zeta potential of ciprofloxacin, doxorubicin and blank nanoparticles respectively

The average Zeta potential of drug-loaded Blank liposomes, Ciprofloxacin, and doxorubicin nanoparticles is -4.6.03, -6.07, -7.08 respectively.
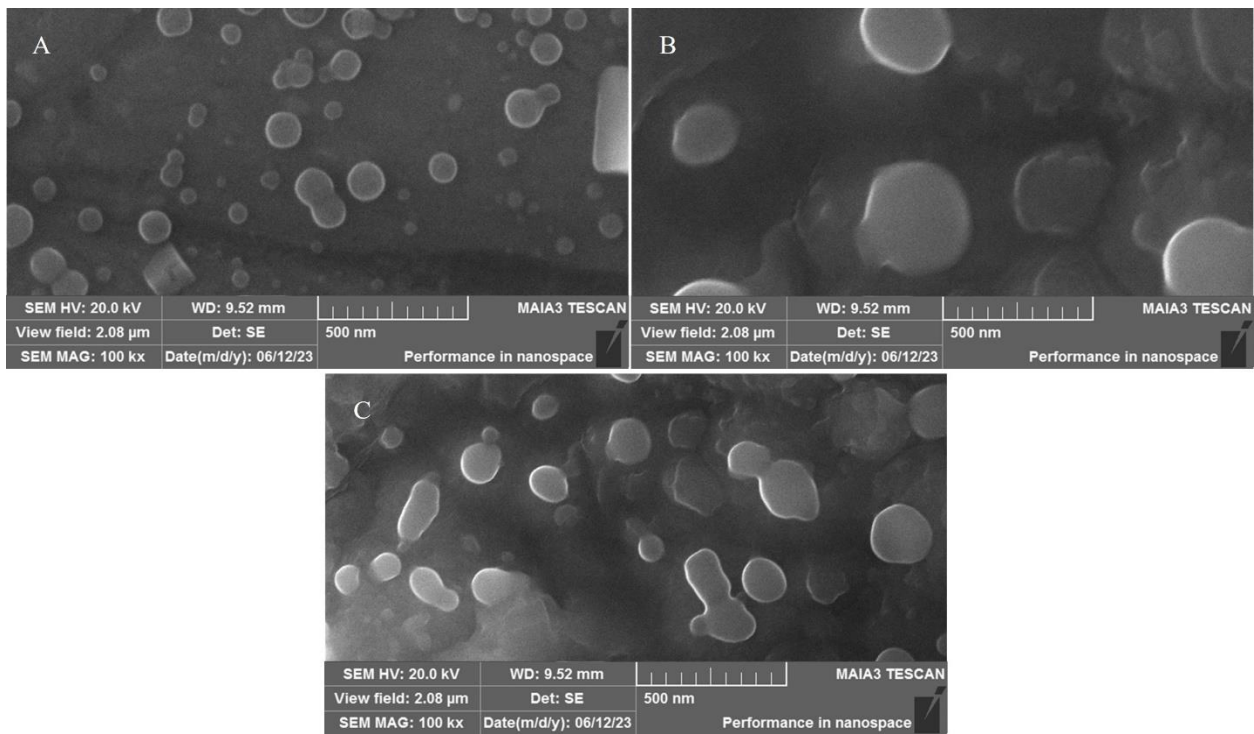
4.1.5 Drug Encapsulation Efficiency and Drug Loading Capacity

The previous formula, which can be found in the material section, was used to determine the encapsulation efficiency, which was calculated to be 77.6% for doxorubicin and 72.4% for ciprofloxacin. This indicates that 77.6% and 72% of the medicine encapsulated inside the doxorubicin and ciprofloxacin nanoparticles respectively.

4.1.6 Drug Release Kinetics

The fact that 49% of the medicine was released from the Doxorubicin liposome nanoparticles and 43% of the medicine was released from the Ciprofloxacin liposome nanoparticles after being monitored for up to 16 hours suggests that doxorubicin and ciprofloxacin LNPs maintain a steady release of the drug throughout time. Because the medicine is kept in the body for such a long period of time, its bioavailability is improved, which eventually results in a greater degree of success intreating illness.
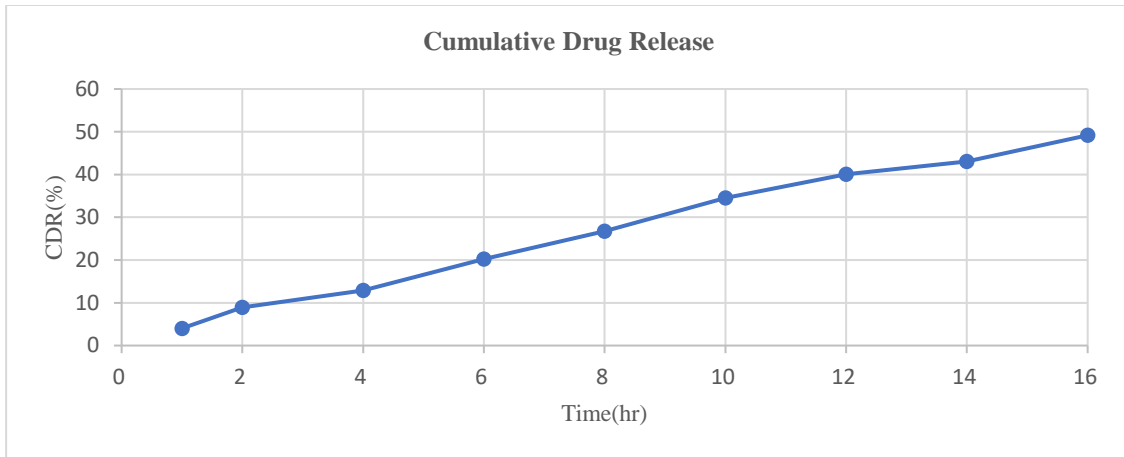
Figure 4.5 Drug Release of doxorubicin LNPs
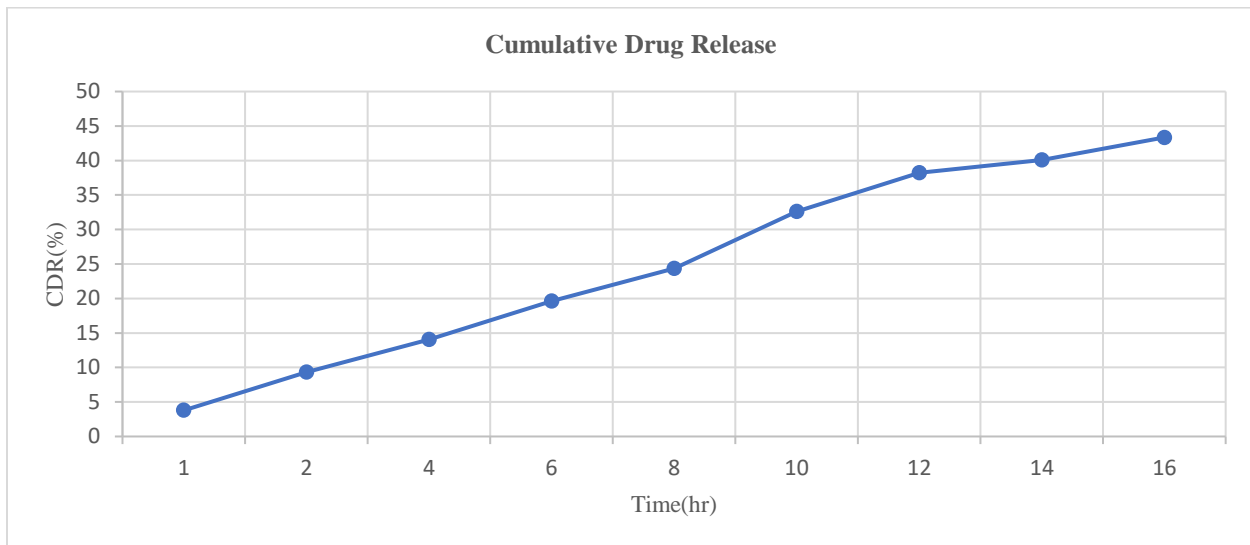
Figure 4.6 show Drug Release of Ciprofloxacin LNPs

4.2 Invitro Assay Result

After careful experimentation, the findings were analyzed. All biological assays were done in triplicates.

4.2.1 Brine Shrimp Assay

After One-way ANOVA analysis, it was observed that the lower concentrations were significantly less cytotoxic as compared to the higher i.e., 80% of free doxorubicin and ciprofloxacin are more toxic same is the case with nanoparticle concentrations higher concentrations are more cytotoxic than lower one. Graph shows very low %age mortality in case of lower concentration of nanoparticles as compared to free drug (p<0.0001).

4.2.2 Hemolytic assay

After One-way ANOVA analysis, it was observed that the lower concentrations significantly cause less red blood cell lysis as compared to the higher i.e., 80% of free doxorubicin and ciprofloxacin are more toxic same is the case with nanoparticle concentrations higher concentrations that is 100% are more cytotoxic than lower one that are 60% dilution. Graph shows very low %age of hemolysis in case of lower concentration of nanoparticles as compared to free drug (p<0.0001).
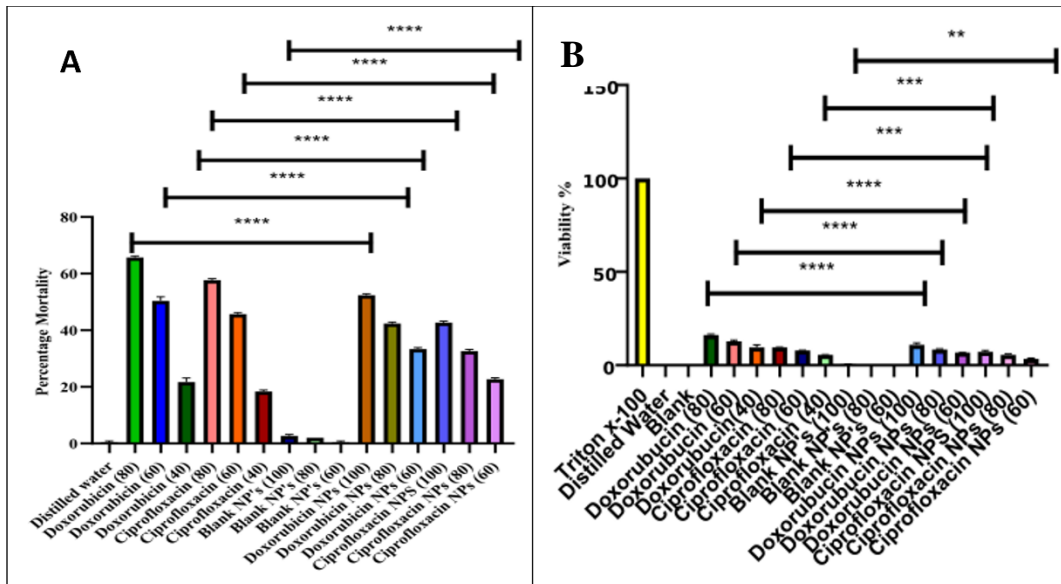
Figure 4.7 Comparison of A) %age mortality of brines shrimp and B) viability of blood different concentrations of free drugs and nanoparticles

4.2.3 Total Reducing Power, DPPH Scavenging Percentage, and Total Antioxidant Capacity

According to One-Way ANOVA, best reducing power was depicted by of 100% of ciprofloxacin nanoparticles was relatively lower than that of ascorbic acid i.e., positive control indicating its excellent reduction potential at this dilution. Decreasing the concentration of compound further to lower concentration decreased the reduction capacity also. Similarly, the reduction potential of nanoparticles was also slightly lower than that of free drugs. Nanoparticles however showed a gradual decline in their reduction potential values with concentration. Both free drugs and nanoparticles showed significant TRP, DPPH, and TAC values in comparison to Ascorbate. All the results were found keeping +ve control as standard. Ascorbate showed significance $P<0.0001$ with all dilutions of nanoparticles.
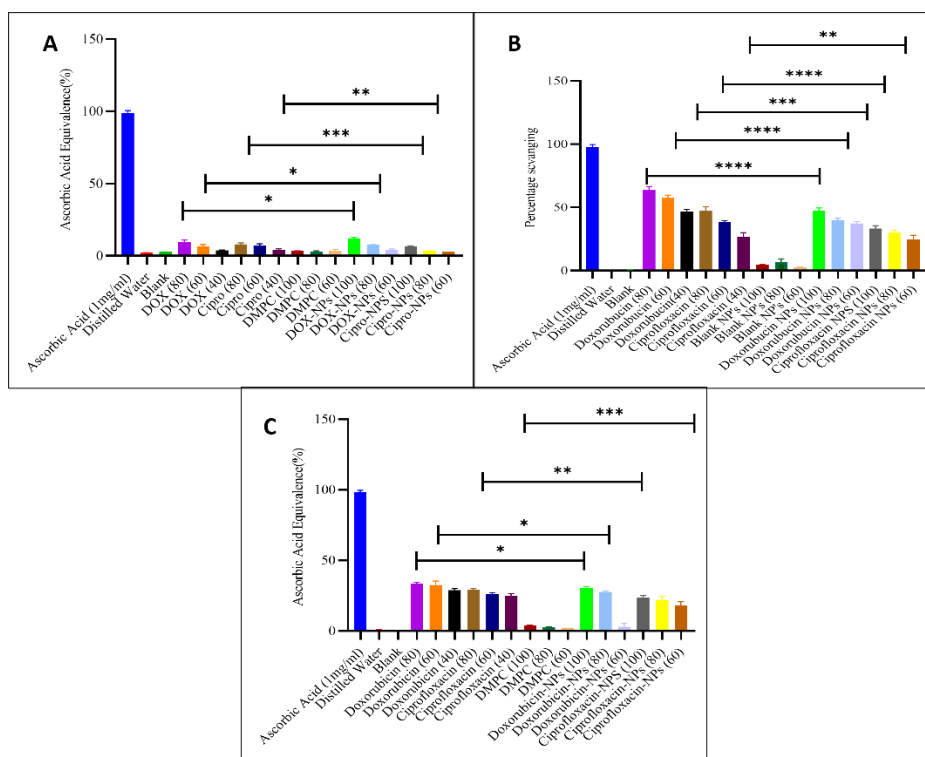
Figure 4.8 Comparison 0f A) and DPPH scavenging percentages B) Total Antioxidant Capacity values, C) Total Reduction Potential value, and DPPH scavenging percentages of free drugs and nanoparticles

4.3 Animal Testing Results

4.3.1   Recovery of Body Weight and Organ Weight

The body weight of rats decreases in all groups during administration of benzene due to disease induction. During treatment, weight recovered to normal level. The weight recovery is higher in nanoparticles treated group as compared to diseased one. The weight decreases during 4 weeks of disease induction and then starts increasing during treatment weeks. The weight of the diseased group does not recover to normal level.
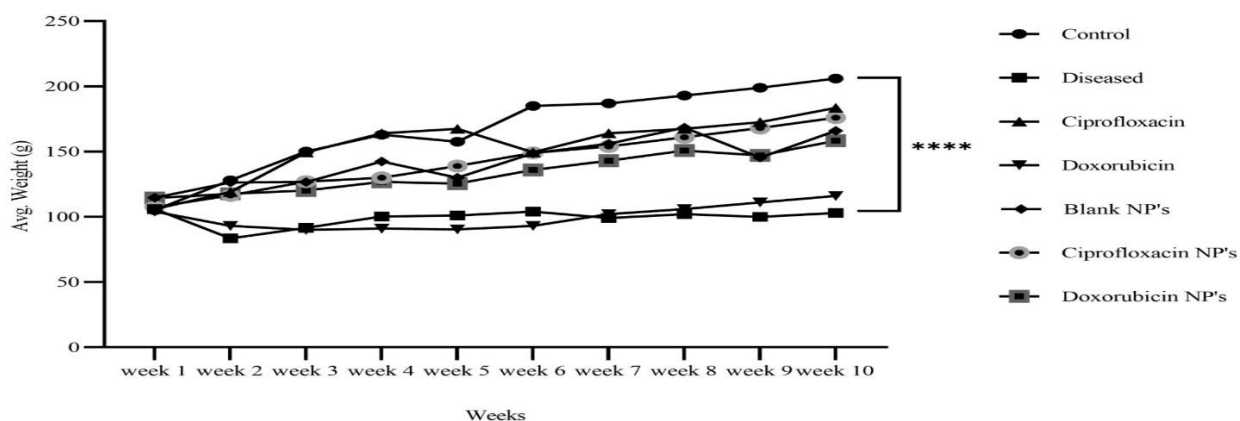
Figure 4.9 Average body weights of different experimental groups of rats.

4.3.2   Recovery of Organ Weight

The relative organ weight of kidney liver and heart increase in diseased group because of inflammation due to benzene induction. Ciprofloxacin and doxorubicin decrease organ weight. Organ weight recovery to normal is significantly higher in rats which were treated with ciprofloxacin and Doxorubicin nanoparticles ($p < 0.0001$)).
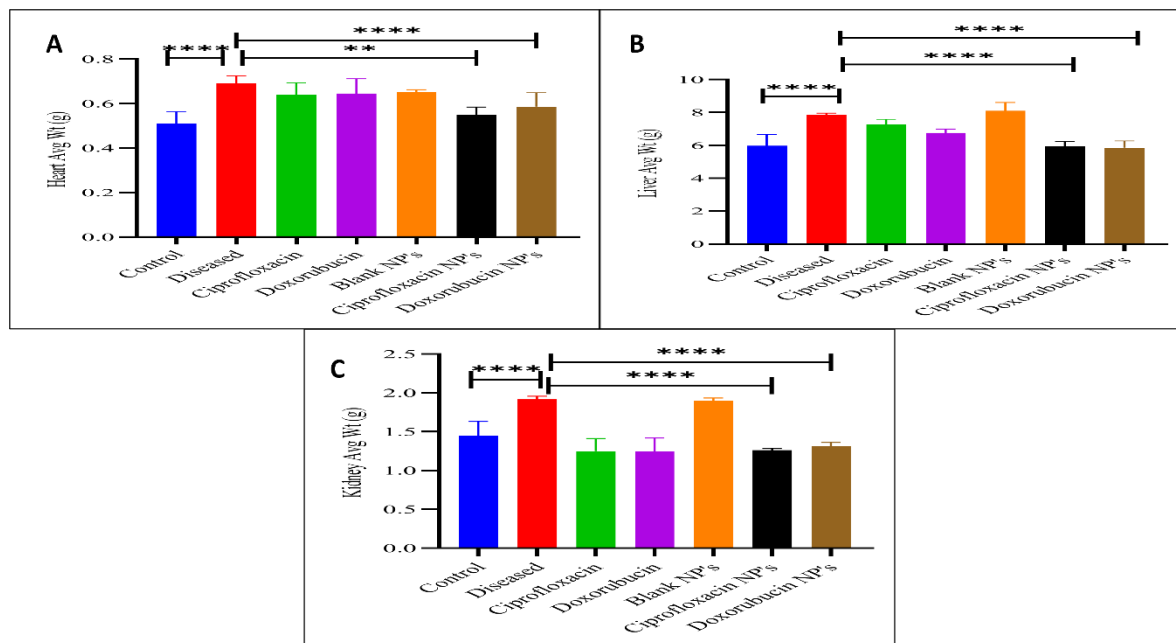
Figure 4.10 show comparison of A)heart, B) kidney and C)liver weight of different experimental groups

4.3.3   Effect on CBC

To analyze the effects of the novel compound on blood cells count, a complete blood count was obtained. White blood cells were counted and visualized by complete blood picture. The overall WBC count rose considerably in group 2 (benzene treated rats), which is indicative of leukemia. In the free drug-treated groups, this aberrant rise was recovered. However, nanoparticles show a more substantial reduction in the number of WBCs. It was observed that the total Red Blood Cells count decreased significantly in groups having benzene induced leukemia. However, treatment with free Doxorubicin and ciprofloxacin exhibited a significant increase indicating restoration of normal RBC count. It was also learned by applying ANOVA over these counts that the efficacy of the nanoparticles of doxorubicin and ciprofloxacin is significantly higher than the free Doxorubicin and ciprofloxacin in restoring normal RBC count. Hemoglobin levels in benzene-treated rats were significantly lower. Doxorubicin and ciprofloxacin were effective in

restoring hemoglobin level. However,hemoglobin levels increased significantly in groups treated with nanoparticles, respectively. A CBC indicated lower platelet counts in benzene-treated rats, which is an indicativeof acute myeloid leukemia. Doxorubicin and ciprofloxacin treated rats had the greatest risein platelet count, however both nanoparticles' medicines were also effective in restoring platelet count.
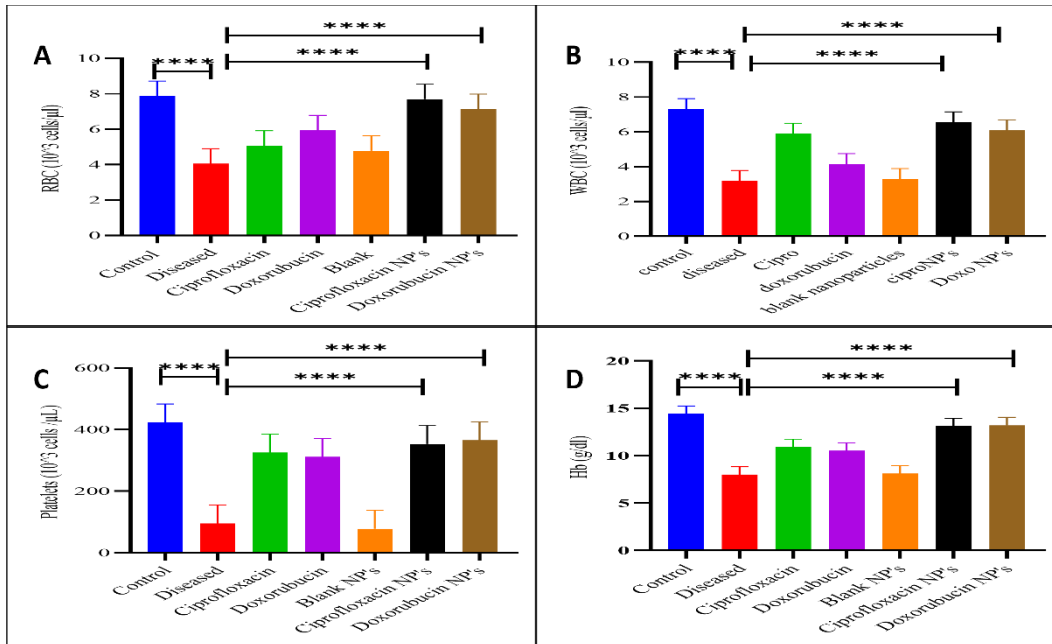
Figure 4.11 show comparison of A)RBC, B)WBC, C)HB, and D)platelets different experimental groups.

Recovery of CBC count

The lymphocytes, Eosinophils Neutrophils, and basophil levels became high in diseased group. The drug-loaded nanoparticles reduced the level of these cells significantly up to the normal values.
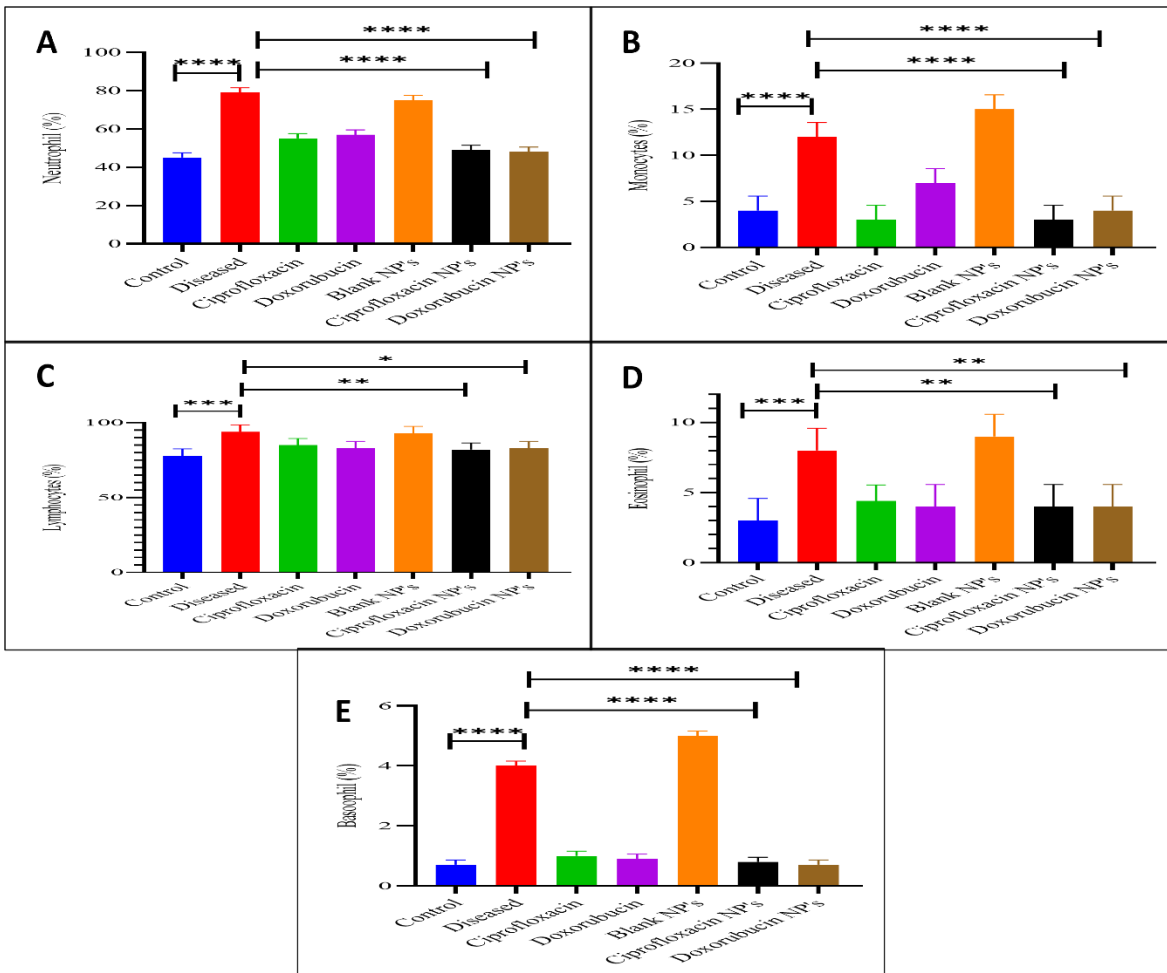
Figure 4.12 show comparison A) Neutrophil, B) Monocytes, C) Lymphocytes, D) Eosinophil, and E) Basophil of different experimental groups.

4.3.4 Microscopic Examination of Blood Cells

The blood cells of the untreated rats had normal morphology, as illustrated in Fig.4.16. The biconcave shape of RBCs remained intact, and the WBCs were mature and well differentiated, as seen by the multilobed nuclei within the cells. However, in the benzene-treated group, a large number of blast cells i.e., immature white blood cells with a high nucleus to cytoplasm ratio, while a low normal cellcount was identified, indicating leukemia. This group's RBCs were also found to be of aberrant shape. The morphology of the doxorubicin-treated group was virtually identical to that of the control group, but a few blast cells persisted. Ciprofloxacin and doxorubicin treated groups restored normal shape of blood cells such as RBCs and WBCs. The RBCs retain their structure, and the multilobed nuclei of the WBCs are still preserved. Meanwhile, the blood smear slides from the Ciprofloxacin and doxorubicin nanoparticles treated therapy group displayed morphology that was much more similar to the normal group, such as regaining regular form and intact morphology of blood cells.
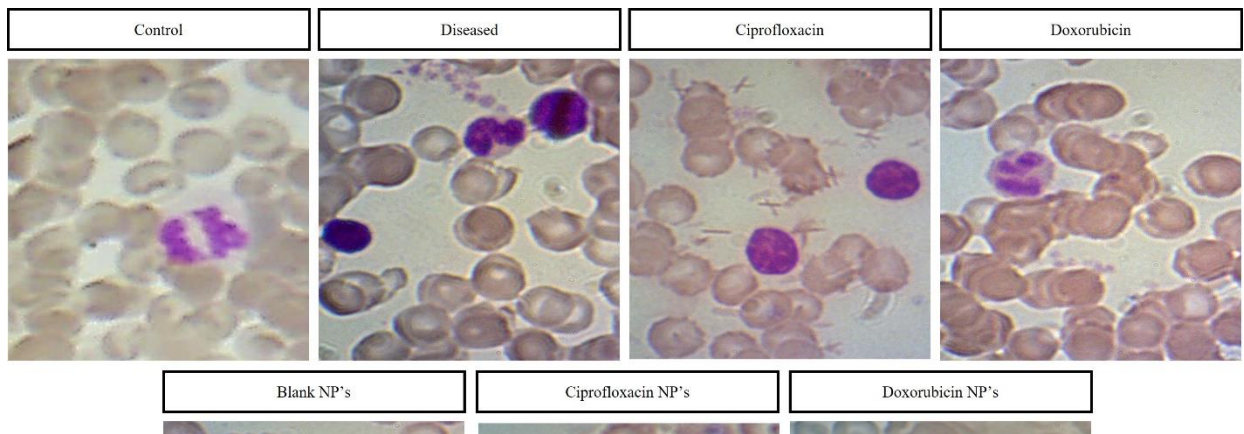
Figure 4.3 shows Microscopic analysis of blood cell of experimal groups
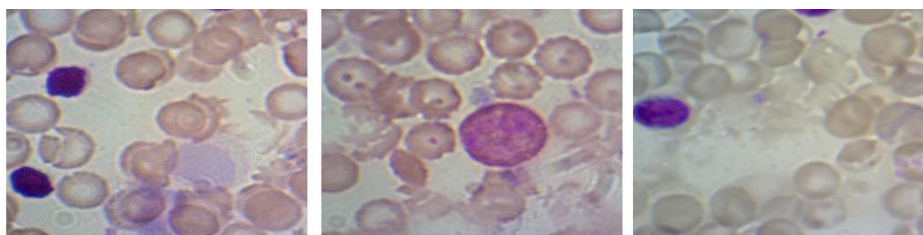
Figure 4.13 show Blood morphology of experimental groups.

4.3.5 Histology Analysis of Organs

Histology of Liver cell

Histological analysis of different organ liver diseases and treated rats was done by hematoxylin and eosin stain. The result shows high deposition of blast cell in liver in the case of benzene-treated diseased rats along with cellular hypertrophy. The morphology of liver is recovered to normal in case of drug-loaded liposome of doxorubicin and ciprofloxacin.
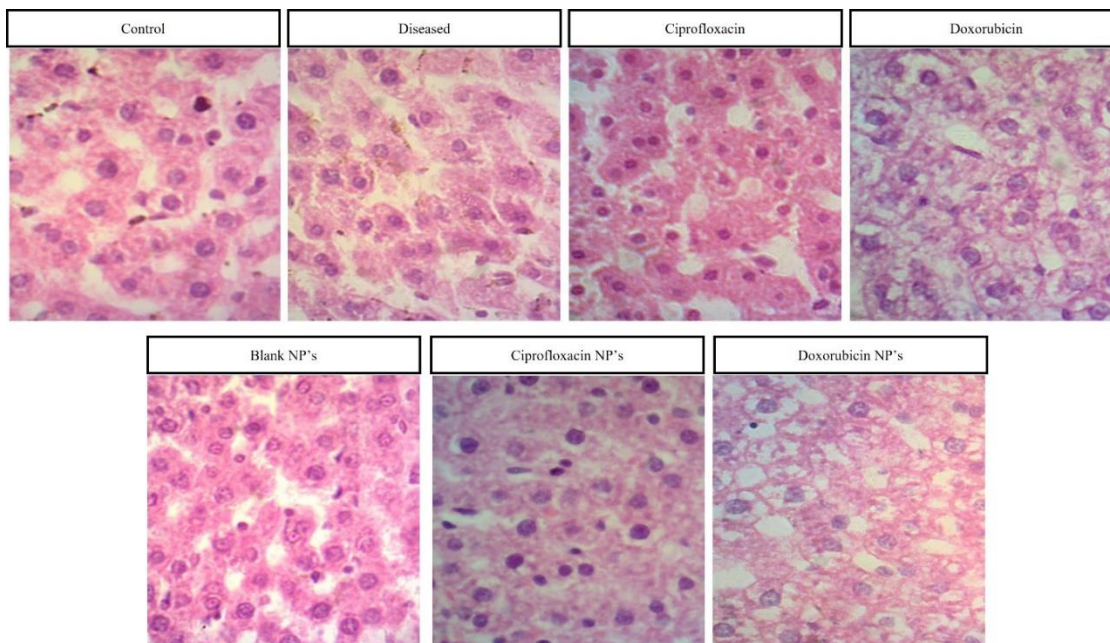
Figure 4.14 show Histology of liver of experimental groups.

Histology of kidney

Histological analysis of different organ kidney diseases and treated rats was done by hematoxylin and eosin stain. The result shows high deposition of blast cell in kidney in the case of benzene-treated diseased rats along with cellular hypertrophy. The morphology of kidney is recovered to normal in case of drug-loaded liposome of doxorubicin and ciprofloxacin.
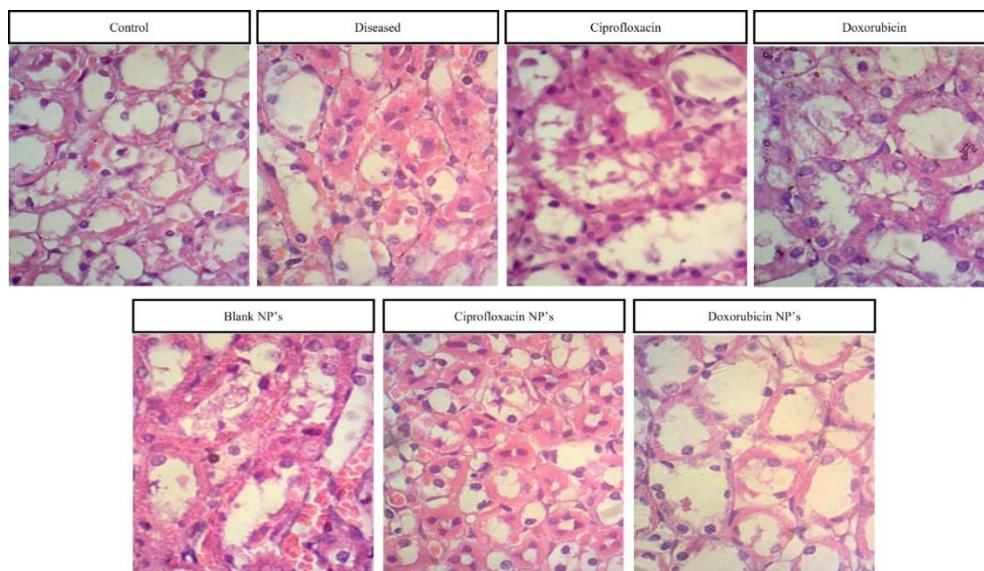
Figure 4.15 show Histology of kidney of experimental groups

Histology of Heart

Histological analysis of different organ heart of diseases and treated rats was done by hematoxylin and eosin stain. The result shows high deposition of blast cell in heart in the case of benzene-treated diseased rats along with cellular hypertrophy. The morphology of heart is recovered to normal in case of drug-loaded liposome of ciprofloxacin. The drug-loaded liposome of doxorubicin shows some toxicity on heart cells.
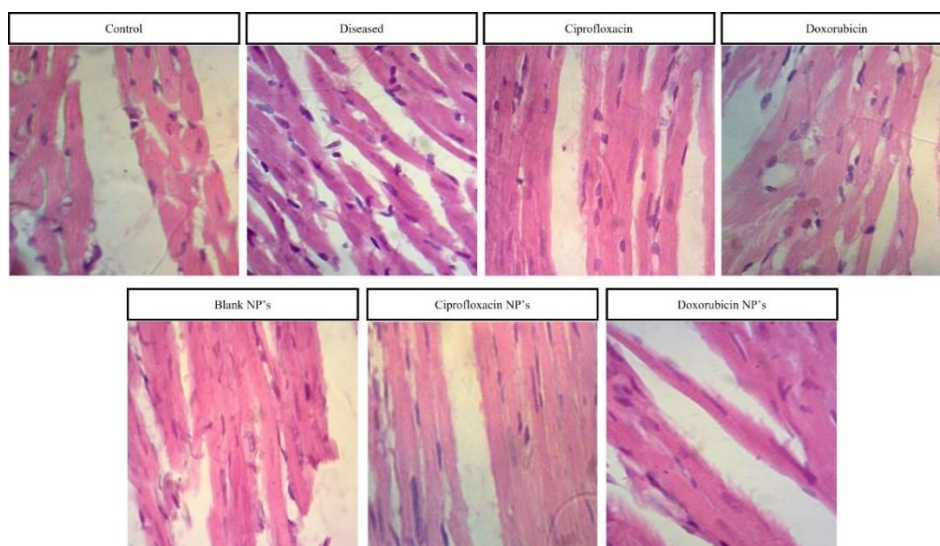
Figure 4.16 show Histology of heart of experimental groups

4.4 Biochemical Assessment

Estimation of Hepatic Biomarkers

The leukemic group of rats treated with benzene revealed significantly higher levels of ALT, AST, ALP, and total bilirubin in comparison to normal group which was taken as control. The group of rats that received free ciprofloxacin and free doxorubicin had low levels of these markers as compared to benzene administrated group. But nanoparticles of ciprofloxacin showed significant reduction in the enzyme levels as compared to benzene group indicating recovery. Restored level of hepatic marker in the case of nanoparticles close to normal group.
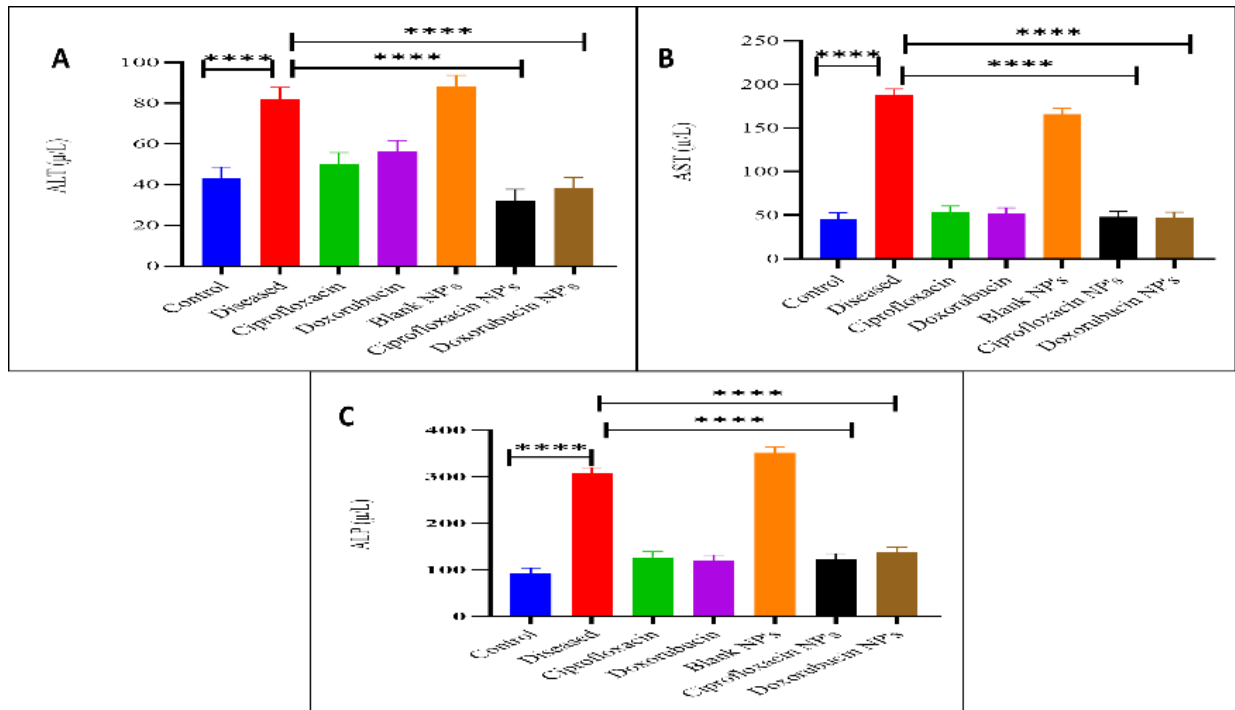
Figure 4.17 Comparison of Serum A)ALT, B)AST, and C)ALP, in different experimental groups

Estimation of Renal Biomarkers

When compared to the control group, the benzene group had a substantial rise in uric acid, urea, and creatinine levels (p < 0.0001). However, doxorubicin and ciprofloxacin therapy resulted in a drop in uric acid, urea, and creatinine level. When nanoparticles of doxorubicin and

ciprofloxacin were administered, uric acid, urea, and creatinine were restored to normal levels with high effectiveness and substantial outcomes.
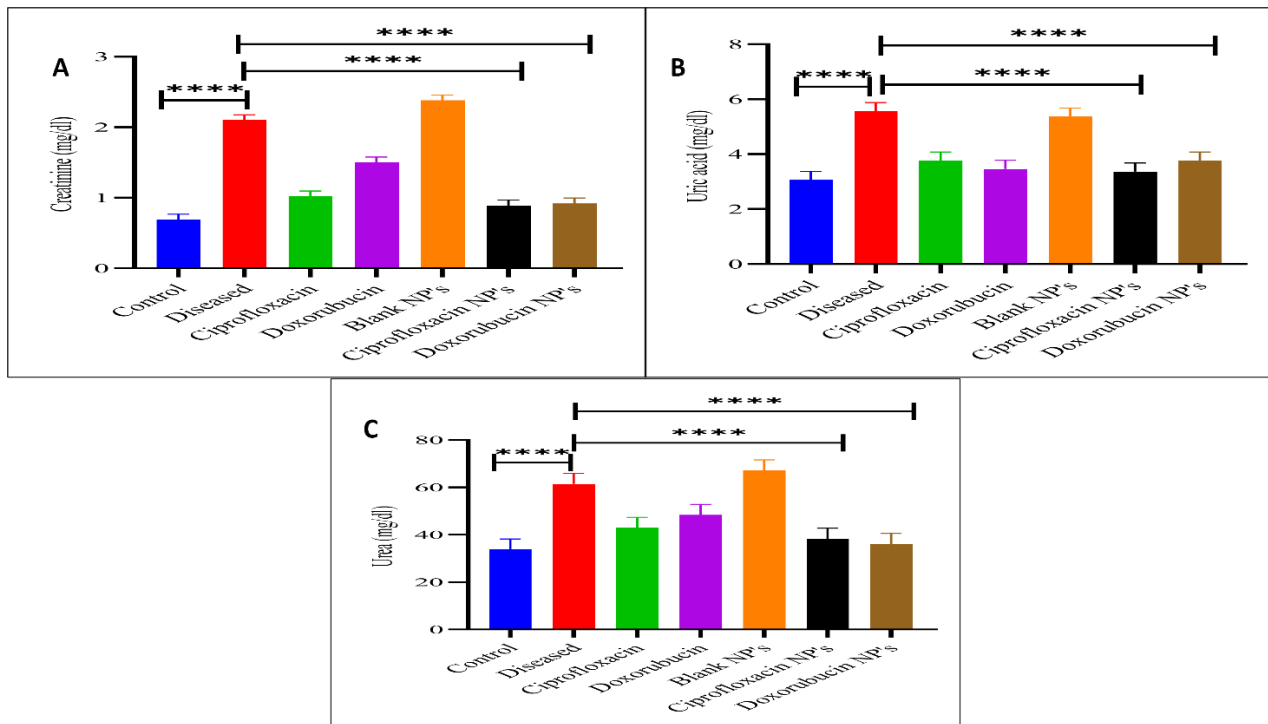
Figure 4.18 Comparison of Serum A) creatinine, B)urea, and C) uric acid, in different experimental groups

CHAPTER 5: DISCUSSION

Alarming uprise in the world population is giving rise to serious diseases. Conventional treatments have led to drug resistance in individuals and are ineffective against the new diseases surfaced. Conventional chemotherapies have recently been shown to be unsuccessful against cancer due to multidrug resistance and a slew of adverse effects that wreak havoc on one's quality of life (Housman et al., 2014). Despite treatment advances, cancer remains a serious concern dueto high fatality and morbidity rates across the world (Hanna et al., 2020). To tackle the rising obstacles in the treatment of leukemia, new therapeutic options with fewer side effects and higher efficacy are required. This brings up the need to use those delivery methods for drugs that can serve as broad-spectrum drugs in the future (Masood, 2016).

The liposomal nanoparticles are efficient delivery agent of drugs for the treatment of AML as it is effective and has fewer side effects. Liposomal nanoparticles have targeted drug delivery, reduced toxicity, improved stability, and reduced drug resistance (Basha et al., 2014).

Ciprofloxacin inhibition results in mitochondrial damage, respiratory chain disruption, depletion of ATP (as demonstrated by Kloskowski et al. in 2012), and suppression of mitochondrial DNA synthesis. In turn, this energy shortage induces cell cycle arrest at the G2/M and/or S stages, which further encourages apoptosis(Beberok et al., 2018). The ciprofloxacin is effective at against cancer at higher concentration, but high concentration of ciprofloxacin causes resistance in cancer cell and cause cytotoxicity. The cytotoxicity of ciprofloxacin has been reported in various Cancer cell lines (Ebisuno et al., 1997).

Liposomal nanoparticles of ciprofloxacin are formed by thin film hydration and nanoparticles are pegylated by PEG 100 to improve internalization of drug (Li et al., 2012). The objective of ciprofloxacin liposomal nanoparticle is to cure AML in benzene induced leukemic rats. To see if

benzene effectively produced leukemia, the leukemic rats were compared to the untreated control rats. In benzene-induced leukemic rats, a significant increase in blast cells and burst red blood cells was detected after Giemsa staining of blood smear slides. Doxorubicin is used as a reference drug. For free drug ciprofloxacin free doxorubicin is considered as reference and for liposomal ciprofloxacin nanoparticle doxorubicin liposomes are considered as reference.

Liposomes containing ciprofloxacin and doxorubicin were loaded by dissolving the compound in a suitable solvent and then using sonication to create a nano-sized formulation. Doxorubicin and ciprofloxacin are hydrophilic; thus both drugs are encased in the pocket of a liposomal vesicle. Nanoparticle size was measured which is smaller for blank liposomes and is greater for both drug-loaded liposomes, respectively. However, measurements of Zeta potential showed values of -4.6.03, -6.07, -7.08 for Blank liposomes, Ciprofloxacin, and doxorubicin nanoparticles respectively. After monitoring liposomes nanoparticles for 16 hours, we found that 49 and 43 percent of the drug had been released from doxorubicin and ciprofloxacin nanoparticles respectively. The liposomal components, doxorubicin and ciprofloxacin loaded liposome nanoparticles, and blank liposomes were analyzed using Fourier transform infrared spectroscopy (FTIR), and the results demonstrated that distinct functional groups were involved, as shown by a drop in peak intensities.

In vitro analysis of free drug and drug entrapped liposomal nanoparticles are performed after careful review of the literature. Antioxidant and cytotoxicity assays were performed. Brine shrimp and hemolytic test are included in cytotoxicity assays. TRP, TAC and DPPH assays were included in the antioxidant activity assessment. The maximum concentration of drug i.e in 100% of free drug stock and nanoparticle is same that is 200 milli molar per 10ml. Cytotoxicity assay showed 50% population killing by ciprofloxacin at concentration of 80%, by doxorubicin at

60%, by liposomes of ciprofloxacin at very high concentration even higher than 100%, and by liposomes of doxorubicin at 100%. Cytotoxic effect was reduced by decreasing the concentration via serial dilutions of 80%, 60%, and 40% for free drugs. 100%, 80%, and 60% for nanoparticles. The graph showed a decrease in percentage mortality upon decreasing the concentration. In the case of Doxorubicin groups, the cytotoxic activity was reduced with concentration, but its decrease was not significantly steep in curve. Doxorubicin forms a complex with iron under high concentrations and then binds to DNA molecules. This binding leads to the inhibition of DNA replication and kills the cells as reported in the literature (Keizer et al., 1990). But as seen in the graph, decrease in iron chelation and DNA damaging effect is observed upon decreasing the doxorubicin concentration as evident by Brine Shrimp Assay and hemolytic assay.

Numerous diseases have been linked with excessive load of ROS in the body and cancer is the most important among them. Antioxidant species tend to protect the body from damage by ROS via scavenging of these reactive species. Antioxidant potential of the free drugs and their liposomes was assessed by Total Reducing Power and Total Antioxidant Capacity assay. Activity trend obtained was similar in all assays and show better results for nanoparticle-based drug as compared to free one.

AML was experimentally produced in rats by administering benzene intravenously for 4 weeks on alternative days, and it was substantiated by blood cell count and morphology. To see if benzene effectively produced leukemia, the leukemic rats were compared to the untreated control rats. In benzene-induced leukemic rats, a significant increase in blast cells and burst red blood cells was detected after Giemsa staining of blood smear slides.

The total white blood cell count in the leukemic group, i.e., those treated with benzenein group 2,

was shown to be higher owing to a mutation in the bone marrow, which disrupts the normal hematological process and results in disordered leukocyte production and differentiation. The groups which were treated with Doxorubicin and Ciprofloxacin resulted in a significant decrease in WBC count. The WBC count in rats treated with ciprofloxacin nanoparticles is approximately equal to normal rats and is comparable with doxorubicin nanoparticles.

Since RBCs rupture in AML and generation of RBCs and platelets in bone marrow is hampered by a significant number of aberrant WBCs, total RBCs and platelets count fell in the benzene-treated group (D'Andrea & Reddy, 2014). Hemoglobin levels were similarly lower in the benzene-treated group, indicating a leukemic state because RBCs drop considerably in leukemia. One of the most common causes of hemoglobin deficiency is severe aplastic anemia (Boddu *et al*., 2018). In the benzene-induced leukemic group of rats, lymphocytes and neutrophils increased, indicating AML. During diagnosis, a higher lymphocyte count is connected to a shorter remission and survival rate (Park *et al*., 2018). Doxorubicin Nanoparticles treatment in the group yielded significant results, since it is a typical medicine in cancer therapy, but it also resulted in high cytotoxicity, which was less in Ciprofloxacin Liposomes as observed in its treated group. Hematological profile also improved in drug-loaded nanoparticles, although Ciprofloxacin liposomes therapy produced a more improved and substantial response against leukemic pathogenicity.

An elevated LDH level in leukemia indicates an accelerated rate of aerobic glycolysis independent of oxygen supply, commonly known as the Warburg effect (Vaupel *et al*., 2019). Higher levels of LDH correlate positively with blast cells, leukocytes, and uric acid levels, but negatively with RBCs and platelets, indicating cytopenia and leukocytosis. Increased LDH levels are connected with cell damage, cell turnover, leukemic cell growth, and chemoresistance,

as well as activation of hypoxia in evasion of apoptosis (Transfus *et al*., 2017). LDH levels were substantially higher in Group which is benzene-treated group than in the untreated group. As a result, LDH is not only a prognostic marker for predicting leukemic cell load, but it is also utilized to classify leukemia, according to the literature (Ding *et al*., 2017)..

Cholesterol levels were substantially higher in leukemic rats but significantly lower in treated groups. All leukemic cells require reprogramming of their cholesterol metabolism to achieve chronic proliferation and drug resistance. Leukemic cells require cholesterol to meet their demand for rapid proliferation, thus they increase their absorption and synthesis of it. This is accomplished by increasing the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and decreasing LDL breakdown by the low-density lipoprotein receptor (LDL-R) indicating increased cholesterol production and absorption (Zhao *et al*., 2019). Since tumor load promotes cell lysis and purines are catabolized into uric acid, an elevated blood uric acid level shows aggressiveness and cellular turnover of leukemic cells (Rasool *et al*., 2015).

Biochemical hepatic markers analysis revealed a substantial rise in ALT, AST, and ALP levels in serum of rats treated with benzene, i.e., the leukemic group, consistent with previous research (Jumaah *et al*., 2021). Group treated with Ciprofloxacin liposomes, on the other hand, exhibited a considerable decrease in these hepatic markers, which is similar to doxorubicin liposomes. Jaundice and cholestasis cause a disruption in hepatic profile in the leukemic group. Transaminase levels rise as a result of leukemic infiltration, indicating liver damage (Akinlolu *et al*., 2018).

The result of invitro analysis demonstrates that free drugs are more cytotoxic as compared to drug-loaded liposomal nanoparticles. So, nanoparticles are safer drug delivery vehicles. The animal model results show ciprofloxacin liposomes are effective against leukemia and its

anticancer activity is comparable with doxorubicin liposomes which is a well-known antileukemic drug.

5.1 Conclusion

It is concluded that Liposomes showed suitable physical characteristics to be used as an effective drug carrier. nanoparticles have demonstrated their viability as a platform for improving drug bioavailability via a variety of administration routes, resulting in optimal therapeutic effects. Because of this, the current study sought to broaden the scope of pertinent research by examining ciprofloxacin's possible protective effects, particularly in the form of nanoparticles, against acute myeloid leukemia. Hence liposomal nanoparticles were synthesized using 'thin film hydration technique'. Ciprofloxacin a class of synthetic antibiotics called fluoroquinolones is well-known for its broad-spectrum activity and is frequently used to treat a variety of illnesses. Their distinct anticancer potential in several cancer cell lines is what distinguishes them, as demonstrated by Kloskowski et al. in 2012. Their ability to block the eukaryotic topoisomerase II enzyme is thought to be responsible for their anticancer effect. The successful synthesis of liposomal nanoparticles was confirmed by different characterization techniques. Pegylation enhanced the stability that resulted the long-term circulation in the body as compared to free drug of ciprofloxacin and doxorubicin. The invitro assays demonstrate that there is significant decrease in toxicity of drug by encapsulation in liposomal nanoparticles but their antioxidant activity is not interrupt by their encapsulation in liposomes. After treating the leukemia rat, the histological, serological, body weights and CBC results demonstrated the substantial betterment in nano particles treated rats.

Ciprofloxacin-loaded liposomal-NPs have the potential to minimize the effect of benzene (AML induction), as proved by differential leukocyte count, morphological analysis, relative organ

weight analysis, and estimation of hepatic biomarkers.

This research is the primary step of using ciprofloxacin liposomal nanoparticles for the treatment of leukemia. Further research is required to find the genetic pathway targeted by ciprofloxacin liposomal nanoparticles to cure leukemia. Ciprofloxacin can be given in combination with some other antileukemic drugs, but this perspective requires more research work.

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