CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Insilico Modeling of Hepatotoxic Drugs Used in Non-small Cell Lung Cancer (NSCLC) and New Drug Dosage Criteria Design

by

Sana Masood

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in the

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First of all, I dedicate this research project to Allah Almighty, The most merciful and beneficent, creator and Sustainer of the earth

And

Dedicated to Prophet Muhammad (peace be upon him) whom, the world where we live and breathe owes its existence to his blessings

And

Dedicated to my parents and Siblings, who pray for me and always pave the way to success for me

And

Dedicated to my teachers, who are a persistent source of inspiration and encouragement for me

CERTIFICATE OF APPROVAL

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Abstract

Lung cancer is the top most cause of morality in U.S despite the reduction of smoking in recent years, approximately about 27.4% from all cancer deaths. The incidence of lung cancer has been decreasing in recent years in men but it is still the second leading cause of morality in males and females following the prostate and breast cancer respectively. Smoking is the most common risk factor for lung cancer, Non-small cell lung cancer is the major cause of morality of lung cancer accounting for 85% of lung cancer. treatment of lung cancer can be classified into chemotherapy, radiotherapy, surgery and immunotherapy and now targeted therapy has also been introduced for lung cancer treatment. As the hepatotoxicity is a major issue in the field of medicinal oncology, a large number of chemotherapeutic drugs produce toxic effect on the liver, resulting in the acute failure of liver.

Therefore, in this study we have performed the hepatotoxicity modeling of drugs used to cure NSCLC to determine their effects on liver. The generalized liver model was developed and simulations were performed, it was observed that, Afitinib, Crizotinib, Erlotinib, Gefitinib, and Paclitaxel produced large hepatotxic effects on the liver, causing liver damage leading to its failure. However, Carboplatin and Cisplatin are non hepatotoxic drugs. While Bevacizumab, Gemcitabine, Lorlatinib, Methotrexate and Pemetrexed are less hepatotoxic drugs.The new dosage criteria was suggested for the hepatotoxic drugs to overcome the liver damage. In future, the new suggested dosage criteria can be used and further confirmed invitro. By reducing the drug dose or drug dose schedule we can reduce hepatotoxic drugs into less or non-hepatotoxic drugs usiing this generalized model.

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Abbreviations

AICART	AminoImidazoleCarboxAmide Ribonucleotide formyl Transferase
AKT	Protein Kinase B
ALK	Anaplastic Lymphoma Kinase
ALT	Alanine Transaminase
ALP	Alkaline Phosphatase
APAP	Acetyl-Para-Aminophenol (Paracetamol or acetaminophen)
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BSEP	Human Bile Salt Export Pump
CALU-6	Human, Caucasian, Lung, Adenocarcinoma
CYP	Cytochrome P450
DHFR	Dihydrofolate Reductase
DILI	Drug Induced Liver Injury
DNA	Deoxyribonucleic Acid
EGF	Endothelial Growth Factor
EGFR	Epidermal Growth Factor Receptor
EXAFS	Extended X-ray Absorption Fine Structure
FRET	Forster Resonance Energy Transfer
GARFT	Glycinamide Ribonucleotide Formyl Transferase
HGP	Human Genome Project
LCC	Large Cell Carcinoma
LTKB	Liver Toxicity Knowledge Base
NMR	Nuclear Magnetic Resonance
NSCLC	Non-Small Cell Lung Cancer

PK/PD	Pharmacokinetics/Pharmacodynamics
PTEN	Phosphatase and Tensin Homolog Protein
QSAR	Quantitative Structure Activity Relationship
RB1	Retinoblastoma Protein
SCC	Squamous Cell Carcinoma
SCLC	Small Cell Lung Cancer
STAT	Signal Transducer and Activator of Transcription Proteins
TB	Tuberculosis
TKI	Tyrosine Kinase Inhibitor
TP53	Tumor Protein 53
TS	Thymidylate Synthetase
ULN	Upper Limit of Normal
USA	United States of America
W	World
WHO	World Health Organization

Chapter 1

Introduction

1.1 Cancer

Cancer is the second major cause of death worldwide. Overall the prevalence of cancer has increased and approximately 1,665,540 people suffered from different types of cancer and 585,720 of them died by 2014 in United States [1]. Therefore, cancer is serious problem affecting the human health all around the world. In males, cancers prevalence is highest in prostate, lung and bronchus, colon and rectum, and urinary bladder respectively. In females, the highest percentage of cancers types occurs in breast, lung and bronchus, colon and rectum, uterine corpus and thyroid respectively. This data indicates that prostate and breast cancers are the major cause of morality in men and women respectively. In children, cancer prevalence is highest in blood cancer and cancers related to brain and lymph nodes respectively [2].

Cancer is a genetic disease of somatic cells which contains multiple abnormalities of both number and structure. The studies of tumor specific translocations in leukemia and lymphomas provided the first direct evidence of cancers which revealed the importance of oncogenes and transcriptional factors genes in cancer. While, hereditary cancer is the second major source of information about cancer genes. Approximately 93% of all human cancers are non-hereditary that is

caused by environmental factors while only 7% are hereditary [3]. Genes associated with human cancer formation are underlined into four classes of genes: 1. Tumor suppressor genes, 2. Proto-oncogenes, 3. DNA mismatch repair genes and 4. Apoptotic genes. Cancer occurs due to a series of successive genetic mutation leading to change in cell function. The genetic mutations include inactivation of tumor suppressor genes and activation of oncogenes. Generally, cancer disturbs the activities of cells leading to dysfunction of important genes that prompts irregular multiplication of cells [4].

Molecular analysis of cancer cells accumulates data that show multiple genetic lesions of various combinations of oncogenes and tumor suppressor genes in a single cancer. Genetic damage of oncogenes results in gain of function and genetic damages of tumor suppressor genes results in loss of function [5]. The report of WHO of 2018 shows that cancer is the second major cause of deaths and morality worldwide causing about 9.6 millions deaths worldwide in 2018. most common cancers are lung, stomach, breast, colorectal, prostate and skin cancer but deaths caused by mostly lung, colorectal, stomach and breast cancer of about 1.76 million, 862000, 783000 and 627000 deaths in 2018 respectively [6].

1.2 General Treatment Options

Cancer is treated by different kind of treatments such as therapy, hormonal therapy, radiotherapy, targeted medical aid, surgery and artificial morbidity. The choice of treatment for a cancer patient is decided by analyzing the state of tumor, phases of sickness and also the general condition of patient. The variety of severities, variety in durations and locations of tumor, drug resistance, cell origin, affectability of drugs and differentiation and comprehension of pathogenesis leads to poor diagnosis, improper visualization and treatment of disease [5].

The interactions between genes and proteins as well as their networks plays a vital role for understanding of cancer's molecular mechanism. Hence, it is fundamental to suggests an idea of systems clinical science which is integration of medicine

and healthcare related fields like clinical science, omics based innovation, system biology, computational biology and bioinformatics respectively which helps in enhancement, treatment and prognosis of cancer [7].

1.3 Bioinformatics in the Research of Cancer

Cancer is a genetic disease in which cells can not follow normal procedure of cell division or cell cycle leading to uncontrollable cell division, incorrect arrangement of chromosomes or have missed large pieces of chromosomes in cancer cell [8]. The medical field search involves a large number of steps that should be fast to perform diagnosis and treatment which requires a lot of efforts. Also the completion of Human Genome Project in 2003 has produced an enormous amount of Biological data which added pressure to apply Bioinformatics in cancer treatment. Therefore, the tools of bioinformatics can be helpful for diagnosis and treatment of cancer are implemented by experts and researchers to extend their research in the field of cancer [9]. Computerized models are one of those applications of bioinformatics that provides biological data and information about number of cancer cells in the body of cancer patient as well as biological state of the cancer patient [10]. In such a way, experts are now able to observe the tumor growth after cancer therapy which was difficult to identify before the bioinformatics. In addition, different studies indicated that gene expressions of cancer cells play vital role, this information helps in efficient treatment [11]. Bioinformatics can also be applied by using databases of cancer cell's expressions and also by the study of drug and tumor responses [12]. Bioinformatics studies ensure the treatment of several cancers types in near future. Bioinformatics also provides information to experts and therapists which helps in the analysis of immune responses for the understanding of controlled and uncontrolled tumors for the efficient treatment of patients [13].

In other words, by the help of mathematical and computational models, bioinformatics explains the effects of radiation and chemotherapy and therapists uses bioinformatics databases and search engines that are readily available to everyone

to find biological data for cancer research and treatment. By the discovery of HGP, bioinformatics can be applied in designing the drug for cancer or any other disease's treatment, to avoid the effects of drug and to develop better drug delivery system [8].

1.4 Modeling and Simulations in Cancer Research

If the protein structure is known, different details of protein and mutations that cause change of protein structure can be determined through different techniques such as NMR, FRET, EXAFS and other biophysical methods but these techniques have some limitations due to their sensitivity, applications and time scales [14]. Therefore, computer aided studies along with molecular studies can provide details that cannot be obtained through experiment. Therefore, molecular modeling approaches are useful in clinically oriented researches, however, these methods are inaccessible to researchers due to lack of understanding of their use, advantages, disadvantages and limitations [15].

Molecular modeling is the studies of application of computer generated models on molecular studies of biomolecule. These computer generated models can be used to stimulate the processes as fast as 10-15 seconds or as slow as few seconds. How accurate the results are and how much detailed the model provides is clearly depends on size of biomolecule and time duration of system. These models help to studies the sub angstrom differences of structures and their influence on binding of drug molecule to its receptor. The differences in size lead to use of different method, so generally, for accurate method to produce meaningful result, there will be need of more time and more computational resources. Therefore, the problem suggests the method of modeling to be used [16].

1.5 Lung Cancer

Lung cancer is the top most cause of morality in U.S despite the reduction of smoking in recent years, approximately about 27.4% from all cancer deaths. The incidence of lung cancer has been decreasing in recent years in men but it is still the second leading cause of morality in males and females following the prostate and breast cancer respectively [17]. Though there have been advancements in treatment of lung cancer in recent years but the survival span for average five years is 15% only [18]. Most of the time, lung cancers are diagnosed at late stage leading to low chances of cure for the cancer but surprisingly there are low chances of cure for stage 1 diagnosis even which increase the need of understanding of molecular alterations that causes poor prognosis and to use this information for better diagnosis and treatment of cancer patient [19].

1.5.1 Risk Factors

Smoking is the most common risk factor for lung cancer because 90% of females and 79% of males having lung cancer are found to be smokers [20]. Passive smoking is the second most common cause of lung cancer in adults for approximately 3000 deaths each year due to exposure to cigarettes smoke depending on intensity and duration of exposure to smoke [21].

In occupational risk factors, the most common factor for lung cancers is exposure to asbestos, the chances of lung cancer reaches to 60% for smokers who are also exposed to asbestos [22]. Other than asbestos, exposure to arsenic, radon, vinyl chloride, nickel, chromium and ionizing radiation are also the most common environmental and occupational risk factor for lung cancer in adults [23]. Patients having non-malignant lung diseases such as TB, chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis have also increased lung cancer rates [18].

1.5.2 Types of Lung Cancer

On the basis of classification lung cancer is characterized into small cell lung cancer represents 15% of lung cancer and non-small cell lung cancer represents 75-85% of lung cancer. NSCLC is further classified into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. For the choice of chemotherapy, this classification was helpful in past decades [24].

1.5.2.1 Small Cell Lung Cancer (SCLC)

Small cell lung cancer is one of the most deaths causing malignancy worldwide with less than 7% rate for average 5-year survival rate. Tobacco exposure is the main cause of high mutations leading to SCLC [25]. In past few decades, decrease in use of cigarettes resulted in decreased incidence of SCLC but SCLC still remains major cause of deaths accounting for 14% of all lung cancers and approximately 30,000 patients annually in U.S. Smoking being the major cause of SCLC with only 2-3% patients of SCLC are non-smokers [26].

The origin of small cell lung cancer is unknown while neuroendocrine cells (NECs) and neuroendocrine progenitors (NEPs) are assumed or considered to be possible source of SCLS [27]. Ki67 protein in case of SCLC is usually greater than 50-70% indicating high proliferation and extensive mitoses and necrosis of cancer cells[28]. In majority of SCLS patients, tumor suppressor genes TP53 and RB1 are mutated and PTEN mutations are present in 10-18% [29].

1.5.2.2 Non- Small Cell Lung Cancer (NSCLS)

Non-small cell lung cancer is the major cause of morality of lung cancer accounting for 85% of lung cancer. NSCLC is further classified into adenocarcinoma, squamous cell carcinoma and large cell carcinoma respectively [30].

Adenocarcinoma is the most common type of lung cancer comprising of about 40% of all type of lung cancer. It originates from small airway epithelial type 2 alveolar

cells that secrete mucus [31]. Smoker and non-smoker males and females affects from adenocarcinoma regardless of their gender and age [32]. Large particles cannot enter in lungs due to addition of filters in cigarettes leading to prevalence of adenocarcinoma in periphery of lungs. Therefore, deeper inhalation of smoke of cigarettes causes peripheral lesions [33]. As compared to other types, adenocarcinoma grows slowly therefore, having greater chance of diagnosis and treatment [30].

Squamous cell carcinoma constitutes 25 to 30 % of all cases of lung cancer. It originates from squamous cells of airway epithelial cells to bronchial tubes of the center of the lungs. SCC is associated with cigarette smoke [34].

Large cell carcinoma is third type of NSCLC comprising 5 to 10% of all lung cancers. In this carcinoma there is no evidence of squamous and glandular secretions therefore it occurs in central parts of lungs, near lymph nodes and into chest walls. LCC is also associated with exposure of tobacco [35].

1.5.3 Treatment Options

Treatment for lung carcinoma depends on different factors such as cancer specific cell type, cancer type, how much it is spread and physical condition of cancer patient. Therefore, on the basis of all these factors, treatment of lung cancer can be classified into chemotherapy, radiotherapy, surgery and immunotherapy and now targeted therapy has also been introduced for lung cancer treatment [36].

For patients with stage 1 to stage 3 NSCLC, surgery is the choice of treatment [37]. Recent studies suggest that chemotherapy before surgery provides better survival rate for NSCLC patients. The tumors that cannot be removed through surgery in case of NSCLC can be cured by radiotherapy and chemotherapy [38]. Patients of nonsquamous carcinoma with advanced stages can be treated with targeted therapies of antivascular EGF agent bevacizumab. Patients treated with bevacizumab combined with chemotherapy has shown increased survival rate than treated with chemotherapy alone [39]. For the treatment of SCLC, chemotherapy

combined with radiotherapy has shown greater survival rate. Palliative and hospice care are very important for chronically ill patients for better end of life treatment. The doctors can suggest the most appropriate option of treatment to be used [18].

TABLE 1.1: Treatment options for Lung Cancer according to stage of the cancer [36].

Stage of Lung Cancer	**Primary Treatment**	**Adjuvant Therapy**	**5 year survival rate (%)**
NSCLC			
I	Resection	Chemotherapy	60-70
II	Resection	Chemotherapy with or without radiotherapy	40-50
IIIA(resectable)	Resection with or without preoperative chemotherapy	Chemotherapy with or without radiotherapy	15-30
IIIA (unresectable) or IIIB (involvement of contralateral or supraclavicular lymph nodes)	Chemotherapy with concurrent or subsequent radiotherapy	None	10-20
IIIB (pleural effusion) or IV	Chemotherapy or resection of primary brain metastasis and primary T1 tumor	None	10-15 but two year survival %

SCLC			
Limited diease	Chemotherapy with concurrent radiotherapy	None	15-25
Extensive Disease	Chemotherapy	None	less than 5

1.6 Purpose

As the hepatotoxicity is a major issue in the field of medicinal oncology, a large number of chemotherapeutic drugs produce toxic effect on the liver, resulting in the acute failure of liver. Therefore, several models should be developed in-silico that predicts the effects of chemotherapies or other medicinal treatment in cancer. Hence, the purpose of this study is to model the liver of human to determine the toxic effects produced by chemotherapies used in NSCLS on liver.

1.7 Problem Statement

As the hepatotoxicity is a major issue in the field of medicinal oncology, the problem statement of this study is to design a liver model to determine the drug induced hepatotoxicity in NSCLC through modeling and simulations and suggestion of new least hepatotoxic dosage regimens.

1.8 Aims and Objectives

- Development of Liver model that can be used in general, for all diseases.
- Estimation of toxic effects of NSCLC chemotherapies on Liver.

- Alteration and suggestion of drugs dosage and regimens on the basis of estimations

1.9 Scope

The drug induced hepatotoxicity is a significant cause of liver damage, resulting in liver transplant to about 50% of cases. However, major population of world cannot afford expenses of liver transplant. Therefore, the developed liver model will be helpful in the selection of those drugs and dosage regimens which do not produce hepatotoxicity, therefore, this study is multi-dimensional in nature because of involving Systems biology and Bioinformatics therefore it has wide range of scope.

Chapter 2

Literature Review

2.1 Hepatotoxicity

Hepatotoxicity refers to chemical-driven liver injury. Drug-induced liver damage is one of major cause of acute and chronic liver disease. The liver is a major organ that plays a central role in clearing chemicals and transforming them hence susceptible of toxicity from these chemical agents. Toxic liver injury reproduce any known pattern of injury such as necrosis, steatosis, fibrosis, cholestasis and vascular injury. Some of the medicinal agents can injure the liver when taken in overdose or even in the therapeutic ranges may lead to liver injury. Chemical agents that are used in laboratories and industries, natural chemicals like microcystins and herbal remedies may also cause hepatotoxicity. Chemicals or chemical agents that induce hepatotoxicity are called hepatotoxins [40].

2.1.1 Drug Induced Hepatotoxicity

According to gastroenterologists, hepatotoxicity is cause of acute liver injury in less than 1% cases but in USA and Europe, drug-induced liver injury has been reported for the most common cause of acute liver failure [41]. In France and Iceland, the annual incidence of DILI is 14-19 per 100,000 individuals [42]. Drug induced

liver injury is major cause of attrition of agents that are uses in development of drugs as well as most frequent cause of drug with-drawls, project termination and restrictions [43]. From 1969 to 2002, 76 drugs had been withdrawn from market from which 12 were accountable for hepatotoxicity. During drug approval, liver signals escape detection leading to post-marketing restrictions, false positive DILI measurements leads to unimportant attrition thus contributes to number of economic issues related to DILI [44]. Patient who uses acetaminophen at single dose greater than 7.5 g, or if plasma concentrations exceeds to 200 or 100 or 8 hours after ingestions, causes acute liver injury in that patient. The licensed dose of APAP is 4g per day for 2 weeks results in elimination of ALT above 3x upper limit of ULN in 1/3 of patients. This dose dependent APAP-induced liver injury is known as intrinsic DILI which is predictable and reproducible in preclinical models [45].

The commencement of idiosyncratic DILI (IDILI) in contrast to intrinsic DILI is responsible for about 10-15% of acute liver injury in USA though it is very rare, and unpredictable [46]. IDILI is caused by change in start and end time of drug usage (weeks to months) and not having the clear dose need [47]. Drug-protein adducts known as neoantigens by histocompatibility complex class II, are formed by drug or its metabolites interacting with host protein, triggers the immunoallergic reaction. Patients having liver injuries like viral hepatitis and inflammatory conditions are more vulnerable to immunoallergic injuries. After the initial injury, other mechanisms like mitochondrial injury, proinflammatory cytokines, endoplasmic reticulum and oxidative stress can further intensify the injury leading to acute DILI. The understanding of host factors that make an individual vulnerable are being focused in ongoing pharmacogenetic research [48].

2.1.2 Models

Pharmaceutical companies have adopted computational modeling approaches to estimate the toxicity, efficacy and mechanisms adopted by pharmaceutical ingredients [49]. In early developmental stages of prediction models, the constructed

models did not show satisfactory predictive power and depended on experimental data for their better performance. Some of the researchers applied molecular signatures, for example ATL, AST and ALP that are commonly evaluated in diagnostic estimation of hepatocellular damage [50]. In recent years, for prediction models, machine learning algorithms have been deployed to gain better predictions [51].

Nonetheless, experimental data have limited use in development of prediction models. So, many researchers have used compound properties and structural features for computational predictions.

Green et al. developed a model using structure-activity relationship for potent hepatotoxic agent or compound. The hepatotoxic compounds were characterized into four classes in association with hepatotoxicity: 1. no evidence, 2. weak evidence, 3. animal hepatotoxicity, and 4. human hepatoxicity. The model yielded a concordance of 56% , sensitivity of 46% and specificity of 73% for hepatotoxicity [52]. The classification model developed by Ekins et al. used Bayesian modeling method integrated with molecular and finger descriptors. the evaluation of this model yieled a concordance of 60% for internal and 64% for external validation respectively [53]. By using QSAR, Rodgers et al. developed a model in which they used adverse effects of drugs on liver as dataset. They applied the information on hepatotoxic enzyme markers but these enzyme markers may fluctuate because of other factors throughout the day [54] .In addition, another model has been developed by using quantitative-structure-activity relationship (QSAR) by Huang et al. in which they used variety of descriptors including fingerprints. The model's performance was very good with an accuracy of 79.1% for internal validation. They also predicted the hepatotoxicity of Traditional Chinese Medicines [55]. Futhermore, an insilico prediction model has been developed by Zhang et al. for DILI in whih they used five machine learning algorithms and 3 different fingerprints. Their model yielded a concordance of 66% by using Support Vector Machine Algorithm and FP4 fingerprints, as well as identification of important substructure patterns associated with liver injury [56]. Regardless of these far-reaching efforts to predict drug-induced liver injury or damage, in contrast to QSAR models available for

mutagens, there are no specific QSAR models for DILI. Furthermore, the information about significant association of substructures with DILI is not enough or less known [57].

2.2 PK/PD Modeling and Simulations with respect to Hepatotoxicity

Pharmacokinetic (PK) and pharmacodynamic (PD) information emerge from the logical prelude of present-day pharmacotherapy. Pharmacokinetics portrays the medication focus time courses in body fluids as a result of the organization of explicit medication measurements, where as pharmacodynamics is the forecast of watched impacts in light of the fact that of a particular grouping of a medication. The reason for PK/PD-demonstrating is to associate pharmacokinetics and pharmacodynamics in order to develop and evaluate portion fixation reaction connections and along these lines delineate and predict the impact time courses of a medication portion [58]. By and large, in light of the essential physiological process, PK/PD displaying should be favored at plausible occasions. The all-encompassing use of PK/PD displaying is believed to be useful for medication progression what's more, what's more, associated pharmacotherapy will without a doubt improve the present state of therapeutics.

2.3 Drugs Used for Treatment of NSCLC

We have selected Non-small cell lung cancer due to its highest morality rate and retrieved all the drugs that are used for the treatment of different stages of NSCLC through literature. The detailed information of the drugs is given below.

2.3.1 Afatinib

Afatinib (an EGFR TKI) is a second- line chemotherapy , approved in April 2016, used for the treatment of patients with non-small cell lung cancer specifically squamous cell carcinoma progressing on or after a platinum-based chemotherapy [59]. Afatinib is a noval oral drug that is permanent ErbB family blocker having wide range of activity of tumor cell lines possesses an ErbB signalling network which is hyperactivated [60][61]. Afatinib has shown clinical adequacy in phase III clinical trials in NSCLC patients. Afatinib in 2013, was allowed for first line treatment of NSCLC with EGFR positive mutation [62]. Study of LUX Lung 3 and LUX Lung 6 reveals that progression free survival rate has increased with treatment of afatinib as compared to standard chemotherapy in advanced non-small cell lung cancer patient with positive EGFR mutation [63][64]. The suggested oral dose of afatinib is 40mg per day, though the maximum dose is 50 mg once a day and minimum dose is 20 mg once a day depending upon the tolerance of patients [62].

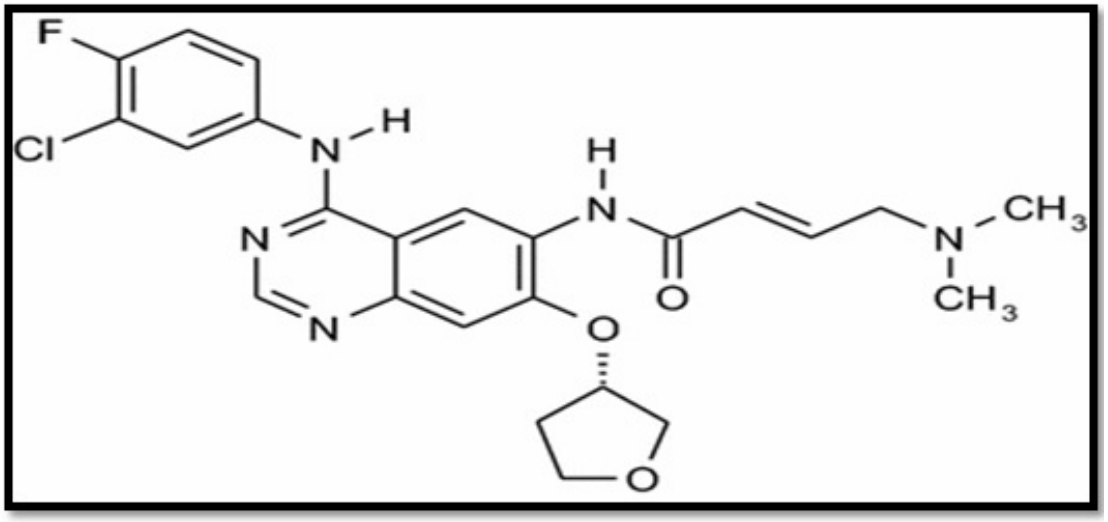

FIGURE 2.1: Chemical structural formula of Afatinib retrieved through [62]

2.3.2 Bevacizumab

Bevacizumab is approved protein based therapies which is a monoclonal antibody that binds to and targets the vascular endothelial growth factors to inhibits the

formation of new blood vessels known as neoangiogenesis [65]. Bevacizumab derived from murine antibody A.4.6.1 when inducted in murine xenograft models, it obstruct the growth of number of human cancer cells as well as CALU-6 that is an NSCLC model [66][67][68]. According to studies, reduction in metastases has been observed in addition of inhibition of growth of cancer tumor [69]. Bevacizumab when combined with carboplatin or paclitaxel increases the rate of response upto 31.4 % from 18.8 %, average progression time increases from 4.2 months to 7.4 months and survival rate increases from 13.2 to 14.2 months respectively as compared to alone chemotherapy of bevacizumab [70].

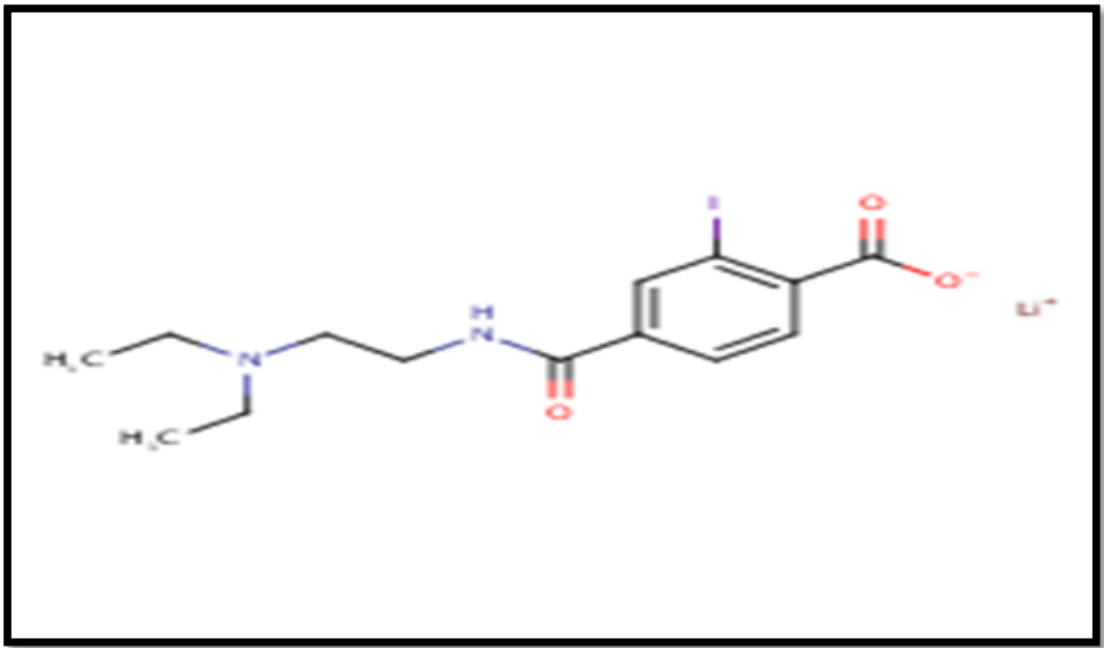

FIGURE 2.2: trutural formula of Bevacimuab retrived through SwissADME web tool.

2.3.3 Crizotinib

Patients having metastatic non small cell lung cancer that occurs due to mutation in expression of anaplastic lymphoma kinase (ALK) can be treated by crizotinib that is tyrosine kinase inhibitor [71]. ALK is a gene that inhibits apoptosis and increase the cell growth so crizotinib is inhibitor of tyrosine kinase that decrease the phosphorylation of ALK [72]. It is approved as a single agent rather use in combine therapy [73]. The suggested oral dose of crizotinib is 200-250 mg twice a

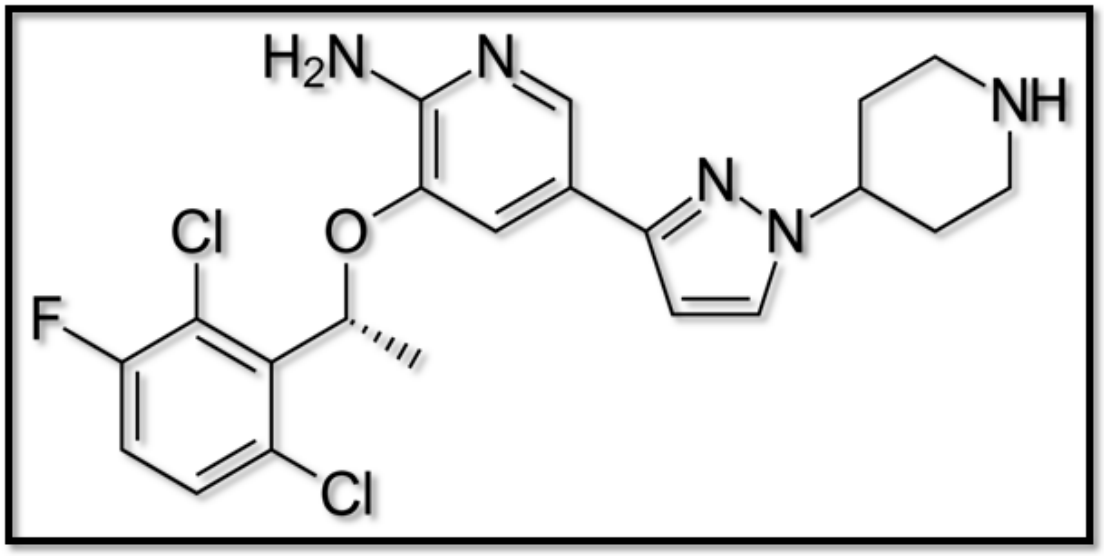

FIGURE 2.3: Structural formula of Crizotinib retrieved through [75]

day for the cycle of 28 days. In case of 8 weeks of continuous usage, the disease control rate exceeds to 87% including 57% patients shows tumor shrinkage and 33 % patient shows stable disease during the period of treatment. However the survival rate is 72% among the patients treated with crizotinib [74].

2.3.4 Erlotinib

Erlotinib is a novel drug that inhibts the activity of HER1 and EGFR tyrosine kinase in pateints suffering from Non-small cell lung cancer. It is reversible and direct inhibitor enzyme that decrease the autophosphorylation of HER1/EGFR in cancer cells with 20nmol/L inhibitory concentration. Erlotinib prevents cell proliferation depending on EGFR at nanomolar concentrations and it also blocks the G1 phase of cell cycle [76]. In mice, erlotinib decreases the autophosphorylation of HER1/EGFR by more than 70% for over 12 hours in human tumor xenografts when orally administered. Daily oral administration of erlotinib in xenografts of human head and neck cancer and squmous cell carcinoma in athmic mice noticeably inhibits the growth of HN5 and A431 respectively [77]. The minimum approved oral dose of erlotinib is 20 mg in the form of tablet and maximum tolerated oral dose is 150 mg tablet respectively [78]. The pharmacokinetic studies

of erlotinib suggests that metabolism of drug is performed by oxidation of cytochromes CYP3A4 and CYP3A5 of liver as well as cytochrome CYP1A1 of lungs respectively [78].

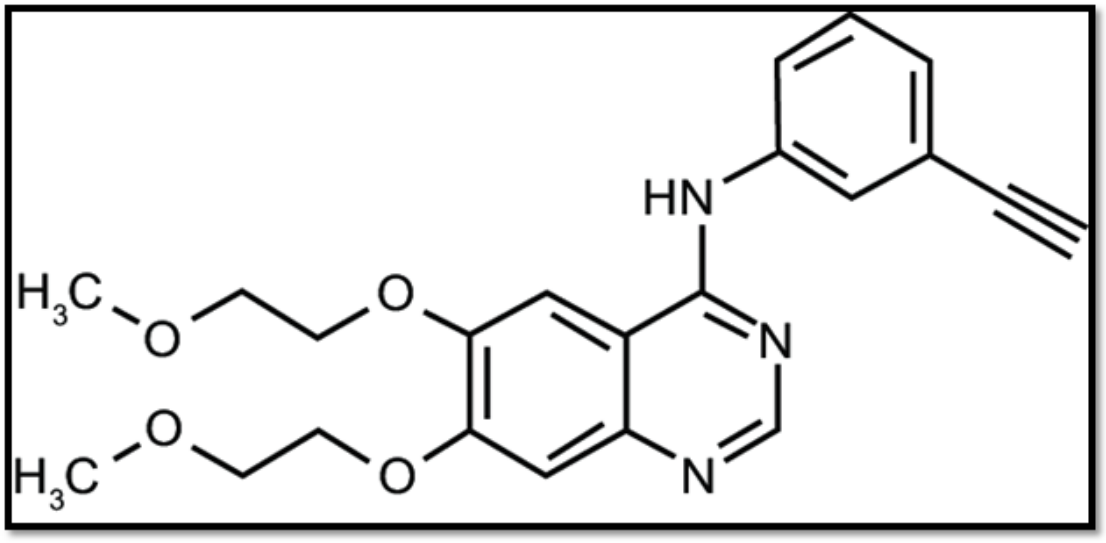

FIGURE 2.4: Structural formula of Erlotinib retrieved through [79]

2.3.5 Gefitinib

Gefitinib is also a tyrosine kinase inhibtor that activates the mutations in EGFR resulting the inhibition of epidermal growth factor receptor in NSCLCs. These mutant EGFRs reportedly activate the AKT and STAT signaling pathways that support cell survial and reduces cell proliferations [80]. The clinical response of Gefitinib in non-small cell lung cancer varies in population, showing higher rates in non smoker, women and patients suffering from bronchioloalveolar cancer and adenocarcinoma histology [81]. The mutations of EGFR induced by gifitinib are located at exons 18-21 that encodes for tyrosine kinase domain and most common mutations are deletions of exon 19 and L858R missense mutation at exon 21 respectively [82][83]. the treatment of metastatic non-small cell lung cancer through gifitinib is consist of 250 mg tablet once a day untill disease development and intolerable toxicity [82].

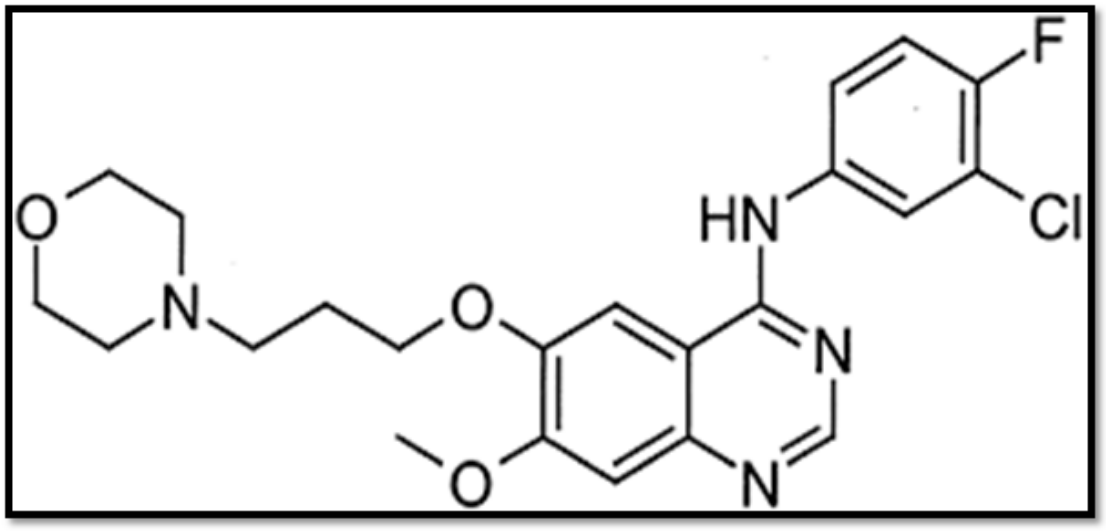

FIGURE 2.5: Chemical structure of Gifitinib retrieved through [84]

2.3.6 Lorlatinib

Lorlatinib is a noval, reversible, oral potent that is ATP viable macrocyclic third-generation tyrosine kinase inhibitor of ALK and ROS1. This potent and selected third generation inhibitor is designed to overcome the mutations causing ALK resistance by going through blood brain barriers [85]. Lorlatinib shows nanomolar effectiveness for wild-type ALK as well as it is effective for ALK-resistant mutants such as ALK G1202R [86]. Lorlatinib is highly selective against ALK that targets a specific residue on ALK domain which is leucine at position 1198 that is present on 25% of kinases respectively [85]. Lorlatinib also shows antitumor activity against various xeograft models that are ALK-positive NSCLC. Moreover, lorlatinib inhibts the activity of ROS1 and for in vitro and in vivo it keeps activating against ROS1 G2032R [87]. The approved oral dose for lorlatinib ranges from 25-100 mg once a day until disease progression, unbearable toxicity, patients unwillness or death [88].

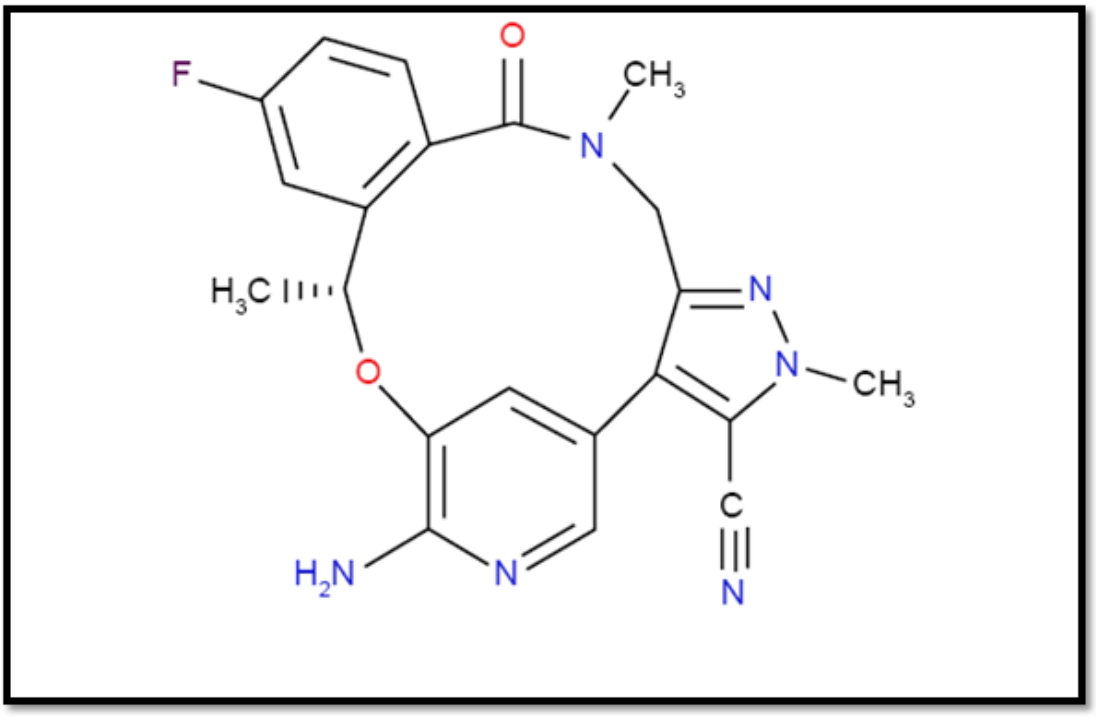

FIGURE 2.6: Chemical structure of lorlatinib obtained from [89]

2.3.7 Pemetrexed

Folate serves as coenzyme in many metabolic pathways for the synthesis of DNA. Folate is necessary for synthesis of purine and pyrimidine base, upon which proliferation of cancer cells is dependant [90]. Therefore, Pemetrexed is an analog of folate which reduces the folate activity by resulting in disruption of activity of those enzyme that require folate such as DHFR, GARFT and TS enzymes respectively [91][92]. Dihydrofolate reductase (DHFR) is required for purine and pyrimidine synthesis is the main target of methotrexate, however, thmidylate synthase which is required for synthesis of thymine is the primary target of pemetrexed. In addition , AICART is folate dependant enzyme which is responsible for protein synthesis and cell growth of tumor cell is the secondary target of pemetrexed [93]. Phase I studies of pemetrexed dose escalation shows that adminstration of pemetrexed as a single agent, dose of 600 mg/m2 for more than 10 min after every 21 days is optimal for the studies of phase II [94][95].

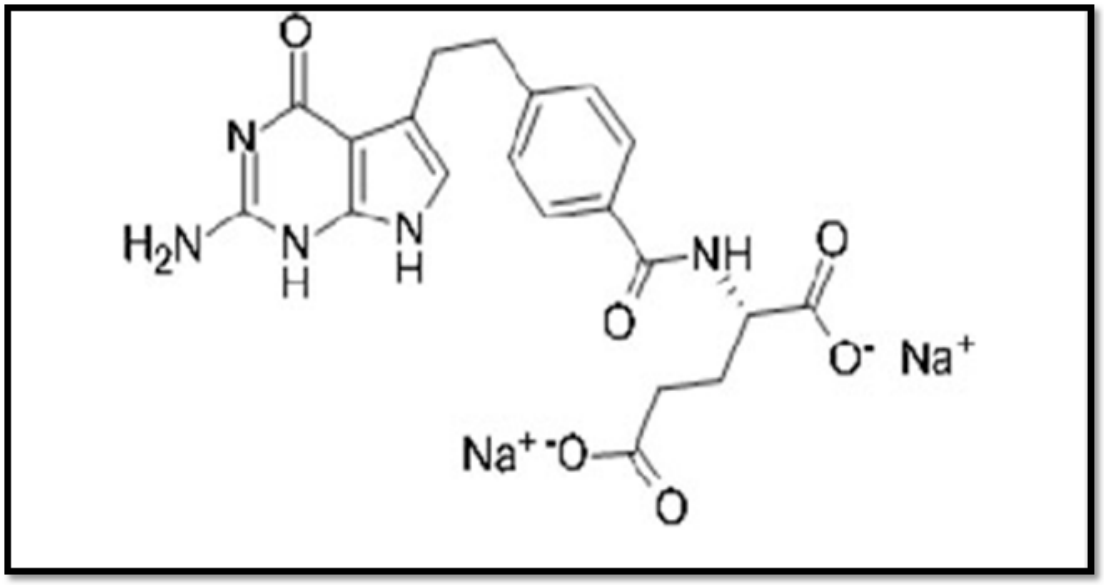

FIGURE 2.7: Structure of pemetrexed obtained from [96]

2.4 Liver

The liver is the most significant organ of the body, being domed in shape, playing role in the metabolism of nutrients and compounds. It also helps in the excretion of several waste metabolites [97]. Basically, it manages the influx and efflux of substances from the digestive system and distributes them to blood circulatory system, dysfunction of the liver can cause death of an organism within minutes. The upper region of liver is linked with diaphragm whereas, its lower posterior region is located above the abdomen, it is linked to intestine, pancreas, gallbladder, and esophagus [98]. It weighs around 1500 g accounting for 2.5% of overall body weight and is situated in the upper right corner of the stomach area, acting as a route for venous blood enriched with several nutrients. The liver performs more than 500 metabolic functions, bringing about blend of items that are discharged into the circulation system [99].

2.4.1 Basic Structure of Liver

The liver is divided into 4 parts: right, left, caudate, and quadrate. The left and right regions are the biggest, while the caudate and quadrate are little and found posteriorly. Two ligaments, attached to liver are noticeable anteriorly. Superiorly, the falciform tendon iseparates the right and left areas beneath the falciform ligament, a round ligament is attached, appearing as a protrusion from liver. The gallblader is also attached to the lower region of right lobe of liver [99]. The blood vessels, hepatic arteries, portal veins, lymphatics, nerves and hepatic bile duct supply the blood to liverthrough their junction; hilus. From the hilus, they branch and re-branch inside the liver to shape a framework that move together in a course structure. The diagram of liver obtained through [98] is shown in figure 2.8.

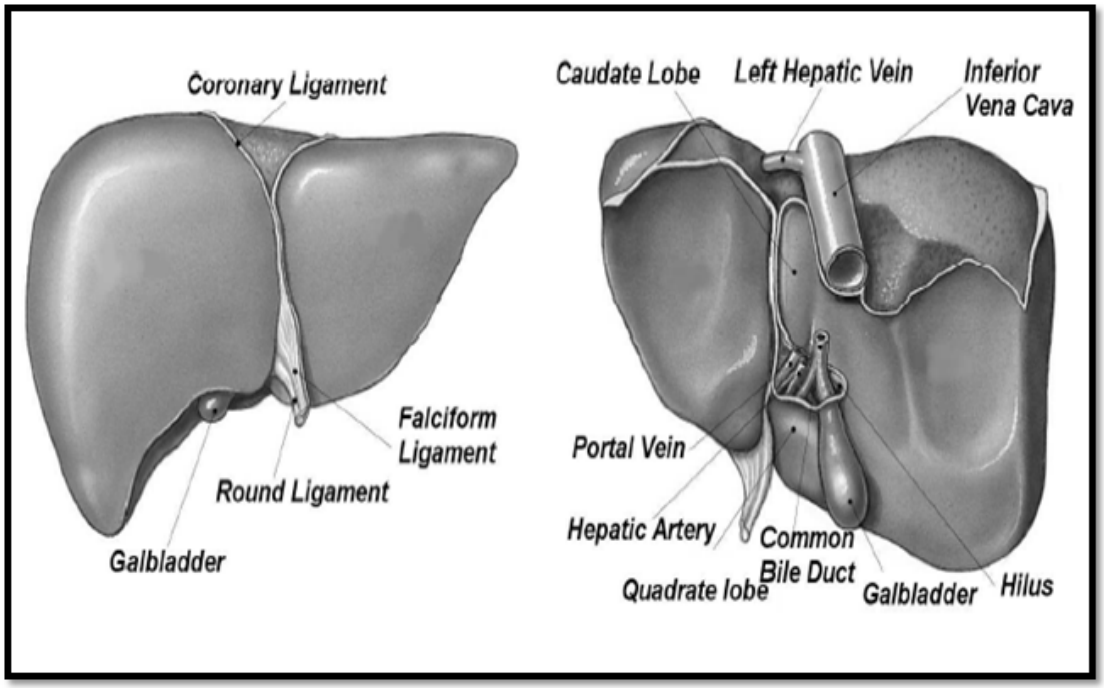

FIGURE 2.8: The structure of liver obtained through [98]

2.4.2 Histology of Liver

The liver lobule is the elementary functional unit of liver having normal size of a sesame seed with hexagonal shape, the primary structures of a liver lobule include

hepatocytes sheets forming the lobule, portal veins at the ends of hexagon, central veins, sinusoids, kupffer cells, interspaces between hepatocytes and sinusoids, and bile canaliculi (small canals) [100].

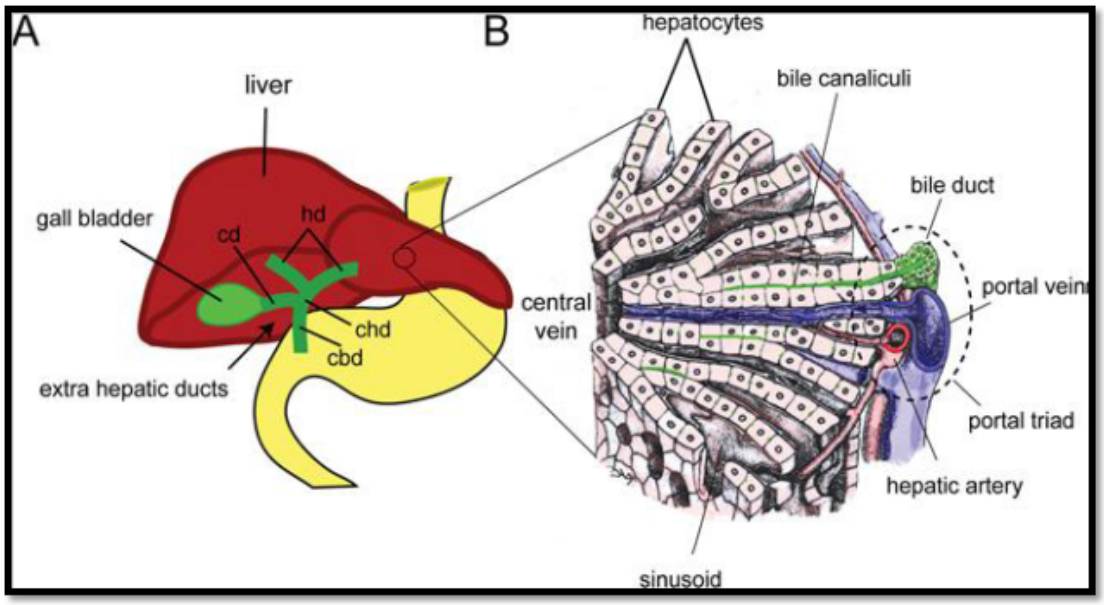

FIGURE 2.9: Design of liver at cell level.

In Figure 2.9 (A) red color demonstrates an adult liver, green color represents additional hepatic ducts and gall bladder and yellow color demonstrate the stomach and intestines. The additional hepatic duct framework comprises of the hepatic ducts (hd), which chanel bile from the liver to common hepatic duct (chd), via cystic hepatic duct (chd) to gall bladder and via common bile duct (cbd) to the duodenum. (B) A schematic representaion of cellular design of the liver demonstrating the hepatocytes (pink in color) that are arranged in hepatic plates which are separated by sinusoid spaces that are radiating along a central vein. Bile canaliculi chanel bile into bile ducts (green in color), that runs parallel to hepatic arteies (red in color) and portal veins (blue in color) which forms the "portal triad.

The portal triads contain three vessels: a hepatic portal arteriole, a hepatic portal venule, and a bile duct. The blood flows in same direction from both arteries and veins because of the presence of sinusoids toward the central vein, leading to the hepatic vein and the inferior vena cava. Secreted bile flows in the opposite direction – through the bile canaliculi causes the bile to flow in opposite direction

away from the central vein, toward the portal triad, and excreting through the bile duct. During the flow of blood through the sinusoids and the interspaces between hepatocytes and sinusoids, it results in the storage of nutrients in hepatocytes, whereas Kupffer cells engulf the bacteria and broken blood cells [101].

2.4.3 Distribution of Blood Pressure in Liver

Blood pressure in the vessels and the distribution of pressure inside the liver, is basically comparative for many species. Pressure of the hepatic artery, beginning from the plummeting aorta and the celiac trunc, is viewed as equivalent to the pressure inside aorta. This incorporates a high pulsatile pressure somewhere in the range of 120 and 80 mmHg with a recurrence equivalent to the pulse. Vessel consistence causes a slow diminishing in throb as the hepatic supply route branches and re-branches inside the liver. Once at the sinusoidal area, pulsation sufficiency diminishes to for all intents and reaches to zero and pressure drops to roughly 2-5 mmHg [102]. On the other hand, pressure in the vein, starting from vessels of the stomach related tract, has no pulse and a pressure of 10-12 mmHg. In the sinusoids, both venous and hepatic blood vessel pressure is 3-5 mmHg. Subsequently, the pressure drop inside the liver is considerably less in the entrance venous framework than in the blood vessel framework. The pressure drop from the gathering focal veins to the vena cava is then around 1-3 mmHg, fluctuating marginally with breath [100].

2.4.4 Distribution of Blood Flow in Liver

Total blood flow of human liver shows about 25% of the output from heart, up to 1500 ml/min. Hepatic blood flow is subdivided in 25-30% for the hepatic artery (500 ml/min) and the for the portal vein (1000 ml/min). Expecting a human liver weighs 1500 g, complete blood flow in liver is 100 ml/min per 100 g liver [103]. Contrasting this standardized blood flow rate with different species, it very well may be inferred that all out liver blood flow is 100-130 ml/min per 100 g

liver, autonomous of the species. The proportion of blood vessel: blood stream is species-subordinate. The hepatic artery starts straightforwardly from the aorta, and is in this way immersed with oxygen [104].

It represents 65% of complete oxygen supply to the liver. The hepatic artery likewise assumes a vital job in liver and connective tissue perfusion. It likewise makes the bile duct secure. The blood from the entrance vein is nutrient rich gotten from the digestive system and permits the hepatocytes to play out their roles. Blood from the hepatic artery and the vein enters in the sinusoids [105].In any case, late examinations by others just as our own perceptions have uncovered that there are both normal and separate channels for blood vessel and for entrance of blood. The hepatic artery perfuses the liver vascular bed in a dotted design, while the vein perfuses the liver consistently. The liver can manage chiefly blood vessel stream by sphincters, arranged at the in-and outlets of the sinusoids. A standout amongst the most vital triggers for sphincter work is the requirement for steady oxygen supply. In the event that the rate of oxygen conveyance to the liver differs, the sphincters will respond and the proportion of blood vessel: entryway blood stream changes [106].

Chapter 3

Material and Methods

3.1 Selection of Disease

The key step in the research domain involves identification of problem to be studied. For current research project, disease was selected as a problem for which investigation was conducted. Diseases exist in wide range of verities, for example infectious diseases, heart disease, autoimmune diseases, liver disease and cancer. All diseases have their morbidity and mortality ratios but cancer has been found to be associated with leading cause of deaths around the globe with major burden of diseases. Therefore, cancer disease was selected for this research. Cancer have many types but the highly ranked and common cancers are lung, breast, skin and colorectal cancers. Among all, lung cancer is second most leading cause of moraility and deaths worlwide. Small Cell lung Cancer and Non-Small lung cancer are the two types of lung cancer causing 15% and 85% deaths respectively. Therefore we have selected NSCLC because of its greater impact and morality worldwide. This step of disease selection was carried out thoroughly by literature review, for which Google Scholars, PubMed, research gate and many other literature databases were visited.

3.2 Selection of FDA Approved Drugs

Drugs have the ability to cure, inhibit or lower the effects of certain disease but drugs are also vulnerable to induce acute liver injury known as hepatotoxicity or Drug-induced liver injury [107]. This hepatotoxicity of chemical agent provides reason for a certain drug are withdrawn from market at the late stages of its discovery [108]. Thus the drugs that are used in the treatment of Non-Small Cell Lung Cancer were retrieved from literature and were validated by DrugBank, PubChem, ChemSpider to investigate their hepatotoxicity in order to suggest safe, less hepatotoxic and more effective drugs. The criteria set for the retrieved drugs include: (i) those drugs that were already available in market and are consumed, (ii) under investigational drug that were net introduced commercially in the market and (iii) drugs subjected towards clinical trial [110].

3.3 Dosage Criteria Identification

Drug dose taken at certain frequency level is termed as dosage e.g one tablet three times a day. For each of the retrieved drug, dosage criteria were identified using DrugBank database. The dosage of drug and its inflow and outflow in the processing compartments of human body have both favorable and adverse effects. Therefore, dosage criteria have been identified through DrugBank to check the effect of drugs at certain dose on the different compartments of liver and to suggest them as either hepatotoxic or non/less hepatotoxic. DrugBank (http://www.drugbank.ca) is basically an online database readily available to use, provides information about biochemical and pharmacological design of drugs, their targets, the mechanism of action of drugs, their in-silico design, discovery of drug target, prediction of drug metabolism, prediction of drug interaction and general pharmaceutical information [111].

3.4 Phyiochemical Properties of Drugs

Physicochemical properties of drugs are requisite to determine the proper formation and delivery mechanism of a drug [112]. Thus a necessary step to determine the physiochemical properties of collected drugs was done through DrugBank and validation was performed by Protox and PubChem. ProTox, a server to predict median oral lethal doses (LD50 values) and toxicity classes in rodents. It also specify possible toxicity targets on the basis of collected protein–ligand-based pharmacophores, hence providing the suggestions for the toxicity development mechanism [113].

3.5 Parameters Identification and Estimation

The parameters for different parts of human liver were investigated and retrieved through literature. These parameters were included blood flow rate in liver and weight of all compartments of liver. These parameters were estimated in matlab through ODE solver. The physiochemical parameters for drugs in the form of drug dosage, drug absorption rate, drug clearance rate, mol weight etc. were investigated and retrieved through pkCSM. The pkCSM is a web server developed on the basis of graph based signatures which helps in prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties in development of valuable drug [114].

3.6 Mathematical Formulation

For each part of liver model, ODE's (ordinary differential equation) were developed. Mathematical modeling is the conversion of problems into mathematical formulas which in turn helps in analysis of problem and providing a better solution for that particular problem [115]. ODE's based modeling provide kinetic information of biological system. Hence, these are widely used in system biology

helping in explanation of time-varying effects of biological process and stability of biochemical agents that are not known in experiment [116].

3.7 Development of Liver Model

The key step of this research work was to develop a liver model for the purpose of determining hepatotoxic effects of drugs in the MATLAB software. The model was developed on the basis of analysis of human liver anatomy. The model comprised certain compartments such as; Right Lobe, Left Lobe, Coronory ligament, Falciform Ligament, Ligamentum Teres, Gall Bladder and Linked Part of Pancrease. MATLAB is a high through-put software that is used for technical computation, providing integrated environment of visualization, programing, simulations, and computation. Matlab promotes an advanced environment of programming language. MATLAB is superb tool for teaching and research [117].

3.8 Induction of Drug Dosage into Model

The dosage criteria identified for each retrieved drug through Literature [118] have been inducted into the newly build liver, simulation time and days were set and simulations were performed.

3.9 Hepatotoxicity Modeling

Any injury of liver that is driven by means of drug or some chemical is known as Hepatotoxicity. Hepatotoxicity modeling was performed in the Matlab simbiology tool box of MATLAB. It provides system for simulations and sensitivity analysis. It uses ODE's to stimulate drug profiles depending on time and its efficacy [119].

3.10 Analysis of Simulation Results

The results of simulation were analyzed, and least/non hepatotoxic drugs were suggested to be use in future. However for the remaining hepatotoxic drugs new dosage criteria were designed and the drugs were validate by performing simulations. The applied methodology for this research is shown below in the form of flow chart.

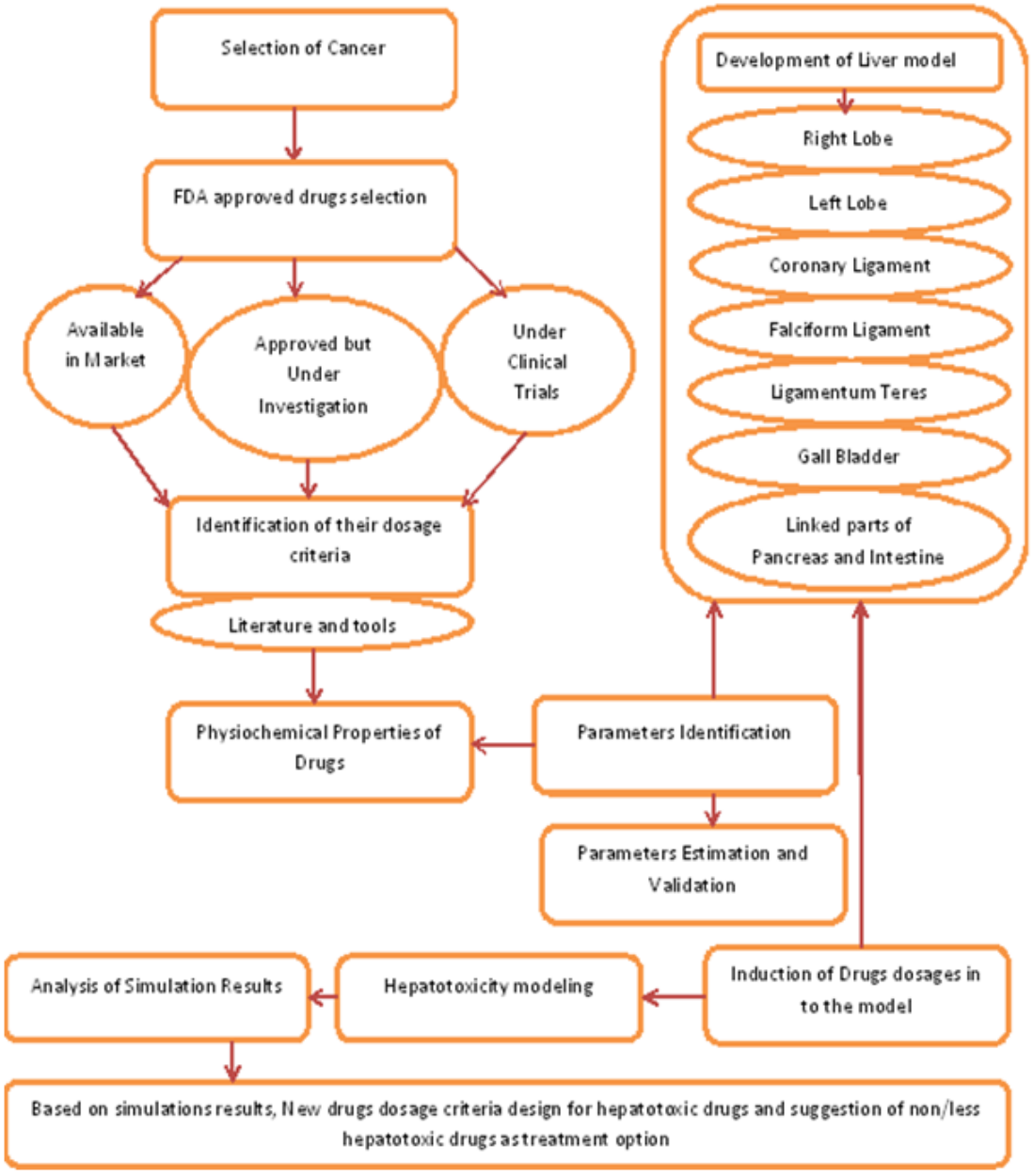

FIGURE 3.1: Flow chart of Methodology

Chapter 4

Results and Discussions

4.1 Collection of Drugs Dataset

The drugs that are used for the treatment of non-small cell lung cancer were retrieved through literature and their dosage, class, type of cancer, mode of intake and their status have been accessed through database of Drug Bank. the detailed information is given below in table 4.1.

Table 4.1: The information of all drugs that are used for treatment of different types of NSCLC

Drug Name	**Dosage**	**Status**	**Class**	**Mode of Intake**
Afatinib	Tablet 20-40 mg	Approved	Dia zanaphthalenes	Oral
Bevacizumab	Injection 100mg / 4mL-400mg/16mL Solution 25 mg	Approved, Investigational	Carboxylic Acids and Derivatives	Intravenous

Carboplatin	Solution 10 mg	Approved	Carboxylic acids and derivatives	Intravenous
Cisplatin	Injection 1mg/mL Liquid 1-5 mg	Approved	Protein	Intravenous
Crizotinib	Capsule 200-250 mg	Approved	Pyridines and derivatives	Oral
Erlotinib	Tablet 25-150 mg	Approved, Investigational	Dia zanaphthalenes	Oral
Gefitinib	Tablet 250 mg	Approved, Investigational	Dia zanaphthalenes	Oral
Gemcitabine	Powder for solution 100-1000mg	Approved	Pyrimidine nucleosides	Intravenous
Lorlatinib	Tablet 25-100 mg	Approved, Investigational	Macro lactams	Oral
Methotrexate	Tablet 2.5 mg, Injection 25 mg/1mL	Approved	Carboxylic acids and derivatives	Oral, Subcutaneous
Paclitaxel	Injection 100-200 mg/mL	Approved, Vet Approved	Prenol lipids	Intravenous
Pemetrexed Disodium	Injection, Powder 100-1000 mg	Approved, Investigational	Carboxylic acids and derivatives	Intravenous

The drugs given in Table 4.1 are being used for non-small cell lung cancer along with other types of lung cancer. We have selected all the drugs either used alone or in combination for treatment of all types of non-small cell lung cancer. Their approved drug dosages have been retrieved through drug bank. Some of the drugs are developed specifically for the treatment of non-small cell lung cancer while others can be used for the treatment of other types of cancer. The most common drugs used for the treatment of NSCLC belongs to carboxylic acids and derivatives class and the diazanaphthalenes class respectively.

4.2 Parameters Identification

To understand the fundamental processes of each component of the designed liver model, a few parameters were required to determine the reaction kinetics, their mechanisms and the behavior of model components [115]. The parameters of liver model were obtained from literature [120][106][121] and summarized in table 4.2. Moreover, the parameters for drugs have been retrieved through pkCSM web tool and summarized in table 4.3.

TABLE 4.2: Parameters estimation for components of Liver Model.

Components	**Parameters**
Liver weight	1500 g (1.5 kg)
Hepatic flow	500 ml/min
Portal vein flow	1000 ml/min
Total liver flow	100 ml/min per 100 g liver
Pancreas weight	60-100 g
blood flow rate in Gall-bladder	50 ml/50 g
Flow rate in systemic circulation	2500 ml/min

The significant parameters of drugs considered for this research or study are absorption rate, clearance rate, toxicity (hepatotoxicity) and LD50 respectively. The absorption rate, rate of drug clearance, LD50, and hepatotoxicities of drugs were calculated through PKCSM tool, shown in table 4.3.

TABLE 4.3: The absorption and Clearance rates of selected drugs from liver

Drug	**Absorption**	**Total Clearance**	**Unit**	**Hepato-toxicity**	**LD50 value**
Afatinib	90.314	0.593	Numeric (log ml/min/kg)	Yes	2.622 mol/kg
Bevacizumab	76.662)	0.906	Numeric (log ml/min/kg)	Yes	1.315 mol/kg
Carboplatin	22.203	1.099	Numeric (log ml/min/kg)	No	1.623 mol/kg
Cisplatin	44.62	0.533	Numeric (log ml/min/kg)	No	2.481 mol/kg
Crizotinib	92.006	0.571	Numeric (log ml/min/kg)	Yes	3.515 mol/kg
Erlotinib	94.511	0.446	Numeric (log ml/min/kg)	Yes	2.43 mol/kg
Gefitinib	90.992	0.937	Numeric (log ml/min/kg)	Yes	2.859 mol/kg

Gemcitabine	68.247	-0.058	Numeric (log ml/min/kg)	Yes	2.026 mol/kg
Lorlatinib	100	0.397	Numeric (log ml/min/kg)	Yes	2.807 mol/kg
Methotrexate	9.947	0.37	Numeric (log ml/min/kg)	Yes	3.221 mol/kg
Pemetrexed	0	1.851	Numeric (log ml/min/kg)	Yes	2.683 mol/kg
Paclitaxel	100	-0.36	Numeric (log ml/min/kg)	Yes	2.776 mol/kg

The clearance rate is the most important parameters which is described as volume of body fluid from which a drug removed from body by discharge per unit time and bio transformation. Clearance rate basically give details about fate of drug in human body. The accurate prediction of clearance rate is basic for medication admission in human body [122]. Total clearance rate is sum of clearance from all the organs i.e sum of renal, hepatic and lung clearance rate respectively.

It can be observed that all the drugs have low total clearance which increases the half life of drug. Intestinal absorption refers rate of drug absorption depending on drug dosage, greater the dosage higher will be absorption which is main characteristic of an effective drug. From above data, it has been observed that Carboplatin and Cisplatin are not involved in hepatotoxicity so we verified these drugs by performing hepatotoxicity modeling by using our developed model which provides

validity for these two drugs not involved in drug induced liver injury which in turn also validate our designed model.

The above mentioned parameters were inducted to the liver model to perform PK/PD simulations to verify different dosages for selected drugs.

4.3 Detailed Model of Liver

Model-develpment based approach is basically remodeling in a manner in which researchers works and facilitate them to develop the clinical labortary tasks on their computer screens [115]. To access the hepatotoxic effects of drugs, the detailed model of the liver was developed. The model consists of several compartments i.e left lobe, right lobe, falciform ligament, Gall bladder, ligamentum teres and linked pancrease, consisting of single drug dose and its elimination route from the liver, the model is shown in figure 4.1.

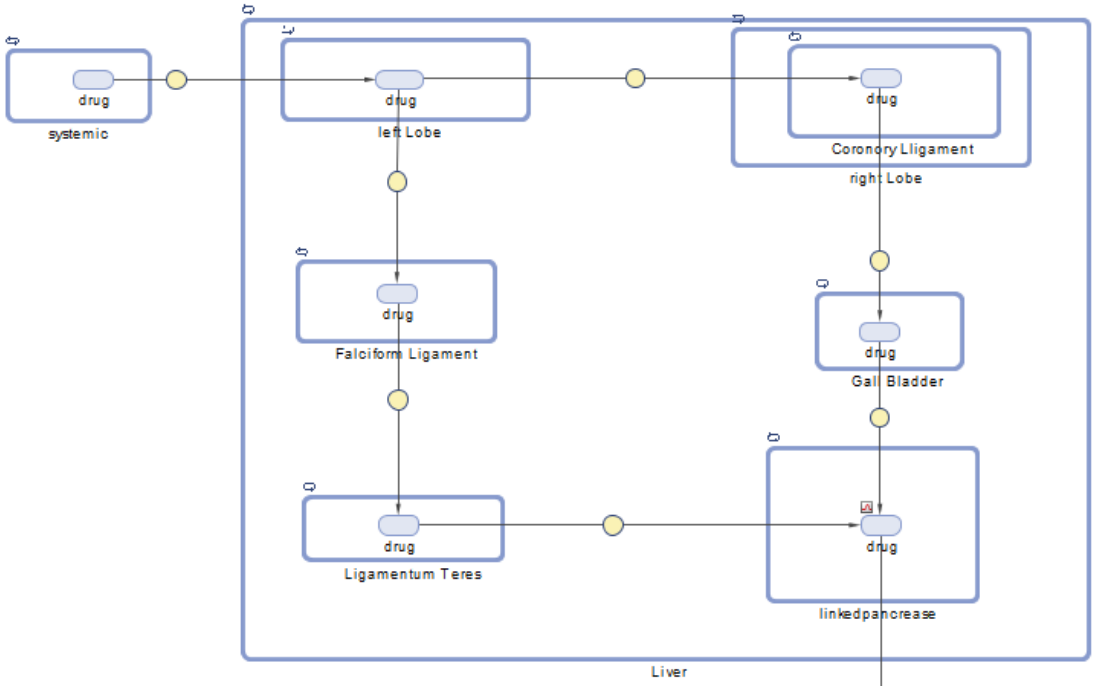

FIGURE 4.1: The PK/PD model of liver showing its all compartments

Here in the figure 4.1, all the linked portions of other organs are connected with the liver. The drug enter into the liver through systemic compartment , spread

to all the areas of liver and produce hepatotoxic effect. The circles represents the reactions of drug with the compartments (left lobe, right lobe, falciform ligament, Gall bladder, ligamentum teres and linked pancrease). The model is represented in the terms of mathematical formulations is as under:

$$\frac{d(Drug)}{dt} = \frac{1}{Central} \times (kaCentral \times Dose - (keCentral \times Drug) \times Central) \quad (4.1)$$

$$\frac{d([CoronoryLigament].drug)}{dt} = ([hepaticflow] \times [portalvein] \times [leftLobe].drug \times kactivity) - ([hepaticflow] \times [CoronoryLigament].drug) \quad (4.2)$$

$$\frac{d([leftlobe].drug)}{dt} = ([totalflow] \times flow_rate \times systemic.drug \times kactivity) - ([hepaticflow] \times [leftLobe].drug) - ([hepaticflow] \times [portalvein] \times [leftLobe].drug \times kactivity) \quad (4.3)$$

$$\frac{d([Falciformligament].drug)}{dt} = -([hepaticflow] \times [FalciformLigament].drug) + ([hepaticflow] \times [leftLobe].drug) \quad (4.4)$$

$$\frac{d([LigamentumTeres].drug)}{dt} = -([hepaticflow] \times [LigamentumTeres].drug) + ([hepaticflow] \times [FalciformLigament].drug) \quad (4.5)$$

$$\frac{d([GallBladder].drug)}{dt} = -([totalflow] \times [GallBladder].drug) + ([hepaticflow] \times [CoronoryLligament].drug) \quad (4.6)$$

$$\frac{d(linkedpancrease.drug)}{dt} = [hepaticflow] \times [LigamentumTeres].drug) + ([totalflow] \times [GallBladder].drug) - (kcl * linkedpancrease.drug)) \quad (4.7)$$

$$\frac{d(systemic.drug)}{dt} = -([totalflow] \times flow_rate \times systemic.drug \times kactivity) \quad (4.8)$$

In the above equations kactivity refers to the constant rate of drug activity, ka shows the absorption rate of drug, the drug clearance rate is represented by kcl. The central variable shows the central compartment of liver, total flow and flow rate are used for blood.

4.4 Hepatotxicity Model

In order to determine the effects of drugs on the liver, the hepatotoxic model was also designed and integrated with the liver model, shown in figure 4.2. In figure 4.2, the HT represents the toxic effects of drugs on liver and E shows the effect on liver function due to hepatotoxicity induced by the drugs.

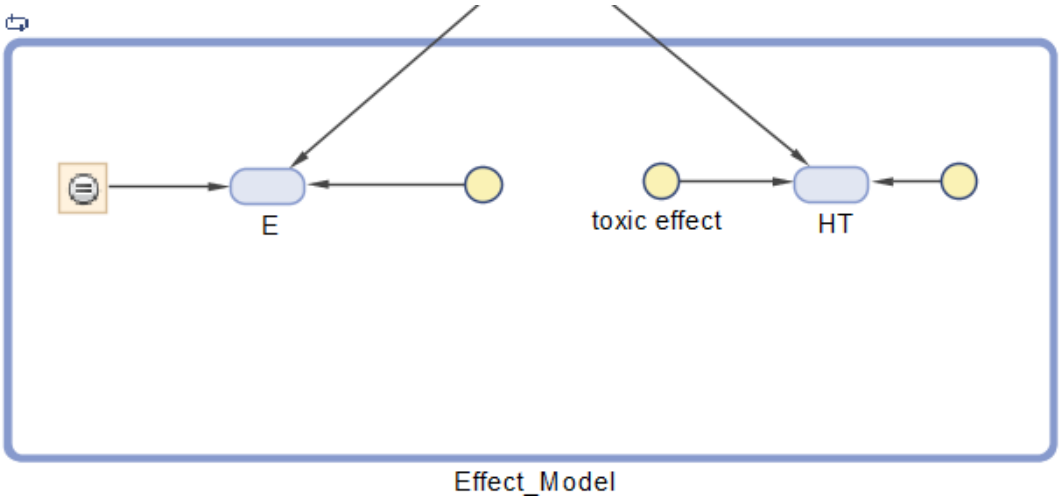

FIGURE 4.2: The hepatotoxicity model.

The differential equations produced for the Hepatotoxicity model are following

$$\frac{d(E)}{dt} = ((kcl \times linkedpancrease.drug) + kE) \tag{4.9}$$

$$\frac{d(HT)}{dt} = ((phli2 \times E^2 \times systemic.drug \times k2_1 \times E \times E^2 \times HT \times linkedpancrease.drug) + (kcl \times linkedpancrease.drug) + kHT) \tag{4.10}$$

In these equations, E shows effect of drug, hepatotoxic effect is represented by HT, and phil2 is model integration parameter.

4.5 Hepatotoxicity Modeling of Selected Drugs

Pharmacodynamics is the study of effect of drug dose on human body. Pharmacodynamic modeling describes the connection of drug exposure to human body and it helps to decide that how much dose of a drug is required for the treatment of disease as well as tells us to what extent it will help to obtain the proper effect of that drug [123]. Hepatotoxicity modeling is basically Pharmacodynamic modeling

of drugs to determine their effects on liver, in which the drug doses were entered into developed liver model, the simulations times were set according to dosing schedule mentioned in literature and drug bank. Pharmacokinetic estimates of all drugs were set accordingly and then graphs were generated to observe the effects of every drug on liver. The resultant graphs of hepatotoxicity modeling are shown in figures 4.3 to 4.14 respectively. In figure 4.3 to 4.14, the x axis represents the time in days and y axis shows concentration of drugs in mol/liter (Blue lines) and hepatotoxic effects (Red lines).

4.5.1 Hepatotoxicity Modeling of Afitinib

Afatinib is the is the first drug chosen for this research as it is used for the treatment of NSCLC. The standard dose for Afatinib is 40 mg which was introduced into the liver model on the first day of treatment and repeated once a day for cycle of 28 days. The results of hepatotoxicity modeling of Afatinib is shown in figure 4.3.

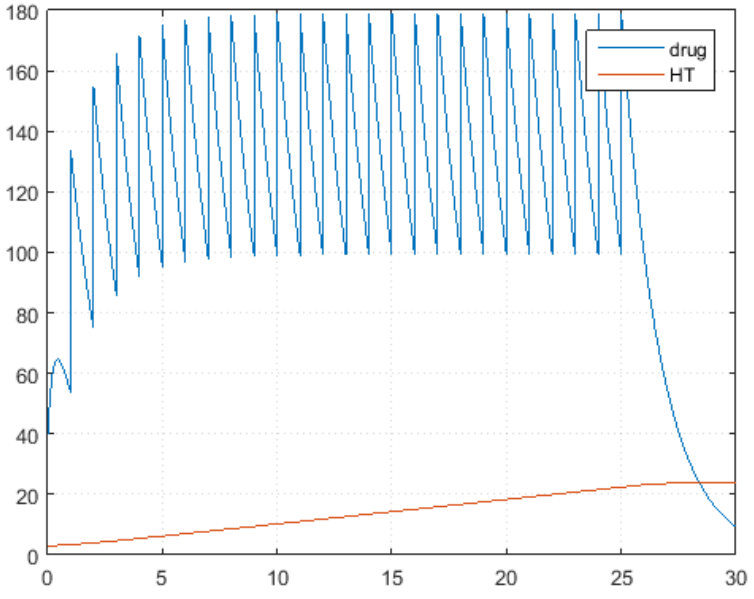

FIGURE 4.3: The hepatotoxicity modeling of Afatinib to determine the effects of its concentration on liver

From figure 4.3, the x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). it can be observed

that initial value for hepatotoxicity was 0 and it gradually increased day by day as drug could not completely eliminated from body till its next dose which lead to liver injury. Though the drug had been given for 28 days, it completely cleared from body till 30th day which leads to high concentration of drug into the liver which ultimately affect it by causing toxicity which cannot be reversed.

4.5.2 Hepatotoxicity Modeling of Bevacizumab

The standard dose for Bevacizumab is 25 mg which was introduced into the liver model on the first day of treatment with repetition of once a week i.e. every 7th day for a cycle of 3 weeks. The results of hepatotoxicity modeling of Bevacizumab is shown in figure 4.4.

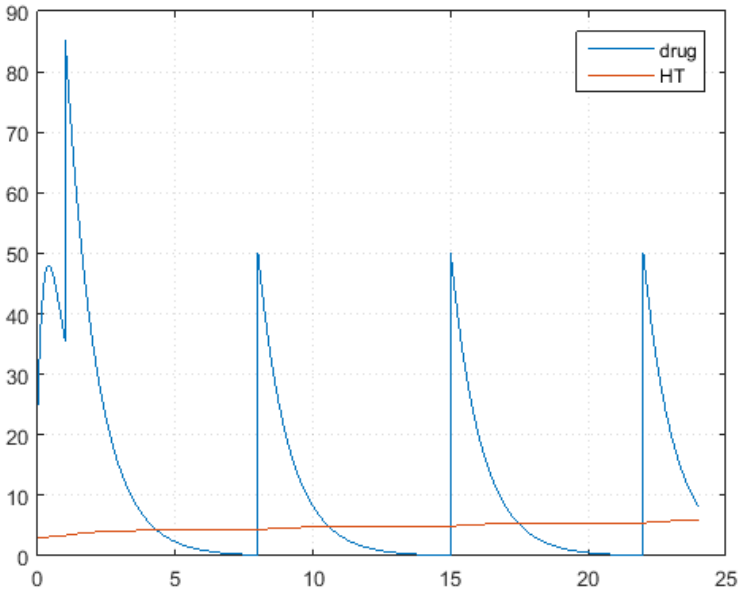

FIGURE 4.4: The hepatotoxicity modeling of Bevacizumab to determine the effects of its concentration on liver

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). It can be observed from the figure 4.4 that initial value for hepatotoxicity was 0 at first day of induction and it gradually increases day by day and reached to 10 mg.m2 at the completion of

dosage cycle. Though the drug has been given for 21 days, it completely cleared from body till 24th day after the last dose causing increase in toxicity in liver day by day. It can also be seen that after the first administration of Bevacizumab dose into the liver, its concentration declined with time and drug has been eliminated from compartment before second dose administration. Bevacizumab did not accumulate upon continuous dosage and exposure of drug did not changed with administration of every dose.

4.5.3 Hepatotoxicity Modeling of Carboplatin

Carboplatin is used for the treatment advanced non-small cell lung cancer and given intravenously in the form of injection in combination with Paclitaxel [124]. The standard dose for Carboplatin is 10 mg which was introduced into the liver model on the first day of treatment with repetition of once after 21 days for a cycle of 4 weeks. The results of hepatotoxicity modeling of Carboplatin introduced alone in model is shown in figure 4.5.

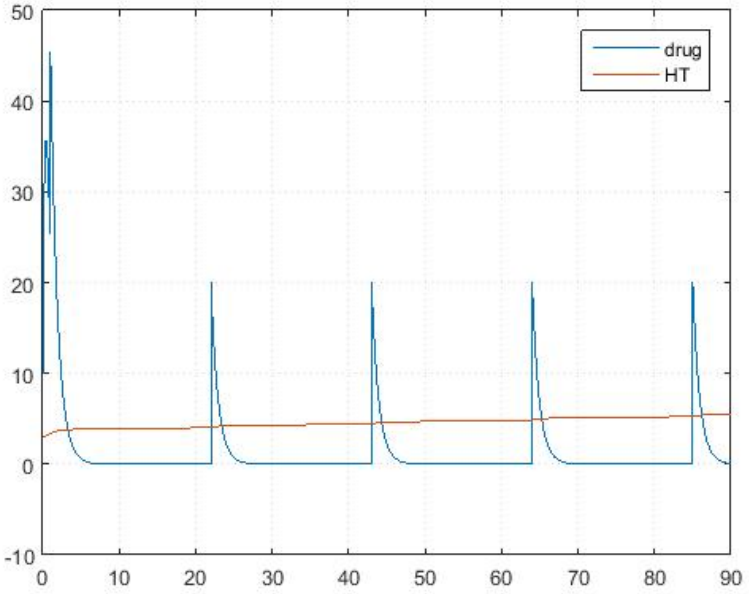

FIGURE 4.5: The hepatotoxicity modeling of Carboplatin to determine the effects of its concentration on liver

In figure 4.5,the x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines), it can be observed that initial value for hepatotoxicity was 0 at first day of induction and it gradually increases day by day but it did not exceed from 5mg/m2 until the completion of cycle. The resultant graph shows that Carboplatin non hepatotoxic drug for the treatment of non small cell lung cancer.

It can also be seen that after the first administration of Carboplatin dose into the liver, its concentration declined with time and drug has been eliminated from compartment before second dose administration. Carboplatin did not accumulate upon continuous dosage and exposure of drug did not changed with administration of every dose.

4.5.4 Hepatotoxicity Modeling of Cisplatin

Cisplatin is used for the treatment advanced non-small cell lung cancer and small cell lung cancer respectively. The standard dose for Cisplatin is 5 mg which is inducted into the liver model on the first day of treatment with repetition of once after 21 days for a cycle of 4 weeks. The results of hepatotoxicity modeling of Cisplatin is shown in figure 4.6. In figure 4.6 the x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines).It can be determined from the figure 4.6 that initial value for hepatotoxicity was 0 at first day of induction and it gradually increases day by day but did not exceed from 5mg/m2 until the completion of cycle because of firstly the dose is very low i.e. 5 mg and secondly the dose gap is high which helps in complete clearance of drug from liver before administration of next dose. The resultant graph shows that Cisplatin non-hepatotoxic toxic drug for the treatment of non small cell lung cancer. Cisplatin did not accumulate upon continuous dosage and exposure of drug did not changed with administration of every dose.

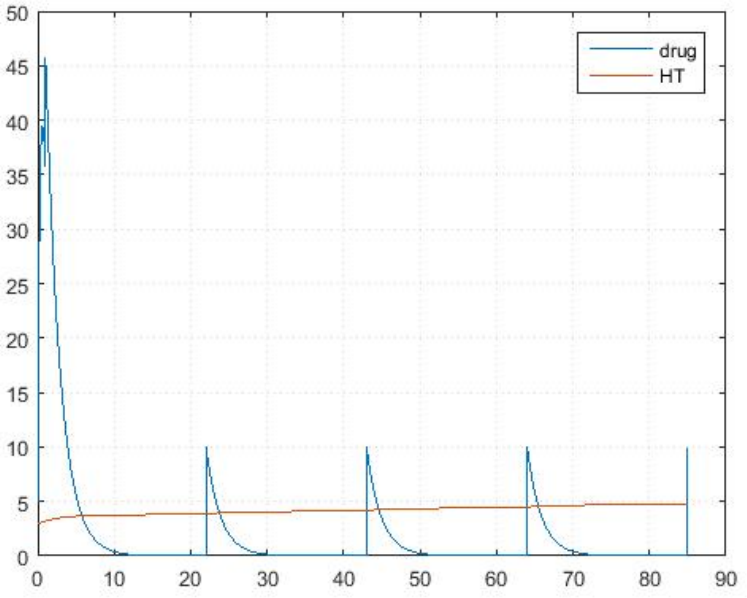

FIGURE 4.6: The hepatotoxicity modeling of Cisplatin to determine the effects of its concentration on liver

4.5.5 Hepatotoxicity Modeling of Crizotinib

The standard dose for Crizotinib is 200-250 mg which is inducted into the liver model on the first day of treatment and repeated twice a day for cycle of 28 days. The results of hepatotoxicity modeling of Crizotinib is shown in figure 4.7.

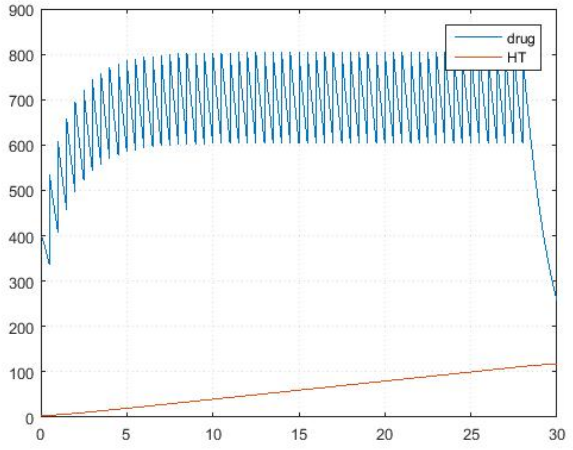

FIGURE 4.7: The hepatotoxicity modeling of Crizotinib to determine the effects of its concentration on liver

Here the x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines).From figure 4.7, it can be observed that initial value for hepatotoxicity was 0 and it gradually increases day by day exceeding 100 mg/m2 at the end of one cycle, which is very high for normal human liver to cause acute liver failure. Though the drug has been given for 28 days, it completely cleared from body till 30th day which leads to high concentration of drug into the liver during whole cycle which ultimately affect it by causing hepatotoxicity which cannot be reversed.

4.5.6 Hepatotoxicity Modeling of Erlotinib

The standard dose for Erlotinib is 150 mg which is inducted into the liver model on the first day of treatment and repeated thrice a day i.e. after every meal, for cycle of 28 days. The result of hepatotoxicity modeling of Erlotinib is shown in figure 4.8.

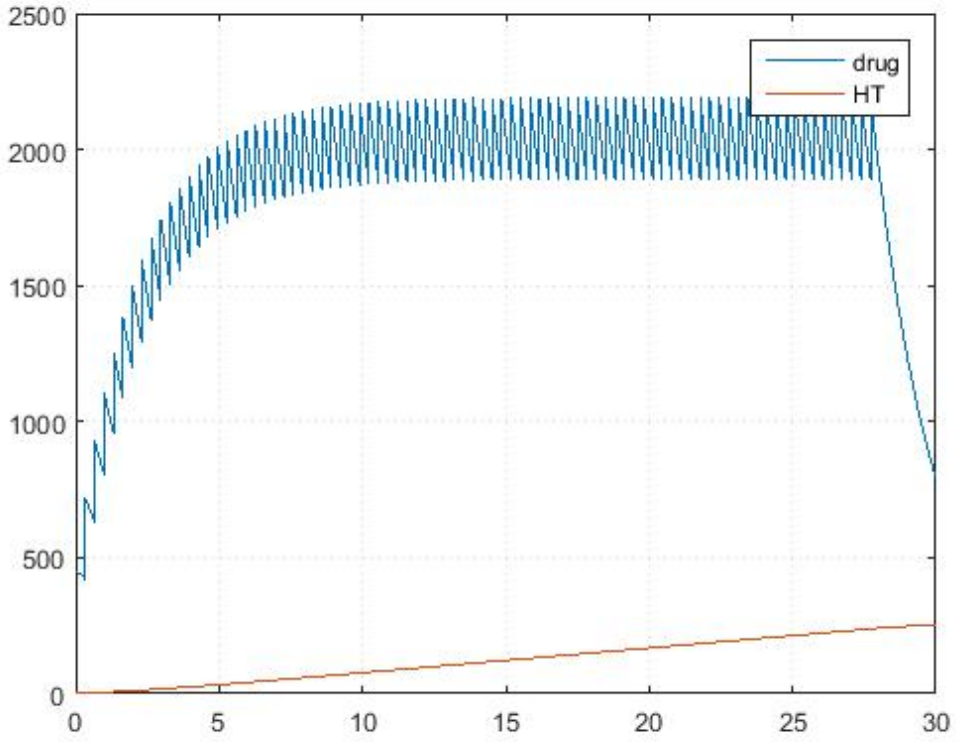

FIGURE 4.8: The hepatotoxicity modeling of Erlotinib to determine the effects of its concentration on liver

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). It can be observed from the figure 4.8 that initial value for hepatotoxicity was 0 and it gradually increases day by day till reaches more than 200 mg/m2 at the end of cycle which is extremely high to cause acute liver failure. Though the drug has been given for 28 days, it completely cleared from body till 30th day which leads to high concentration of drug into the liver during whole cycle which ultimately affect it by causing toxicity which cannot be reversed.

It can also be seen that the concentration of Erlotinib gradually increases in liver with time reaches to 2000 mg at the end of one cycle. The drug accumulates into liver because it does not clear out completely before the adiminstration of next dose. The drug exposure is very high due to continuous dosage once a day i.e. three times in one day which leads to drug induced liver injury.

4.5.7 Hepatotoxicity Modeling of Gefitinib

Gefitinib is used for the treatment of metastatic non-small cell lung cancer and the standard dose for Gefitinib is 250 mg which is inducted into the liver model on the first day of treatment and repeated once a day for a cycle of 28 days. The result of hepatotoxicity modeling of Gefitinib is shown in figure 4.9.

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). From figure 4.9, it can be observed that initial value for hepatotoxicity was 0 and it gradually increases day by day till reaches more than 150 mg/m2 at the end of cycle which is very high to cause acute liver failure. Though the drug has been given for 28 days, it completely cleared from body till 30th day which leads to high concentration of drug into the liver during whole cycle which ultimately affect it by causing toxicity which cannot be reversed.

It can also be seen that the concentration of Gefitinib gradually increases in liver with time reaches to 800 mg.m2 at the end of one cycle. The drug accumulates

into liver because it does not clear out completely before the adiminstration of next dose. The drug exposure is very high due to continuous dosage which leads to drug induced liver injury.

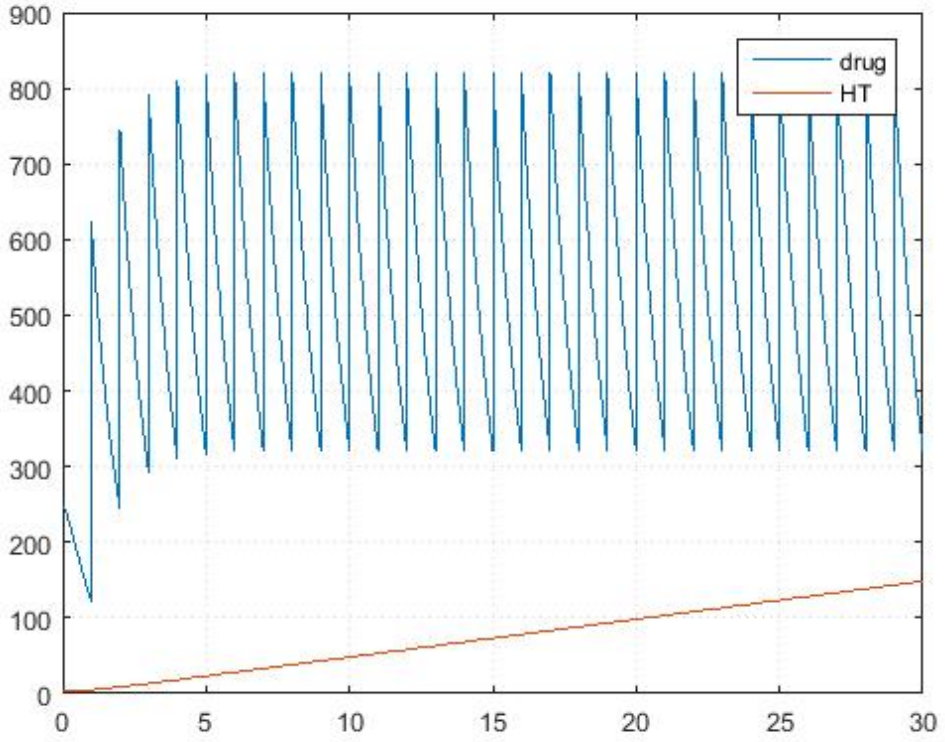

FIGURE 4.9: The hepatotoxicity modeling of Gefitinib to determine the effects of its concentration on liver

4.5.8 Hepatotoxicity Modeling of Gemcitabine

The standard dose of Gemcitabine used for the treatment of NSCLC (stage IV) is 1000 mg which is inducted in liver model at the first day of treatment with repetition of once a week for a 28 days cycle respectively. The results of hepatotoxicity modeling of Gemcitabine is shown in figure 4.10.

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). It can be observed from the figure 4.10 that initial value for hepatotoxicity was 0 at first day of induction and it gradually increases day by day but it can be observed that toxicity in liver did

not exceed from 50mg.m2 until the completion of cycle because though the dose is high but the drug eliminates from liver before administration of next dose. The resultant graph shows that Gemcitabine is hepatotoxic drug for the treatment of non small cell lung cancer. Gemcitabine did not accumulate upon continuous dosage and exposure of drug did not changed with administration of every dose.

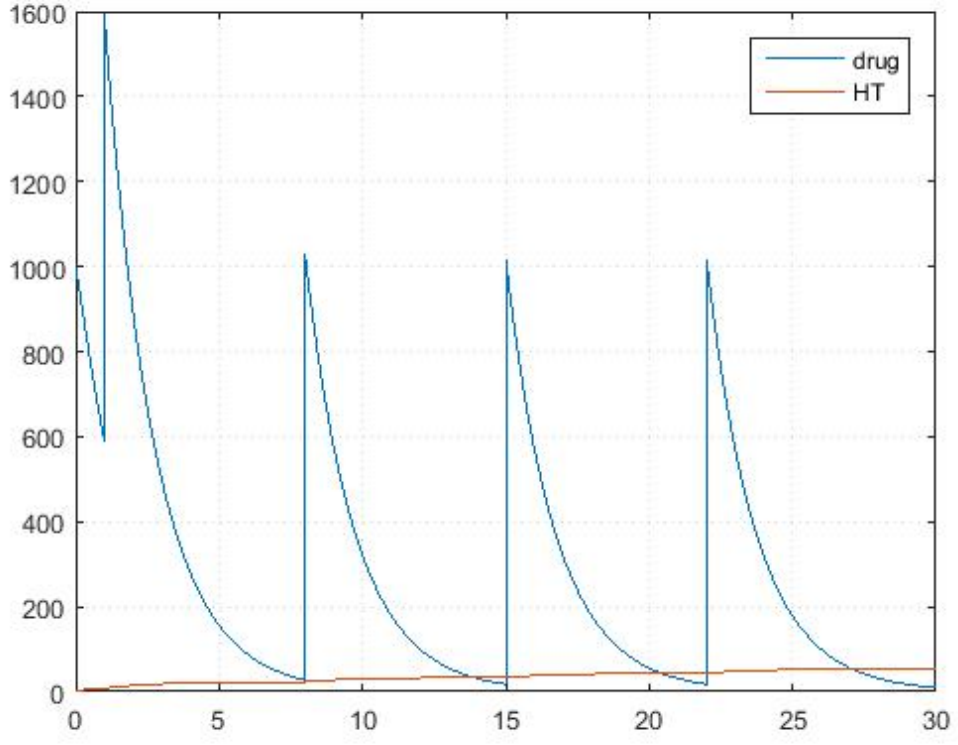

FIGURE 4.10: The hepatotoxicity modeling of Gemcitabine to determine the effects of its concentration on liver

4.5.9 Hepatotoxicity Modeling of Lorlatinib

Lorlatinib is used for the treatment of metastatic non-small cell lung cancer and the standard dose for Lorlatinib is 100 mg which is inducted into the liver model on the first day of treatment and repeated once a day for a cycle of 28 days. The results of hepatotoxicity modeling of Lorlatinib is shown in figure 4.11.

In figure 4.11,The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines), it can be observed that

initial value for hepatotoxicity was 0 at first day of induction and it gradually increases day by day but it can be seen that toxicity in liver did not exceed from 50mg/m2 until the completion of cycle. The resultant graph shows that Lorlatinib is hepatotoxic drug for the treatment of non small cell lung cancer but less hepatotoxic as compared to other drugs.

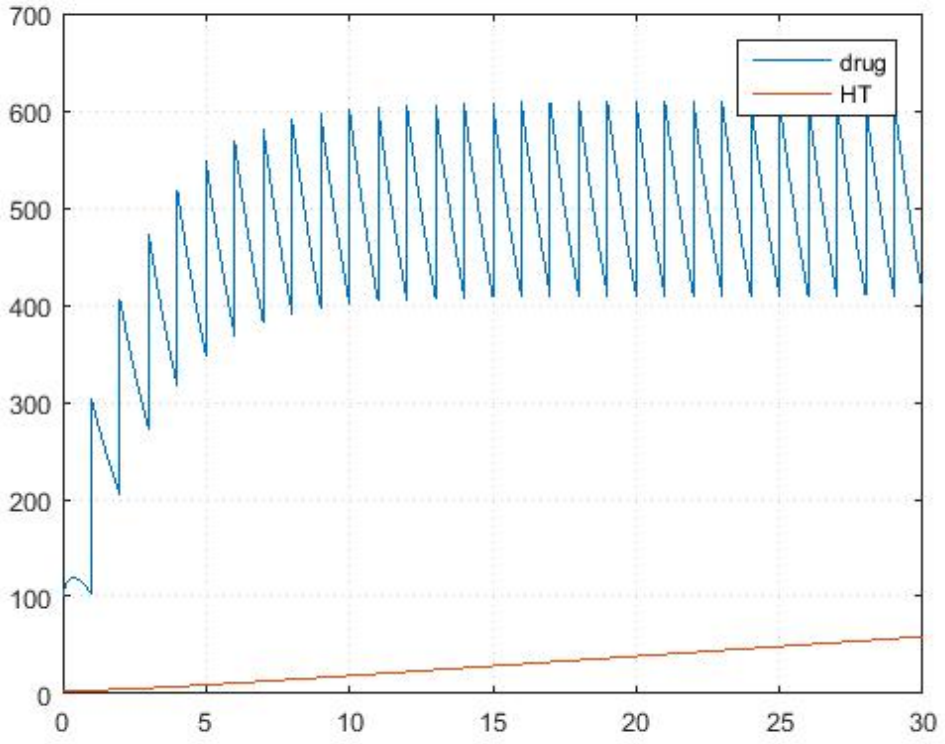

FIGURE 4.11: The hepatotoxicity modeling of Lorlatinib to determine the effects of its concentration on liver

4.5.10 Hepatotoxicity Modeling of Methotrexate

Methotrexate is used for the treatment of squamous cell carcinoma which is one of the type of non-small cell carcinoma that constitutes 25 to 30% of all cases of lung cancer [59] . The standard dose for Methotrexate is 2.5 mg which is inducted into the liver model on the first day of treatment and repeated once a week for a cycle of 4 weeks. The results of hepatotoxicity modeling of Methotrexate is shown in figure 4.12.

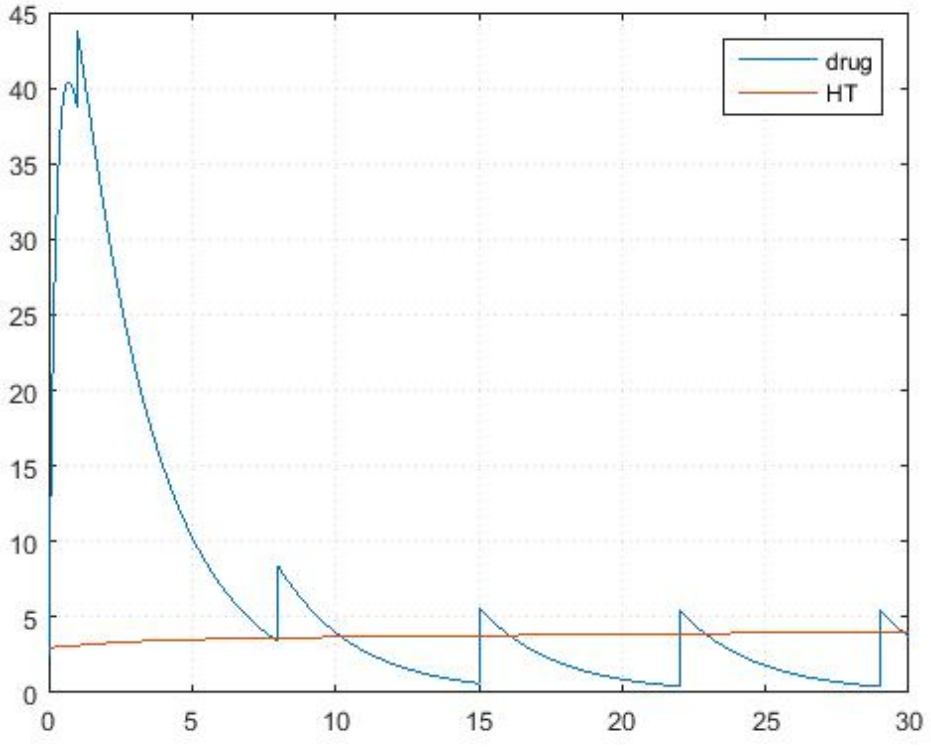

FIGURE 4.12: The hepatotoxicity modeling of Methotrexate to determine the effects of its concentration on liver

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). It can be observed from the figure 4.12 that the value for hepatotoxicity did not change from first day of induction to the completion of cycle. The resultant graph shows that Methtrexate falls in the category of less toxic drug for the treatment of squamous cell carcinoma.

4.5.11 Hepatotoxicity Modeling of Paclitaxel

Paclitaxel is used for the treatment non-small cell lung cancer and given intravenously in the form of injection in combination with Carboplatin or Cisplatin [124]. The standard dose for Paclitaxel is 135 mg which is introduced into the liver model on the first day of treatment with repetition of once a day for a cycle of 3 weeks. The results of hepatotoxicity modeling of Paclitaxel introduced alone in model is shown in figure 4.13.

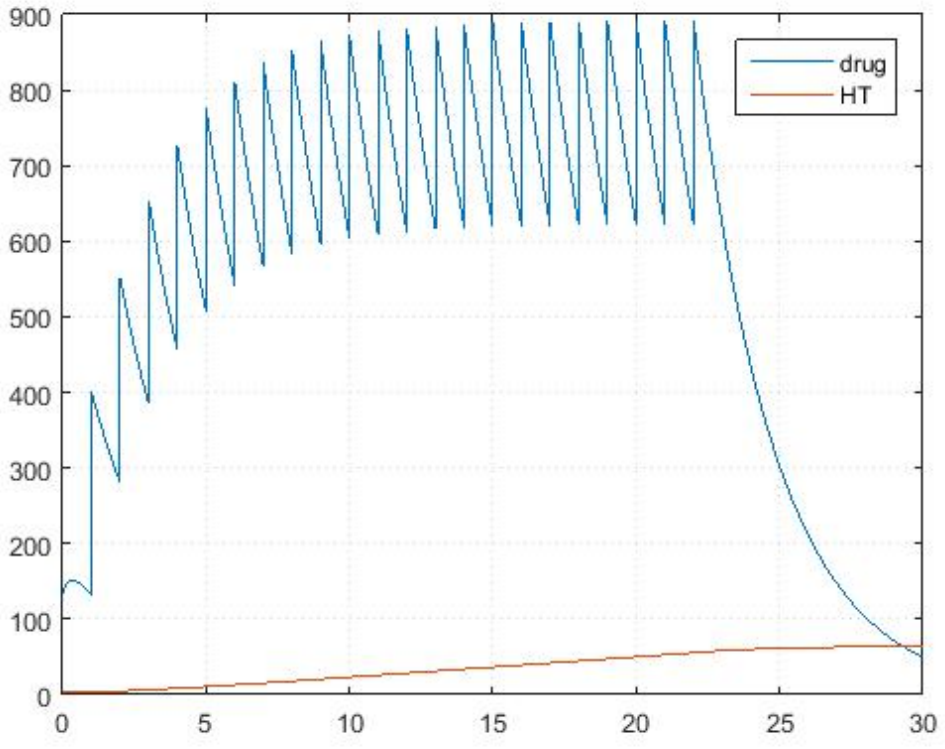

FIGURE 4.13: The hepatotoxicity modeling of Paclitaxel to determine the effects of its concentration on liver

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). In figure 4.13, it can be observed that initial value for hepatotoxicity was 0 and it gradually increases day by day till reaches more than 80 mg/m2 at the end of one cycle which can cause liver injury. Though the drug has been given for 21 days, it completely cleared from body till 30th day which leads to high concentration of drug into the liver during whole cycle which ultimately affect it by causing toxicity which cannot be reversed.

It can also be observed that the concentration of Paclitaxel gradually increases in liver with time reaches to 800 mglm2 at the end of one cycle. The drug accumulates into liver because it does not clear out completely before the adiminstration of next dose. The drug exposure is very high due to continuous dosage which leads to drug induced liver injury.

4.5.12 Hepatotoxicity Modeling of Pemetrexed

The standard dose for Pemetrexed is 500 mg which is inducted into the liver model on the first day of treatment with repetition of once after 21 days cycle of 4 cycles. The results of hepatotoxicity modeling of Pemetrexed introduced alone in model is shown in figure 4.14.

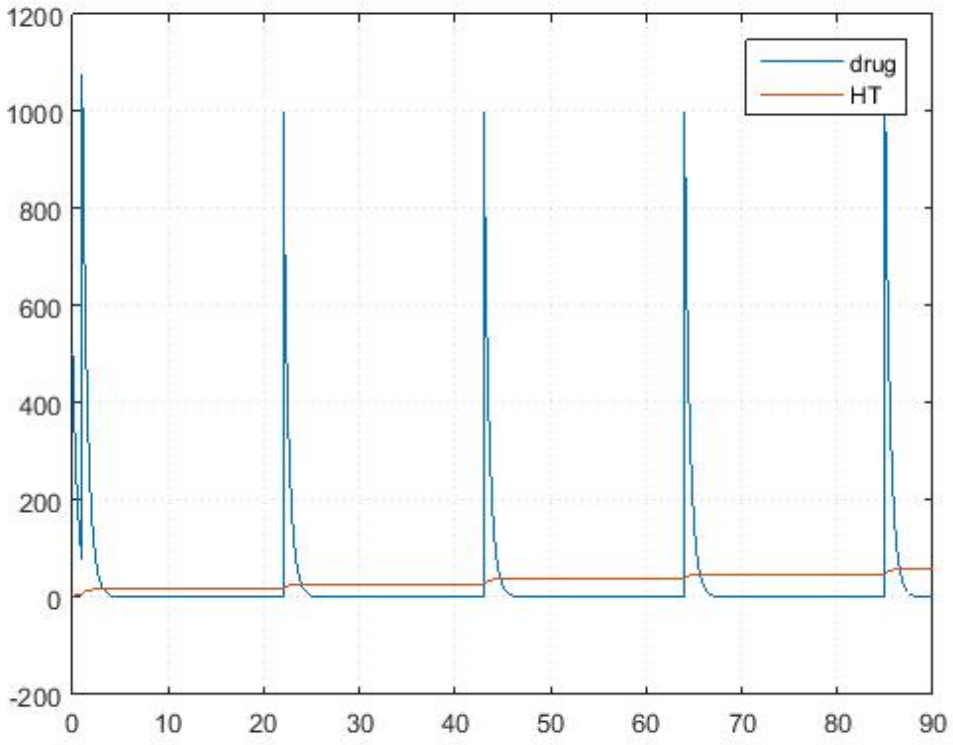

FIGURE 4.14: The hepatotoxicity modeling of Pemetrexed to determine the effects of its concentration on liver

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). It can be observed from the figure 4.14 that initial value for hepatotoxicity was 0 at first day of induction and it gradually increases day by day but did not exceed from 50mg/m2 until the completion of cycle because though the dose is high but the drug absorption rate is very low and eliminates from liver before administration of next dose because of huge gap between doses . The resultant graph shows that Pemetrexed is less toxic drug for the treatment of non small cell lung cancer as compared to other

drugs. Pemetrexed did not accumulate upon continuous dosage and exposure of drug did not changed with administration of every dose.

4.6 Validation of Model

It can be observed from table 4.3 that Carboplatin and Cisplatin are not involved in causing hepatotoxicity. We introduced these drug's dosages along with their dose schedule into our designed liver model and performed hepatotoxicity modeling. The resultant graphs shown in figure 4.5 and 4.6 declared that Carboplatin and Cisplatin induced less than 5 mg/m2 hepatotoxicity which validates our liver model and helped us to determined the hepatotoxicity of all the other drugs used for the treatment of Non-Small Cell Lung Cancer.

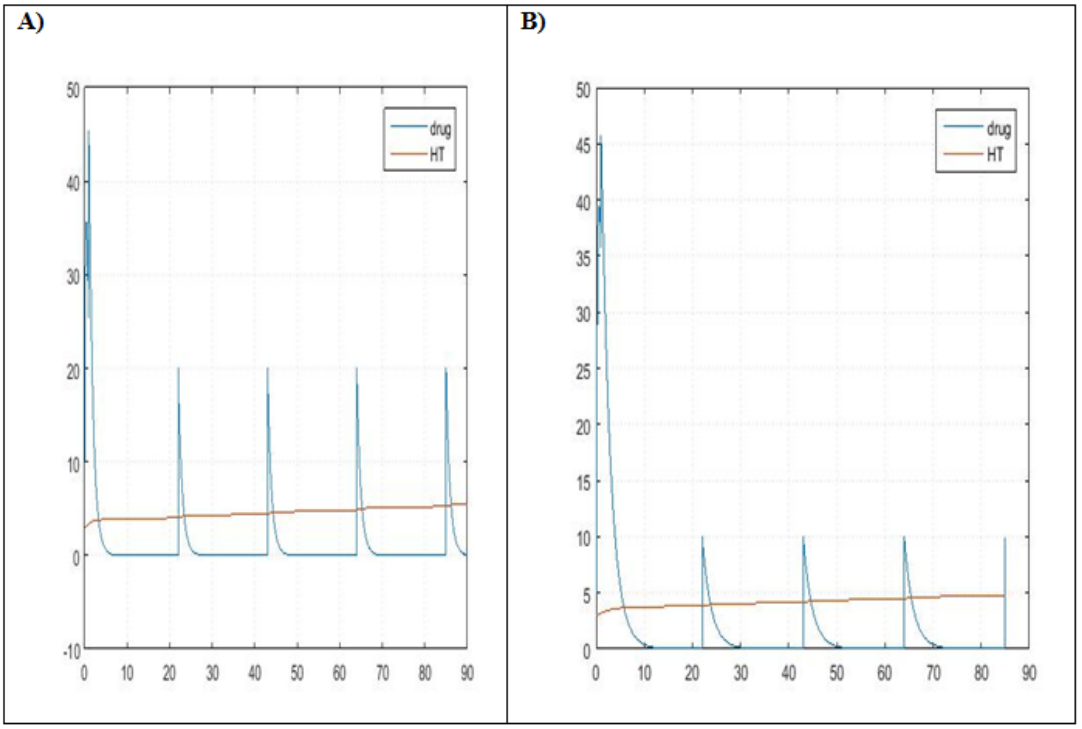

FIGURE 4.15: The hepatotoxicity modeling of Carboplatin and Cisplatin. The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). A) The hepatotoxicity modeling of Carboplatin shows 00 hepatotoxicity less than 5mg/m2. B) The hepatotoxicity modeling of Cisplatin shows hepatotoxicity less than 5mg/m2.

4.7 Suggestion of New Dosage Criteria to Reduce Hepatotoxicity

It can be observed from figures 4.3, 4.7, 4.8, 4.9 and 4.13 that Afitinib, Crizotinib, Erlotinib, Gefitinib and Paclitaxel are involved are in causing hepatotoxicity in Non-small cell lung cancer patients during treatment leading to acute liver failure. In order to reduce hepatotoxicity we have tried new drug dosages for Afitinib, Crizotinib, Erlotinib, Gefitinib and Paclitaxel and inducted them in our designed liver model. The results of hepatotoxicity modeling of new drug dosages of all these five drugs are shown in figure 4.16 to 4.20 respectively. In figure 4.16 to 4.20, the x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines).

The general dose for Afitinib ranges from 20 to 40 mg but standard dose of Afitinib uses for the treatment of NSCLC is 40 mg once a day for a cycle of 28 days respectively. The graph in figure 4.3 shows that this drug dose causes hepatotoxicity more than 20 mg/m2 for one cycle. Therefore, we used the minimum dose of Afitinib i.e. 20 mg and inducted in liver model on the first day of treatment and repeated once a day for a cycle of 28 days. The result of hepatotoxicity modeling of new drug dosage of Afitinib is shown in figure 4.16.

In figure 4.16, The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). It can be observed that initial value for hepatotoxicity was 0 and it gradually increases day by day till reaches 15 mg/m2 at the end of cycle. Therefore by just reducing the drug dose to minimum dose, the hepatotoxicity reduces up to 10 mg/m2per cycle. The hepatotoxicity can be further reduced by reducing dose schedule from once a day to after four days but this may slow down the treatment process to a very great extent. Therefore it can only be suggested if this much slow treatment is acceptable by doctors and researchers.

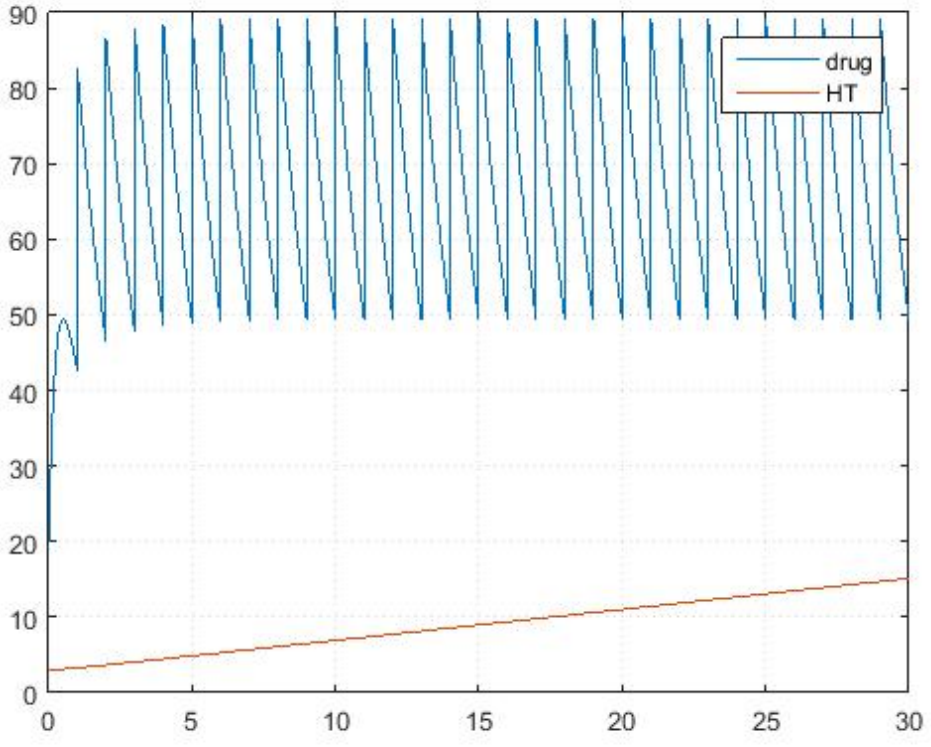

FIGURE 4.16: The hepatotoxicity modeling of suggested drug dose of Afitinib to determine the effects of its concentration on liver

The standard dose for Crizotinib uses for the treatment of NSCLC is 200 mg twice a day for a cycle of 28 days respectively. The graph in figure 4.7 shows that this drug dose causes hepatotoxicity more than 100 mg/m2 for one cycle. Therefore, we reduced the dose of Crizotinib from 200 mg to 100 mg and inducted in liver model on the first day of treatment, repeated twice a day for a cycle of 28 days. The result of hepatotoxicity modeling of new drug dosage of Crizotinib is shown in figure 4.17.

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). The figure 4.17 shows that initial value for hepatotoxicity was 0 and it gradually increases day by day till reaches 60 mg/m2 at the end of cycle. Therefore by just reducing the drug dose from 200 mg to 100 mg, the hepatotoxicity reduces upto 50 mg/m2 per cycle. The hepatotoxicity can be further reduced by reducing dose schedule from twice a day to after two days but this may slow down the treatment process to a very

great extent. Therefore it can only be suggested if this much slow treatment is acceptable by doctors and researchers.

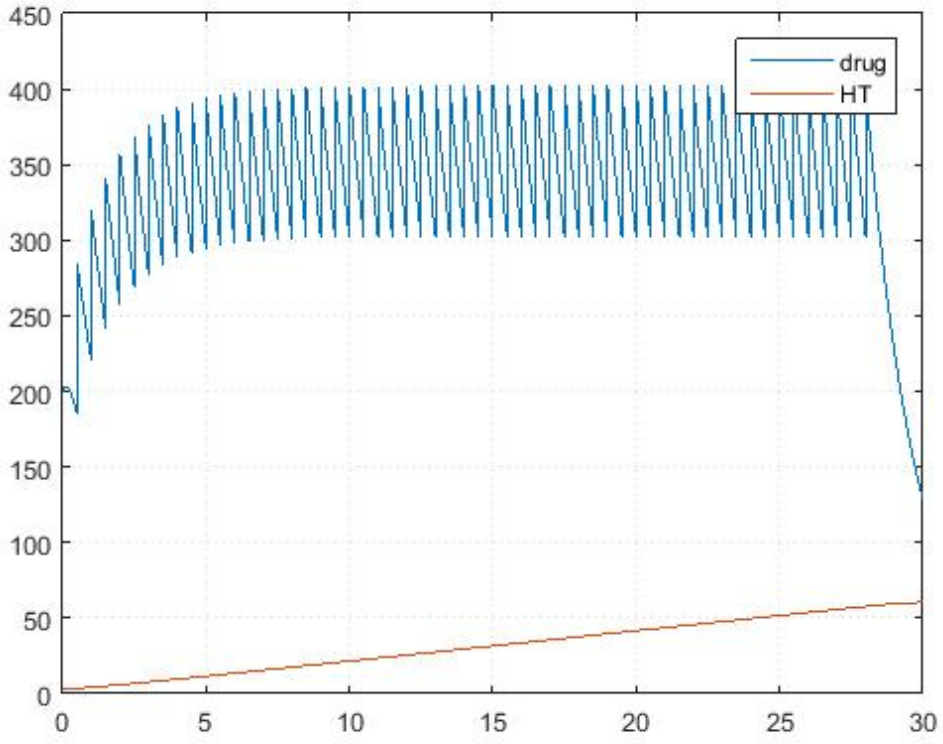

FIGURE 4.17: The hepatotoxicity modeling of suggested drug dose of Crizotinib to determine the effects of its concentration on liver

The general dose for Erlotinib ranges from 25 to 150 mg but standard dose of Erlotinib uses for the treatment of NSCLC is 150 mg thrice a day for a cycle of 28 days respectively. The graph in figure 4.8 shows that this drug dose causes hepatotoxicity more than 200 mg/m2 for one cycle. Therefore, we used the same dose of Erlotinib i.e. 150 mg but reduces the dose schedule from thrice a day to once a day and inducted in liver model on the first day of treatment and repeated once a day for a cycle of 28 days. The result of hepatotoxicity modeling of new drug dosage of Erlotinib is shown in figure 4.18.

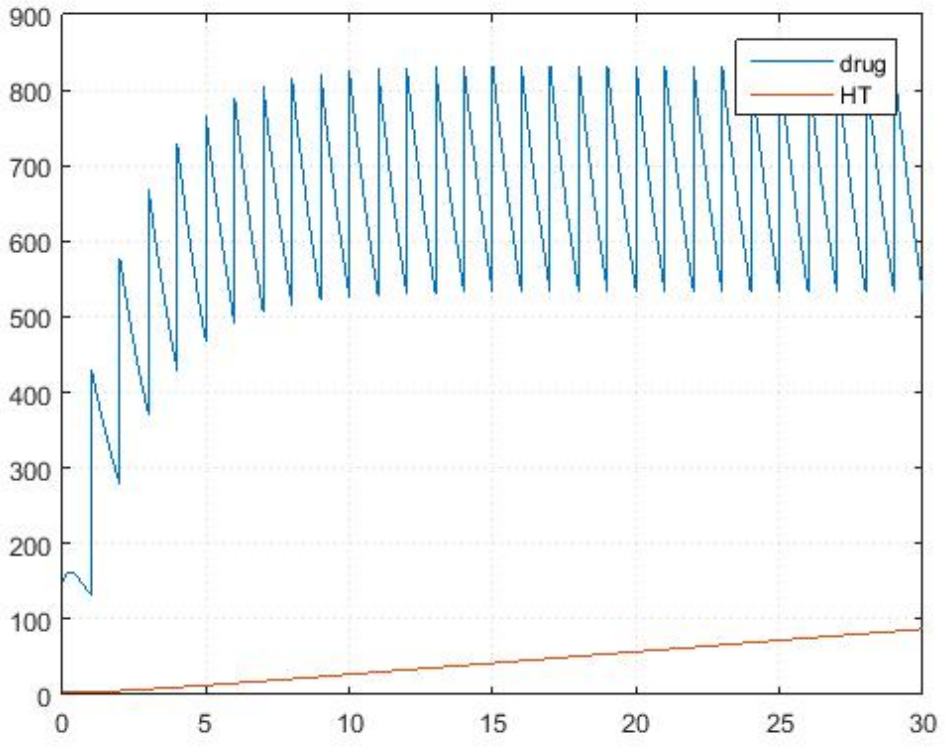

FIGURE 4.18: The hepatotoxicity modeling of suggested drug dose of Erlotinib to determine the effects of its concentration on liver

In figure 4.18,The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines), it can be observed that initial value for hepatotoxicity was 0 and it gradually increases day by day till reaches 90 mg/m2 at the end of cycle. Therefore by just reducing the drug dose schedule from thrice a day to once a day, the hepatotoxicity reduces from 200 to 90 mg/m2 per cycle. The hepatotoxicity can be further reduced by reducing drug dose but this may slow down the treatment process to a very great extent. Therefore it can only be suggested if this much slow treatment is acceptable by doctors and researchers.

The standard dose of Gefitinib uses for the treatment of NSCLC is 250 mg once a day for a cycle of 28 days respectively. The graph in figure 4.9 shows that this drug dose causes hepatotoxicity more than 150 mg/m2 for one cycle. Therefore, we reduce the dose of Gefitinib from 250 to 100 mg and inducted in liver model on the first day of treatment and repeated once a day for a cycle of 28 days. The

result of hepatotoxicity modeling of new drug dosage of Gefitinib is shown in figure 4.19.

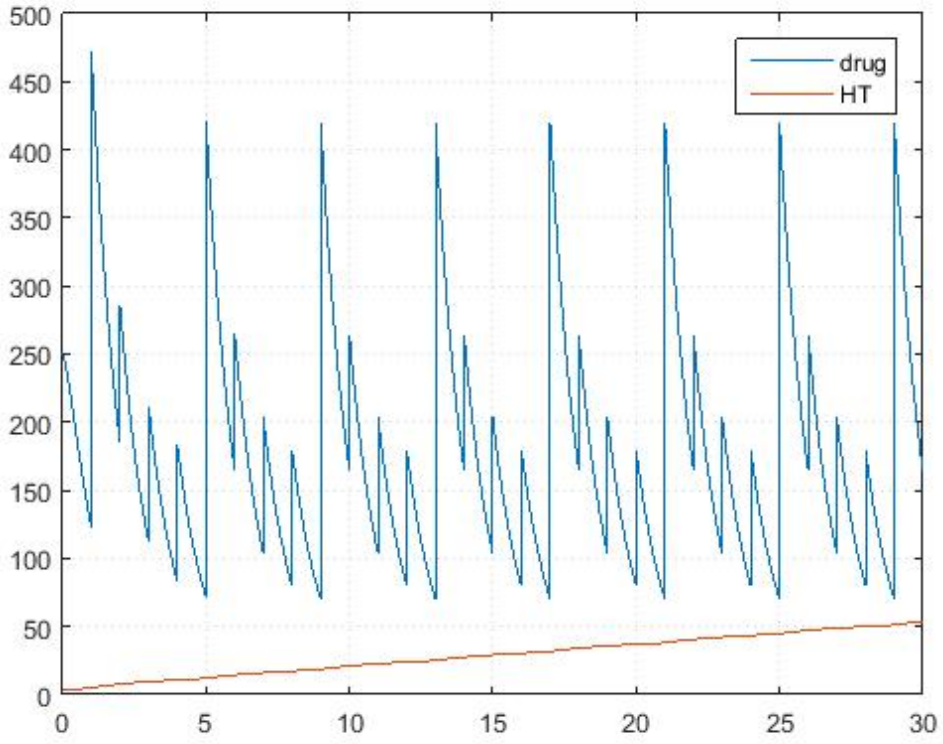

FIGURE 4.19: The hepatotoxicity modeling of suggested drug dose of Gefitinib to determine the effects of its concentration on liver

In figure 4.19, The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines), it can be observed that initial value for hepatotoxicity was 0 and it gradually increases day by day till reaches 50 mg/m2 at the end of cycle. Therefore by just reducing the drug dose from 250 to 100, the hepatotoxicity reduces from 150 to 50 mg/m2 per cycle. The hepatotoxicity can be further reduced by reducing drug dose schedule but this may slow down the treatment process to a very great extent. Therefore it can only be suggested if this much slow treatment is acceptable by doctors and researchers.

The standard dose of Paclitaxel uses for the treatment of NSCLC is 135 mg once a day for a cycle of 3 weeks respectively. The graph in figure 4.13 shows that this drug dose causes hepatotoxicity more than 80 mg/m2 for one cycle. Therefore, we

reduce the dose of Paclitaxe from 135 to 100 mg and inducted in liver model on the first day of treatment and repeated once a day for a cycle of 21 days. The result of hepatotoxicity modeling of new drug dosage of Paclitaxe is shown in figure 4.20.

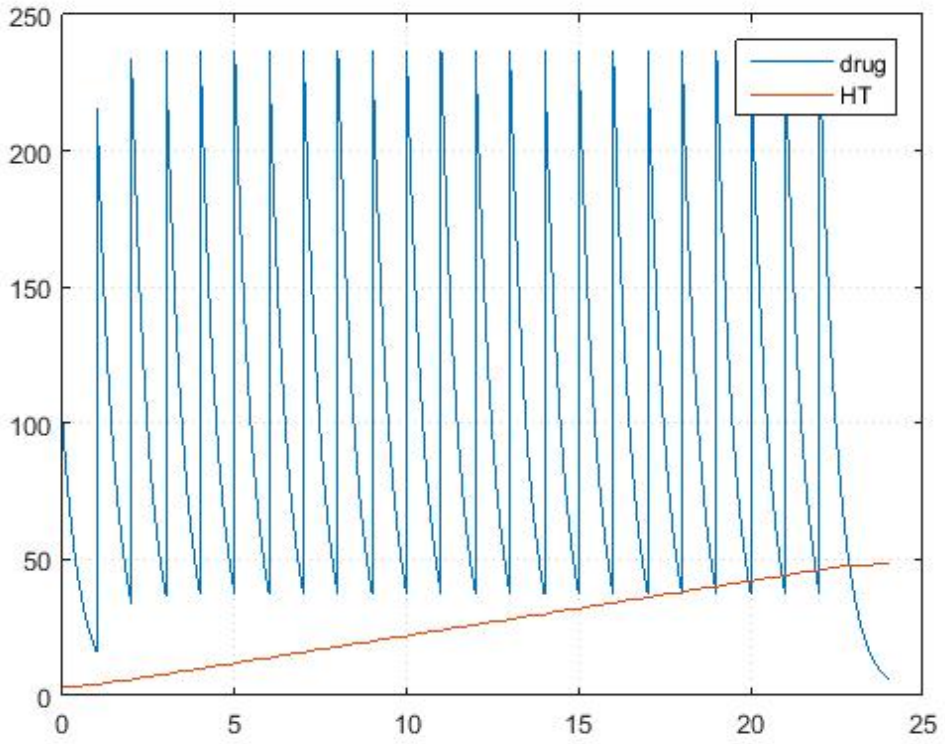

FIGURE 4.20: The hepatotoxicity modeling of suggested drug dose of Paclitaxel to determine the effects of its concentration on liver

The x axis represents the time in days and y axis shows concentration of drugs (Blue lines) and hepatotoxic effects (Red lines). The figure 4.20 shows that initial value for hepatotoxicity was 0 and it gradually increases day by day till reaches 50 mg/m2 at the end of cycle. Therefore by just reducing the drug dose from 135 to 100, the hepatotoxicity reduces from 80 to 50 mg/m2 per cycle. The hepatotoxicity can be further reduced by reducing drug dose schedule but this may slow down the treatment process to a very great extent. Therefore it can only be suggested if this much slow treatment is acceptable by doctors and researchers.

Chapter 5

Conclusion and Future Recommendations

Cancer is the leading cause of deaths worldwide and the most common types of cancers that are causing morality worldwide are lung cancer, breast cancer, prostate cancer, colorectal cancer and skin cancer etc. Lung cancer is most common type of cancer leading to approximately 9.6 million deaths per year worldwide. There are two types of lung cancer , small cell lung cancer and non-small cell lung cancer. Non-small cell lung cancer is causing 75-85% of deaths caused by lung cancer worldwide. Treatment options for non-small cell lung cancer are chemotherapy, radiotherapy, surgery etc. For chemotherapy in the treatment of NSCLC, there are approximately 12 drugs that are used with standard dosages approved from FDA. Liver is a major organ that plays a central role in clearing chemicals and transforming the chemical agents hence susceptible of toxicity from the drugs used for the treatment of a disease. Drug-induced liver damage is one of major cause of acute and chronic liver disease. Pharmaceutical companies have adopted computational modeling approaches to estimate the toxicity, efficacy and mechanisms adopted by pharmaceutical ingredients. PK/PD modeling and simulations helps in estimating the safety and feasibility of medicines for better use in the treatment of cancer or any other disease by reducing hepatotoxicity leading to inhibition of liver failure.

To determine the effects of all the drugs used for the treatment of NSCLC on liver, we designed a liver model based on old data and performed hepatotoxicity modeling on these 12 drugs individually. The hepatotoxicity results of all these drugs shows that Carboplatin and Cisplatin are non hepatotoxic drugs while Bevacizumab, Gemcitabine, Lorlatinib, Methotrexate and Pemetrexed are less hepatotoxic drugs. In addition, Afitinib, Crizotinib, Erlotinib, Gefitinib and Paclitaxel are highly hepatotoxic drugs that are used for the treatment of non-small cell lung cancer. All these drugs other than Carboplatin and Cisplatin are involved in causing hepatotoxicity leading to drug induced liver injury in non-small cell lung cancer patients. By performing hepatotoxicity modeling on Carboplatin and Cisplatin by using our designed model, we validate the designed liver model by proving the non- hepatotoxicity of these drugs.

By reducing the drug dose or drug dose schedule we can reduce hepatotoxic drugs into less or non-hepatotoxic drugs. Therefore, we suggested new drug dosages and new dose schedule and inducted them into liver model, that helps in reducing hepatotoxicity of highly hepatotoxic drugs converting them into less hepatotoxic drugs.

Hence we suggest the mentioned drug doses and drug schedule of Afitinib, Crizotinib, Erlotinib, Gefitinib and Paclitaxel for further studies and implementing them for the treatment of Non small cell lung cancer in case of chemotherapy with these drugs. The designed liver model is generic which can be used for research of all the different drugs used for treatment of different diseases and different types of cancer.

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