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The Effect of Norfloxacin on Pharmacokinetics of Carbamazepine at Steady State in Rabbits

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﴿ وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ ﴾

(105) سورة التوبة

صدق الله العظيم

Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, and that this thesis (*or any part of it*) has not been submitted for higher degree to any other university or institution.

Signed.....

Faten Abed Al Raziq Abu Mhadi

Date.....

Dedication

To my parents…,

To my brothers and my sister … ,

To my instructors and supervisors … ,

To my friends…,

To all those who helped me to accomplish such

afascinating work … ,

faten

Acknowledgement

I am deeply grateful to my supervisor **Dr. Issam Abushammala** for his valuable assistance and patience which he showed in guiding me during the preparation and development of this study.

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Abstract

Title: The Effect of Norfloxacin on Carbamazepine's Pharmacokinetic parameters at Steady State in Rabbits.

Introduction: Carbamazepine (CBZ) is one of the most commonly prescribed antiepileptic drugs. Carbamazepine is rapidly absorbed with a bioavailability of 75–85 % and plasma protein binding is 75 %. It is extensively metabolized in the liver, primarily by CYP3A4, to carbamazepine-10, 11-epoxide. CBZ initial half-life elimination values range from 25-65 hours, decreasing to 12-24 hours on repeated doses, because of autoinduciton effect (Brunton,Chabner and Knollman, 2011). Metabolism of drug by cytochrome system can lead to several drug-drug interactions, which result in decrease pharmacological action, drug toxicity and adverse drug reaction. Norfloxacin (NFX) is an antibiotic, which exhibited a moderate CYP3A4 inhibitory effect (Mclellan et al., 1996).

Aim: To investigate the effect of NFX on pharmacokinetics of CBZ at steady state.

Methodology: An in vivo drug-drug interaction, a randomized, crossover design study was conducted in six healthy male rabbits between NFX and CBZ. The study was carried out on two periods, first period CBZ was administered alone as single oral dose daily (40 mg/kg) for 10 days. In the second period, CBZ was administered as a single oral dose (40 mg/kg) for three consecutive days. On the fourth day a single dose of NFX (11.4 mg/kg) was given orally along with CBZ for the following seven days to each rabbit, after a washout period (10 days). Serial blood samples were collected over a period of 24 hours after the last dose of CBZ. Chemiluminescent enzyme immunoassay (CLEIA) was used to measure CBZ concentration in serum. Pharmacokinetic parameters as $C_{max,}$ t_{max} AUC_{0-24}, $AUC_{0-\infty}$, $t_{½}$ and the constant rate of elimination K_e were determined in the two period.

Result: Six rabbits were enrolled in the study which exhibited good tolerability to CBZ and NFX formulations. No statistical differences were found based on ANOVA. The mean values of PK parameters for first and second periods were as follows: $\mathbf{C_{max}}$ 9.970 versus 8.400 µg/ml, **AUC0-∞**; 154.1 versus 166.8 µg.h/ml, $\mathbf{AUC_{0-24}}$**;** 130.3 versus 113.6 µg.h/ml, $\mathbf{k_e}$**;** 0.0587 versus 0.0419 h^{-1}, $\mathbf{t_{max}}$; 4.330 versus 4.580 h and $\mathbf{t_{½}}$**;** 12.78 versus 21.34 h for the first and second periods, respectively.

Conclusion: No significant differences in PK of CBZ was found when CBZ was administered alone or in combination with NFX (P> 0.05).

Keywords: Caebamazepine, Norfloxacin, Drug interaction, Pharmacokinetic, CYP3A4, Cytochrome P450**.**

الملخص العربي

العنوان : تأثير NFX على محددات حركية دواء الCBZ عند حالة استقرار مستويات العقار في الدم في الأرانب.

المقدمة: Carbamazepine (CBZ) يعتبر واحد من أكثر الأدوية إستخداماً لعلاج الصرع، يتم امتصاصه في الجهاز الهضمي بسرعة إتاحة حيوية بنسبة 75-85٪ وارتباطه مع بروتين البلازما هو 75٪. ويتم أيضه في الكبد بواسطة أنزيم CYP3A4 إلى (carbamazepine-10, 11-epoxide). تتراوح فترة نصف العمر ما بين 25-65 ساعة وهذه الفترة تقل إلى 12-17 ساعة مع الاستخدام المتكرر بسبب التحفيز الذاتي لعملية الأيض. عملية الأيض بواسطةCYP450 قد تؤدي إلي تفاعلات بين الأدوية والتي قد تؤدي إلى تقليل المفعول الدوائي و سمية الدواء والأعراض الجانبية. (NFX) Norfloxacin هو مضاد حيوي له تأثير إحباطي متوسط لأنزيم CYP3A4.

الهدف : بحث تأثير NFX على محددات حركية الدواء CBZ عند حالة استقرار مستويات العقار في الدم.

منهجية العمل : دراسة التداخلات الدوائية في الجسم. تم اختيار ست أرانب ذكور بطريقة عشوائية والدراسة كانت بطريقةcrossover. تم إجراء الدراسة على مرحلتين في المرحلة الأولى تم إعطاء الأرانب CBZ جرعة واحدة يومية (40 mg/kg) بالفم لمدة 10 أيام وفي المرحلة الثانية تم إعطاء الأرانب CBZ جرعة واحدة يومية بالفم (40 mg/kg) لمدة 3 أيام متتالية وفي اليوم الرابع تم إعطاء الأرانب NFX جرعة واحدة يومية بالفم (11.4 mg/kg) مع CBZ لمدة 7 أيام متتالية.

جمعت عينات دم على مدار 24 ساعة بعد الجرعة الأخيرة وثم تحليلها باستخدام CLEIA (التحليل الكيميائي الضوئي المناعي للأنزيم). تم تحديد محددات حركية الدواء في الأرانب والتي **شملت** $\mathbf{C_{max}}$, $\mathbf{t_{max}}$ $\mathbf{AUC_{0-24}}$, $\mathbf{AUC_{0-\infty}}$, $\mathbf{t_{½}}$ و**ke** في المرحلتين.

النتائج : متوسط القيم لمعاملات حركية الدواء في المرحلة الأولى والثانية على النحو التالي:

$\mathbf{C_{max}}$; 9.970 versus 8.400 µg/ml, **AUC0-∞;** 154.1 Versus 166.8 µg.h/ml, $\mathbf{AUC_{0-24}}$; 130.3 versus 113.6 µg.h/ml, $\mathbf{k_e}$; 0.0587 versus 0.0419 h^{-1}, $\mathbf{t_{max}}$; 4.33 versus 4.580 hours and $\mathbf{t_{½}}$;12.77 versus 21.33 h.

الاستنتاج : الأرانب الستة التي استخدمت في الدراسة كانت لها قوة تحمل جيدة للأدوية أثناء فترة الدراسة.لا توجد أي اختلافات إحصائية لمعاملات حركية الدواء CBZ عند إعطاءه لوحده أو مع NFX ($P> 0.05$).

الكلمات المفتاحية: Carbamazepine، Norfloxacin، التداخلات الدوائية، معاملات حركية الدواء, CYP3A4, CYP450.

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List of Abbreviation

ADME	Absorption, Distribution, Metabolism and Elimination.
Ah	Aryl hydrocarbon.
AEDs	Anti-epileptic drugs.
ANOVA	Analysis of Variance.
AUC	Area under the plasma concentration-time curve.
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from zero to infinity.
AUC_{0-t}	Area under the plasma concentration-time curve from zero to time.
CAR	Constitutive androstane receptor.
CBZ	Carbamazepine.
CL	Total body clearance.
C_{max}	Maximum plasma concentration.
C_t	The plasma concentration at time t.
CLEIA	Chemiluminescense Immunoassay.
Conc.	Concentration.
CYP	Cytochrome P450.
CV	Coefficient of variation.
DDIs	Drug-drug interactions.
DNA	Deoxy ribose nucleic acid.
DR	Delayed Release.
ER	Extended Release.
h	Hour.
IGE	Idiopathic generalized epilepsy.
GABA	Gamma-aminobutyric acid.
K_e	Elimination rate constant.
L	Liter.
MDR	Multidrug resistance.
µg	Micrograms.
ml	Milliliters.
µl	Micro-liter.

NADPH	Nicotinamide adenine dinucleotide phosphate.
NTI	Narrow Therapeutic Index.
NFX	Norfloxacin.
PK	Pharmacokinetics.
PXR	Pregnane x receptor.
P-GP	P-Glycoprotein.
PPAR	Peroxime proliferative activated receptor.
SPSS	Statistical Package of Social Science.
SSRIs	Selective serotonine reuptake inhibitors.
SD	Standard deviation.
T_{max}	Time to maximum plasma concentration.
$t_{½}$	Half life of elimination.
ABCC2	ATP- binding cassette subfamily Cmember 2.

Chapter 1

Introduction

1.1 Carbamazepine

Carbamazepine (CBZ), 5H-dibenz[b,f]azepine-5-carboxamide, is one of the most commonly prescribed antiepileptic drugs. It is used on a long-term basis for control of generalized tonic clonic seizures and psychosis (Landmark, 2008; Patsalos, 2013). The structure of CBZ is given in figure 1.1.

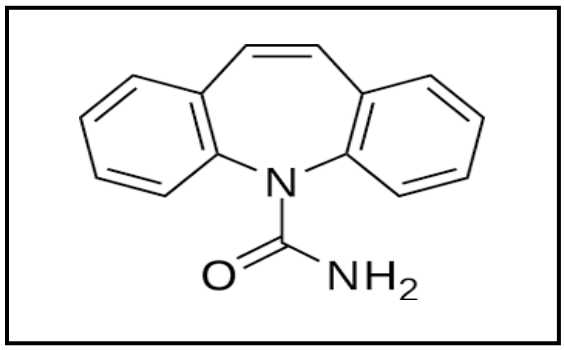

Figure 1.1:Chemical structure of carbamazepine

Physically, it is white to off-white crystalline powder, having molecular mass 236.269g/mol and melting point of about 190.2°C. It is freely soluble in chloroform, dimethylformamide, ethylene glycol monomethyl ether or methanol and slightly soluble in ethanol or glacial acetic acid and water (British Pharmacopeia, 2009).

Carbamazepine is rapidly absorbed with a bioavailability of 75–85 % and plasma protein binding is 75%. It is extensively metabolized in the liver, primarily by CYP3A4, to carbamazepine-10, 11-epoxide which is pharmacologically active . (Patsalos, 2013; Punyawudho et al., 2009).

1.2 Cytochrome P450 (CYP450)

The cytochrome P450 system is an evolutionary system to deal with the breakdown of endogenous and exogenous chemicals in the body. It is a family of isosymes responsible for metabolism of several drugs. There are 55 isoforms of cytochrome. Of the CYP enzymes, CYP3A4 is not only the most prevalent CYP enzyme in the liver, but is used by more than 50% of medications on the market for their metabolism (Ogu and Maxa, 2000; Martin, 2001).

CBZ is metabolized primarily by CYP3A4. Other isoenzymes that contributed to the metabolism are CYP2C8, CYP2B6,CYP2E1, CYP1A2, and CYP2A6. Less than 2 % of an administered dose is excreted as unchanged carbamazepine in urine (Patsalos, 2013).

CBZ is one of the few drugs that, after multiple doses, can stimulate the synthesis of enzymes that catalyze its own metabolism by a process known as auto-induction. CBZ has short half-life in chronic treatment, due to autoinduction of drug metabolism and narrow therapeutic window (Patsalos, 2013; Punyawudho et al., 2009, Galal et al., 2004). Therapy with carbamazepine is complicated by its complex pharmacokinetics and drug-interaction profile (Punyawudho et al., 2009).

Metabolism of drug by cytochrome system can lead to several drug-drug interactions, which result in decrease pharmacological action, drug toxicity and adverse drug reaction(Ogu and Maxa, 2000).

1.3 Norfloxacin

Norfloxacin is a synthetic, broad-spectrum fluoroquinolone antimicrobial. It has in vitro activity against a broad range of gram-positive and gram-negative aerobes but no activity against anaerobes.

Physically, it is a white to pale yellow crystalline powder, having molecular mass 319.331 g/mol and melting point of about 221°C. It is odorless and has a bitter taste. It is freely soluble in glacial acetic acid and very slightly soluble in ethanol, methanol and water. Its octanol-water partition coefficient is 0.46 (British Pharmacopeia, 2009).

The effect of Norfloxacin (NFX) on human hepatic microsomal fraction that was prepared form a female, who exhibited a high CYP3A4 activity showed inhibition of activity by 64% (Mclellan et al., 1996). NFX also inhibited the metabolism of cyclosporine A in human liver microsomes by 56%, when 1μm of cyclosporine A was incubated in presence of 100 μm NFX (Pichard et al., 1999). NFX had significantly also depressed the N-demethylation of erythromycin in human microsomes (mediated by CYP3A4) and in rat microsomes (mediated by CYP3A4) (Mclellan et al., 1996).

NFX had in vivo affecting pharmacokinetics of some drugs like caffeine, theophylline and cyclosporine. The observed interactions were explained by inhibitory effect on CYP450 enzymes (Ho, Tierney and Dales,1988; McLellan et al.,1995; Carbo et al., 1989).

1.4 Justification

The effect of NFX a moderate CYP3A4 inhibitor as a candidate to influence the pharmacokinetic parameters (PK) of the drugs e.g CBZ at steady state remain uninvestigated. In this research we aim to examine such possible interactions

CYP3A4 isoenzyme plays an important role in metabolism of drugs. Inhibition of CYP3A4 can result in the accumulation of parent drug that can put the patient at increased risk for side effects or possible toxicity. The risk is higher when the drug has low therapeutic index.

1.5 Problem statement

CBZ as anti-epileptic drug is used lifelong. It is a substrate of CYP3A4. Co-administration of CBZ with other drugs, which inhibit the enzyme activity, can result in serious interactions. NFX is an antibiotic, which exhibited a moderate CYP3A4 inhibitory effect.

1.6 Aim

The study aims to investigate the effect of NFX on CBZ pharmacokinetics at steady state.

1.7 Objectives

- To determine pharmacokinetic parameters (C_{max}, t_{max} AUC_{0-t}, $AUC_{0-\infty}$, $t_{½}$, K_e) of CBZ in the first and seconds period.
- Statistical analysis of the pharmacokinetic parameters obtained experimentally in both periods.
- Conduction of drug-drug interaction between Norfloxacin and Carbamazepine

Chapter 2

Literature review

2.1 Cytochrome P450 (CYP450)

CYP450 is a family of isozymes responsible for biotransformation of numerous endogenous compounds such as steroids, bile acid, prostaglandins, fatty acids and leukotrienes and metabolises many of drugs, pollutant and environmental chemicals (Cupp and Tracy, 1998; DiPiro, 1999; Estabrook, 2003).

CYP enzymes are membrane-bound, localised to the endoplasmic reticulum, which are found not only in human beings but also in bacteria, fungi, plants and animals(Meyer, 1996; Nelson, 1999).

The enzymes are heme-containing membrane proteins, which are located in the smooth endoplasmic reticulum of several tissues. Although a majority of the isozymes are located in the liver which is the primary organ for the biotransformation of drugs, extrahepatic metabolism also occurs in the kidneys, skin, gastrointestinal tract and lungs because there are significant quantities of CYP enzymes in these organs (Krishna and Klotz, 1994; Danielson, 2002).

2.1.1 History of CYP450

The capacity of mammalian tissues to oxidize nonpolar xenobiotics was widely recognized as early as the 1950s, but the specific enzymes responsible for the catalysis of such reactions were unknown (Garfinkle, 1958).

The first clues came from spectroscopic studies of microsomal heme proteins. Many ferrous heme proteins form characteristic carbon monoxide complexes when pretreated with a suitable reducing agent. In studies of rat and pig microsomal protein fractions had noted the existence of a membrane-bound reduced pigment exhibiting a Soret peak of around 450nm for the carbon monoxide-bound species (Garfinkle, 1958; Klingenberg, 1958).

This pigment was later identified as a P450 hemoprotein. The unique spectral absorbance peak, from which the CYP450 family takes its name, is thought to result from the trans orientation between the cysteinate and carbon monoxide ligands of the enzyme's associated heme group (Omura and Sato, 1964; Dawson and Sono, 1987).

This peak is a unique feature seen in only four classes of hemoproteins namely, P450s, nitric oxide synthases(Stuehr and Saito, 1992), chloroperoxidases (Griffin, 1991) and protein H450 (Kim and Deal, 1976).

Later, Jim Gillette and Julius Axelrod paved the way for a better understanding of the drug (acetanilide) metabolism, which also gave important momentum to the discovery of the

P450s. They worked on the demethylation of ephedrine and showed that NADPH and oxygen are necessary for the reaction in rabbit liver microsomes (Axelrod, 1955).

The research in the area of the steroid hormone metabolism also contributed to the discovery of P450s, responsible for the C21 hydroxylation of progesterone in microsomes from the bovine adrenal cortex. Ryan and Engel showed in their studies the reversable inhibition of carbon monoxide by light for the first time (Ryan and Engel, 1957).

This observation led later to the discovery of P450 function as an oxidase using molecular oxygen and NADPH for their reactions (Estabrook, Cooper and Rosenthal, 1963; Cooper et al., 1965).

By contrast in eukaryotes, P450s are involved in the biosynthesis of membrane sterols. In animals, they are part of the biosynthesis of signal molecules and steroid hormones as well as vitamin D3 (Werck-Reichhart and Feyereisen, 2000).

2.1.2 Structure of CYP450

The cytochromes P450 constitute a large family of cysteinato-heme enzymes (see figure 2.1) which present in all forms of life (plants, bacteria, and mammals), and play a key role in the oxidative transformation of endogeneous and exogeneous molecules. In all these cysteinato-heme enzymes, the prosthetic group is constituted of an iron(III) protoporphyrin- IX (figure 2.1) covalently linked to the protein by the sulfur atom of a proximal cysteine ligand (Meunier and Bernadou, 2000; Ortiz de Montellano and De Voss, 2002).

The core structure is constant in the proteins that are responsible for the mechanism of electron/proton exchange leading to oxygen activation. This constant core consists of four-helix bundle (D, E, I, and L), three parallel helices labeled D, L, and I and one antiparallel helix E (Lewis and Hlavica, 2000).

A heme-binding loop, a characteristic domain with a conserved cysteine amino acid, serves as a ligand to the heme iron. Another well-conserved domain is found in the helix K on the proximal side of the heme iron, which is necessary to stabilize the core structure (Sligar, Makris and Denisov, 2005; Lewis and Hlavica, 2000).

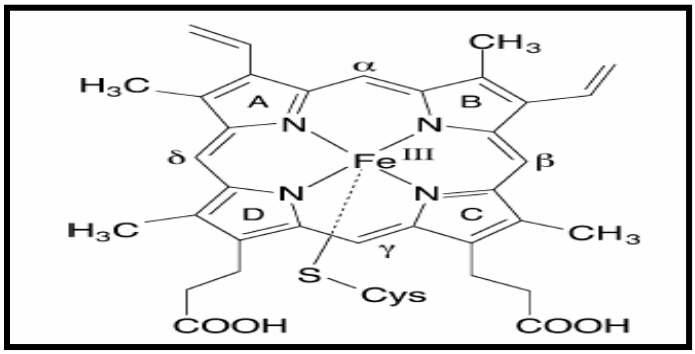

Figure 2.1 :Prosthetic of cysteinato-heme enzymes: an iron- (III) protoporphyrin-IX linked with a proximal cysteine ligand (Lewis and Hlavica, 2000)

Moreover, the central part of the helix I has a conserved amino acid sequence, which is responsible for the proton transfer on the distal end of the heme. Variable flexible regions are involved in many functions, such as substrate binding, amino terminal anchoring, and targeting membrane-bound proteins (Nelson et al., 1996; Lewis and Hlavica, 2000 ; Engman et al., 2001)

2.1.3 Catalytic cycle of the CYP 450

The primary steps in the catalytic cycle for the reactions catalyzed by the CYPs are :

- Substrate binding;
- One-electron reduction of the ferric (Fe+3) enzyme to the ferrous (Fe+2) enzyme;
- Binding of molecular oxygen to the ferrous (Fe+2) iron;
- Transfer of the second electron to the ferrous-oxy-substrate complex leading to the release of water and the formation of an activated oxygen intermediate;
- Catalytic insertion of the activated oxygen into the substrate to form the oxygenated product;
- Release of the oxygenated product resulting in the release of the native ferric (Fe+3) form of the enzyme that can then undergo another catalytic cycle (White and Coon, 1980).

Three of these steps: substrate binding; the binding of molecular oxygen to the ferrous (Fe+2) enzyme and the catalytic step in which the activated oxygen is transferred from the heme iron to the substrate, appear to be particularly susceptible to inhibition (Ortiz De Montellano and Correia,1995; Ortiz De Montellano, 2013).

The catalytic cycle of CYP450 is illustrated in figure (2.2).

Hydrophilic or polar metabolized products are formed at the end of the reaction, catalyzed by P450. The products can be excreted easily in this form. Microsomal CYP requires

an additional enzyme called flavoprotein cytochrome P450 reductase to transfer electrons from NADPH to CYP:

- Adrenodoxin reductase, a flavoprotein which involve in the electron transfer mechanism from NADPH to the iron_sulfur protein.
- Adrenodoxin, which reduces CYP (Nelson et al.,1996; Lewis and Hlavica, 2000; Engman et al., 2001)

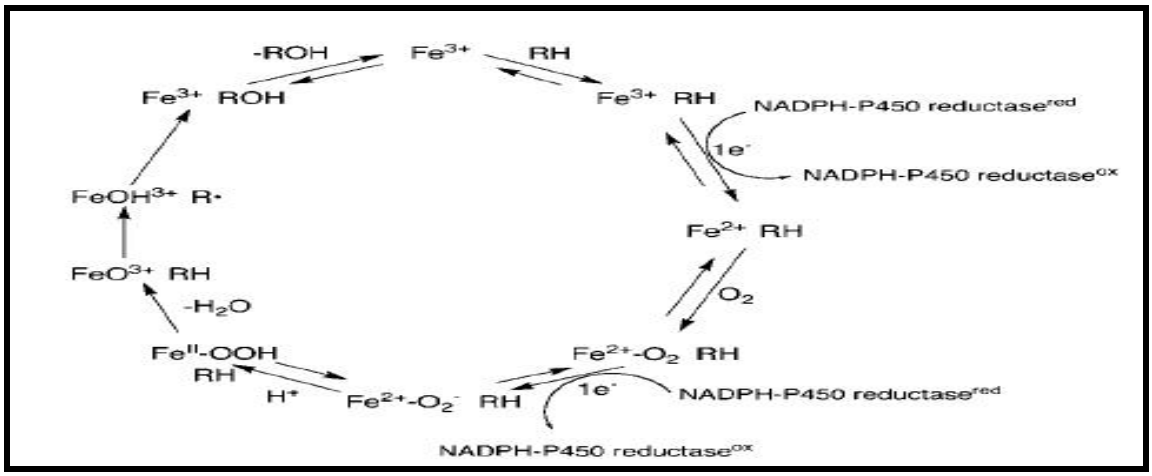

Figure2.2 : Catalytic cycle of CYP enzyme (Guengerich,2008)

The overall reaction catalyzed by CYPs is :

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + NADP^+ + H_2O$$

Where R represents a substrate that serves as a site of oxygenation (Nelson et al.,1996; Lewis and Hlavica, 2000).

2.1.4 Distribution of CYP enzymes in the human body

CYP enzymes are present in most organisms including mammals, birds, fish, reptiles, amphibians, insects, plants, fungi, and bacteria. The finding that CYP enzymes are found in every class of biota suggests that the diverse CYP enzymes identified to date have evolved from a single ancestral gene over a period of 1.36 billion years (Nelson et al., 1996; Guengerich, Wu and Bartleson, 2005; Bandiera, 2001).

In humans and other mammals, CYP enzymes are found in almost all tissues including liver, intestine, lung, kidney, stomach, brain, adrenal gland, gonads, heart, nasal and tracheal mucosa, and skin (Ding and Kaminsky, 2003; Paine et al., 2006).

Concentrations and expression of individual CYP enzymes differ between tissues. Liver contains the largest number and the highest levels of CYP enzymes involved in drug biotransformation (Parkinson, 2001).

CYP enzymes comprise from 4% to 6% of total microsomal protein. In human liver, the total CYP content is lower, with CYP enzymes typically comprising 1.5 – 3% of total microsomal protein, giving a specific content of 0.3 – 0.6 nmol of total CYP per milligram of microsomal protein , or approximately 5 nmol of total CYP per gram human liver. For tissues other than liver, the CYP content, expressed in terms of microsomal protein or tissue weight, is lower (Table 2.1) (Gad, 2008).

The most common P450 cytochrome subfamily expressed in the mucosa of the small intestine is CYP3A (Table 2.2), which represents an average content of approximately 80% of spectrally determined P450 content, followed by CYP2C9 (15%) . CYP1A cytochrome is expressed in the duodenum, together with less abundant levels of CYP2C8-10 and CYP2D6 (Omiecinski, Remmel and Hosagrahara, 1999; Gonzalez, 1997).

Table 2.1: Total CYP content in various human organs (Gad, 2008)

Organ	**Human (nmol/mg Microsomal protein)**
liver	**0.3-0.60**
Adrenal	**0.23-0.54**
Small intestine	**0.03-0.21**
kidney	**0.03**
lung	**0.01**
Brain	**0.10**
Testis	**0.005**
Skin	**Not determined**
Mammary	**<0.001**

Table 2.2: Cyp enzymes detected in various human organs (Gad, 2008)

Organ	CYP Genes Expressed (mRNA or Protein)
Liver	**CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP4A11**
Small intestine	**CYP1A1, CYP2C9, CYP2C19, CYP2D6, CYP2J2, CYP3A4 , CYP3A5**
Nasal macosa	**CYP2A6, CYP2A13, CYP2B6, CYP2C, CYP2J2, CYP3A**
Trachea	**CYP2A6, CYP2A13, CYP2B6, CYP2C, CYP2J2, CYP2S1, CYP3A,CYP4X1**
Lung	**CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C18, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP3A4, CYP3A5, CYP4B1**
Stomach	**CYP1A1, CYP1A2, CYP2C, CYP2J2, CYP2S1, CYP3A4**
Colon	**CYP1A1, CYP1A2, CYP1B1, CYP2J2, CYP2S1, CYP3A4, CYP3A5, CYP4F12**
Kidney	**CYP1B1, CYP2A6, CYP2B6, CYP2E1, CYP2R1, CYP2S1, CYP3A5, CYP4A11, CYP4F2, CYP4F12**
Skin	**CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2S1, CYP3A4, CYP3A5, CYP4A11**
Brain	**CYP1A1, CYP2B6, CYP2D6, CYP2E1, CYP2U1**
Mammary	**CYP1A1, CYP1B1, CYP2C, CYP2D6, CYP3A4, CYP3A5, CYP4Z1**
Placenta	**CYP1A1, CYP2E1, CYP2F1, CYP2S1, CYP3A4, CYP3A5, CYP4B1**

2.1.5 Classification of CYP450

The CYP enzymes are divided into two major classes, those with a specific role e.g, in steroid biosynthesis, bile and arachidonic acid metabolism (CYP4, CYP5, and higher) and those primarily involved in the metabolism of xenobiotics such as drugs, anti-oxidants, and chemicals (CYP1, CYP2, CYP3) (Nelson, 1999).

More than 2700 individual members of the CYP450 superfamily have been identified, and 57 cytochrome P enzymes are recognized in man (Lewis, 2004).

All eukaryotic CYPs except fungal CYP55s are membrane bound; 18 mammalian CYP enzyme structures are known and 15 of these are of human origin; [1A2, 2A6, 2A13, 2B4 rabbit, 2B6, 2C5 rabbit, 2C8, 2C9, 2D6, 2E1, 2R1, 3A4, 7A1, 8A1, 19A1, 24A1 rat, 46A1, 51A1 (Nelson and Nebert, 2011).

The most prominent CYP isoenzymes with regard to the number of substrate drugs are CYP3A4 and CYP2D6, with a smaller number of drugs metabolised by CYP2C9, CYP2C19, CYP1A2, and CYP2E1 (Wrighton and Stevens, 1992; Meyer, 1996).

The gene families CYP1, CYP2, and CYP3 are involved largely in biotransformation of drugs, whereas the remaining 15 families in humans perform endogenous metabolic activities (Table 2.3).

The metabolism of more than 90% of the most clinically important medications can be accounted for by seven cytochrome P (CYP) isozymes (3A4, 3A5, 1A2, 2C9, 2C19, 2D6, and 2E1)(Shapiro and Shear, 2002; Danielson, 2002; Lewis, 2004).

Table 2.3: Cytochrome P450 subfamilies and functions in humans (Danielson, 2002)

Cytochrome P family	Subfamilies	Function
1	A1, A2, B1	Drug metabolism
2	A6, A13, B6, C8, C9, C18, C19, D6, E1, F1, J2	Drug and steroid metabolism
3	A4, A5, A7, A43	Drug metabolism
4	A11, B1, F2, F3, F8, F12	Arachidonic acid and fatty acid metabolism
5	A1	Thromboxane synthase
7	A1, B1	Steroid 7-α-hydroxylase
8	A1, B1	Bile acid biosynthesis and prostacyclin synthase
11	A1, B1, B2	Steroid biosynthesis
17	A1	Steroid biosynthesis (steroid 17-α-hydroxylase)
19	A1	Steroid biosynthesis (aromatase)
20	A1	Unknown
21	A1	Steroid biosynthesis
24	A1	Vitamin D deactivation
26	A1	Retinoic acid hydroxylase
27	A1	Bile acid biosynthesis and vitamin D3 activation
39	A1	Unknown
46	A1	Cholesterol 24-hydroxylase
51	A1	Lanosterol 14-α demethylase

Enzymes of the superfamily cytochrome P450 received their name from Omura and Sato, who identified them as heme proteins in 1962 (Omura and Sato, 1962).

Nomenclature of CYP enzyme system has been established by CYP nomenclature committee. CYP superfamily is subdivided and classified on the basis of amino acid identity, phylogenetic criteria, and gene organization **(** Nelson et al., 1996). The name of cytochrome

P450 is derived from the fact that these proteins have a heme group and an unusual spectrum. The P stands for "pigment" and the 450 for the characteristic absorption maximum at 450 nm (Soret peak) of the carbon monoxide bound form. Naming a cytochrome P450 gene include root symbol "CYP" for humans "Cyp" for mouse and Drosophila (Danielson, 2002).

The symbol CYP is followed by a number for families(e.g. CYP1, CYP2) (in general, groups of proteins with more than 40% amino acid sequence identity), a capital letter for subfamilies A, B, C(more than 55% identity) (Levy, 1995) and arabic number for the individual enzyme or gene , isoenzyme or isoform (e.g. CYP3A4, CYP3A5, CYP1A2) (Nelson et al., 1996). Of the 74 gene families so far described, 14 exist in all mammals. These 14 families comprise of 26 mammalian subfamilies **(**Nelson et al., 1996).

As the human genome project completed, there are 57 genes and more than 59 pseudogenes divided among 18 families of CYP genes and 43 subfamilies have been detected (Wang and Chou, 2010).

2.1.6 Factors affecting expression and function of CYP450

The CYP isozymes are under genetic control and can be expressed to a varying degree in each individual(Evans and McLeod, 2003; Wilkinson, 2004).

Multiple factors, such as smoking, ethanol consumption, environmental factors, disease states, and genetic inheritance, influence the amount and the activity of an individual patient's CYP isozymes, which are summarized in table 2.4 (Shapiro and Shear, 2002; Lewis, 2003).

Table 2.4: CYP450 isozymes and factors that influence their activity (Lewis, 2003).

Cytochrome P isoenzyme	Percent of total CYP	Variability	Percent of drugs metabolized	Activity influenced by
1A1,2	**~13**	**~ 40 fold**	**13**	**Genetic polymorphism; nutrition; smoking; drugs; environmental xenobiotics**
1B1	**<1**		**1**	**Environmental xenobiotics**
2A6	**~ 4**	**~100 fold**	**3**	**Genetic polymorphism; drugs; environmental xenobiotics**
2B6	**<1**	**~50 fold**	**4**	**Drugs**
2C9,19	**~18**	**~100 fold**	**35**	**Genetic polymorphism; drugs**
2D6	**Up to 2.5**	**>1000 fold**	**15**	**Genetic polymorphism; drugs**
2E1	**Up to 7**	**~20 fold**	**3**	**Genetic polymorphism; nutrition; alcohol; environmental xenobiotics**
3A4,5	**Up to 28**	**~20 fold**	**36**	**Nutrition; drugs; environmental xenobiotics**

The following factors may affect expression of CYP proteins and lead to significant alterations in drug metabolism.

2.1.6.1 Age

Activity of CYP enzymes decreases with advancing age in both the sexes. Metabolism of antipyrine (metabolised by at least 10 CYP isoenzymes, CYP1A2, 2A6, 3A4, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6 and 2E1), lidocaine, diazepam and theophylline decreases in the elderly (Sotaniemi et al.,1997)

In vivo activities of CYP1A2, 3A4, 2C9 and 2D6 have been reported to be low at birth, but maximally increased at the young adult stage and decreased in old age (Sotaniemi et al., 1997).

Hepatic metabolism and its relationship with ageing is difficult to understand due to a host of factors that contribute to the enormous variability in hepatic drug biotransformation. Indeed, a high intersubject variability in CYP activity is also observed in a normal, healthy, well-defined population of subjects of similar age. Considerable variability is expected as a result of the following factors: concurrent drug use (including alcohol, drugs of abuse, and caffeine), diseases, environmental exposure (including smoking), gender, genetic differences, liver mass and nutritional intake (Palasz et al., 2012).

As a result, it is not surprising that it is extremely difficult to clearly identify age as a separate variable among all these other factors. For example, smoking influences the rate of metabolism of certain substances and could be a confounding factor in studies of drug metabolism (Anderson, McKnee and Holford, 1997)

Drug metabolism is one of the major determinants of drug clearance in a given individual. Although the relative size of the liver and of hepatic blood flow may affect the rate of drug metabolism during development, the maturation of the drug metabolizing enzymes is probably the predominant factor accounting for age-associated changes in nonrenal drug clearance (Anderson, McKnee and Holford, 1997).

A study conducted in 54 liver samples from healthy donors from 9 to 89 years did not show changes in either microsomal protein content, total P450 nor NADPH cytochrome P450 reductase activity with age (Schmucker et al., 1990).

By contrast, another study carried out in 226 subjects with histopathologic changes of the liver revealed a significant decrease of 32% in total cytochrome P450 content of liver biopsy samples and a decrease of 29% of the in vivo antipyrine clearance in subjects >70 years as compared to young adults (Sotaniemi et al., 1997). Antipyrine has been frequently used in the past to assess in vivo phase I drug metabolism activity in healthy subjects and patients. However,

antipyrine is not a good probe to identify the loss in the activity of one particular enzyme because it is metabolized by multiple CYPs (CYP3A4, 1A2, 2B6, 2C18 and others) (Sotaniemi et al., 1997).

Regarding the hepatic content of individual CYP450 enzymes in humans as a function of age, results are also quite controversial. Shimada et al. (1994) did not observe any change in the content of specific CYP450 isoenzymes as a function of age in livers from 60 subjects between 12 and 73 years (Shimada et al., 1994).

By contrast, another study carried out in 71 individuals showed that, while the content of some isoforms such as CYP1A2 and CYP2C remains constant, total cytochrome P450, CYP2E1 and CYP3A contents as well as the NADPH reductase activity decrease with increasing age (George, Byth and Farrell, 1995).

The activity of CYP complex in rat small intestine was not decreased by the aging process, so the high rate of oxidative metabolic reactions in intestinal mucosa can be maintained till the advanced life stage (Palasz et al., 2012).

2.1.6.2 Hepatic disease

In cirrhotic patients expression of 1A2, 2E1 and 3A isoenzymes was decreased. There are reports of alteration of clearance of drugs metabolised by 3A4 in patients of cirrhosis. Activity of 2C19 was reported to be decreased in patients of liver disease, but activity of 2D6 was unaltered. Levels of 2C subfamily have been reported to be upregulated in patients of hepatic carcinoma. Detailed knowledge of these isoenzymes affected in disease states would be used to enhance the design of rational drug therapy (Murray,1992; Adedoyin et al.,1998).

2.1.6.3 Gender

Gender based differences in metabolic activity of hepatic CYP isoenzymes have been identified in humans. Women exhibit higher baseline 3A4 activity than men and therefore a greater extent of interactions on average. Clearance of diazepam and prednisolone is more in women, but clearance of some drugs like propranolol are more in men**(** Kashuba et al., 1998).

Many drugs metabolized by CYP3A4 show higher clearance in women compared with men, and this difference even persists after correcting for physiologic factors such as body weight (Harris**,** Benet and Schwartz, 1995; Meibohm, Beierle and Derendorf, 2002).

CYP3A4 drug substrates with higher clearance in women include, for example, cyclosporine (Kahan et al., 1986) erythromycin (Watkins et al.,1989) tirilazad (Hulst et al., 1994) and verapamil (Dilger et al., 1999).

2.1.6.4 Hormones

Testosterone deficiency decreases CYP activity which decreased activity of CYP enzymes in the elderly (Liddle, 2000).

Estrogen has been found to decrease oxidation of some drugs e.g. imipramine. Oral contraceptives (estrogen/progesterone combination preparations) were reported to decrease clearance of diazepam and chlordiazepoxide (Kirkwod et al., 1991; Liddle, 2000).

Menstrual cycle phases have variable effect on CYP activity. Theophylline clearance was found to be decreased in luteal phase. Increased metabolism of antipyrine was reported near the time of ovulation (Kirkwod et al., 1991; Tanaka, 1983). Growth hormone deficiency may lead to down regulation of CYP enzymes (Liddle, 2000). Pregnancy may induce CYP enzymes. Increased metabolism of metoprolol was found in pregnant women due to induction of 2D6 isoenzyme (Wadelium et al., 1997).

The growth hormone induces CYP1A2 and, to a lesser extent, inhibits CYP2C19 in elderly men, but it exerts no effects on CYP2D6 and CYP3A4. Although the induction of CYP1A2 may be of some clinical relevance, the small inhibition of CYP2C19 is probably unimportant (Jürgens et al., 2002).

2.1.6.5 Nutrition

Starvation and obesity are known to induce CYP enzymes in rodents, but in humans these conditions inhibit CYP enzymes. Obesity has been reported to increase metabolism of enflurane and sevoflurane in humans **(**O'Shea et al., 1994).

The addition of food supplements containing cruciferous vegetables, such as cabbage and Brussel sprouts to rats, could increase the activity of both CYP1A1 and CYP1A2 by a factor of 70. A further example relates to induction of CYP2B1 by diallyl sulphide in garlic (Brady et al., 1991; Roby et al., 2000).

In recent years, a number of alternative medicines including herbal remedies such as St John's Wort, which is used for mild depression, have gained increasing popularity. St John's Wort is known to interfere with the metabolism of a large number of medicines due to its ability to induce CYP3A4 (Roby et al., 2000).

Another important cause of morbidity due to enzyme inhibition is citrus fruit. The most important of these is grapefruit juice, which contains a number of potent CYP enzyme inhibitors. These include the plant alkaloids naringin, naringenin and bergamottin. In particular, CYP3A4 is inhibited, leading to altered drug disposition of a number of substances including the antihistamine terfenadine, which can result in fatal cardiac arrhythmia (Ameer and Weinttraub, 1997).

2.1.6.6 Inflammation

Acute phase response inflammatory mediators have been reported to suppress CYP activity in humans which lead to abnormally high plasma levels and toxicity of drugs that are metabolised by CYP dependent enzymes and have low therapeutic ratio. An example is oral anticoagulants in herpes zoster, theophylline in acute respiratory viral infection, nifedipine in acute febrile infection and quinine in malaria. Tumour necrosis factor and interleukin- 1, two major inflammatory cytokines probably play a role in inhibition CYP enzymes in rats and mice **(**Chen et al., 1994).

2.1.6.7 Environmental factors

Cigarette smoking is known to induce CYP enzymes. Smoking has been found to increase clearance of phenacetin and theophylline **(**Sarkar and Jakson, 1994).

Charbroiled food can induce CYP enzymes (Smith et al., 1996). Also Alcohol and cigarette smoke Liver enzyme induction in cigarette smokers is complex, due to the multiplicity of substances which can be detected in cigarettes. The polycyclic aromatic hydrocarbons typically induce CYP1A1 and CYP1A2 (Conney, 1982).

CYP1A1 induction has little pharmacokinetic relevance but it is thought that there may be an association with an increased risk of lung cancer (Kawajiri et al., 1990).

Cigarette smoke also contains a number of small molecules, including various alcohols, styrene, acetone and vinyl chloride, which are also inhaled. These are substrates for CYP2E1 and this enzyme is also induced. Nicotine may also play a part in liver enzyme induction as shown in animal studies it induces CYP1A, CYP2B6 and CYP2E1 (Zevin and Benowitz , 1999) .

There is an interesting synergy between alcohol ingestion and cigarette smoking. Although alcohol is primarily metabolised by alcohol dehydrogenase, CYP2E1 accounts for around 20% of its breakdown (Lieber, 1997).

2.1.6.8 Genetic polymorphism

Polymorphisms are generated by non random genetic mutations that occur in at least 1% of a population and give rise to distinct subgroups within that population that differ in their ability to metabolize xenobiotics. Clinically significant polymorphisms have been documented for CYP2D6, CYP2C9, and CYP2C19**.**The 2 isozymes most affected by genetic control are CYP2C19 and CYP2D6 (Belpaire and Bogaert, 1996).

The enzyme activity of CYP3A4 shows wide interindividual variability (up to 60-fold), resulting in therapeutic failure, unpredictable adverse side effects or severe drug toxicity (Nicolas, Espie and Molimard, 2009).

The enormous variation observed in drug metabolism is mainly due to the combined effect of genetic polymorphisms, regulation of gene expression and interaction with drugs or environmental chemicals (Wilkinson, 2004).

Genetic polymorphisms, accounting for as much as 90% of interindividual variability in CYP3A4 activity, are therefore of clinical value in predicting an individual's ability to respond to certain therapeutic agents (Ozdemir et al., 2000). Moreover CYP3A4 polymorphisms can also assist prediction of disease predisposition (Perera, 2010).

Extensive or rapid metabolizers (generally the largest proportion of a population) have heterozygous or homozygous dominant alleles. The large variability (40-fold or greater) in enzyme activity was documented in extensive metabolizers, drug interactions may not manifest in all subjects with this phenotype (Rendic and Di Carlo, 1997; Kroemer and Eichelbaum, 1995).

Poor metabolizers possess variant homozygous autosomal recessive alleles. Poor-metabolizer phenotypes can be at high risk for toxicity from drugs that require CYP inactivation and at high risk for therapeutic inefficacy from prodrugs that need CYP activation. They are at low risk for drug interactions that involve enzyme inhibition or induction because their activity is preemptively compromised and cannot be induced (Rendic and Di Carlo, 1997; Kroemer and Eichelbaum, 1995).

Ultraextensive metabolizers exhibit gene amplification of autosomal dominant alleles (Kroemer and Eichelbaum, 1995).

There is a group of ultra-rapid metabolizers associated with CYP2D6, which results from gene amplification (Chang and Kam, 1999) .

2.2 Inhibition of CYP450

Lipophilic and large molecular size drugs are more likely to cause inhibition **(**Thummel and Wilkinson, 1998).Two characteristics make a drug susceptible to inhibitory interactions: one metabolite must account for >30- 40% metabolism of a drug and that metabolic pathway is catalysed by a single isoenzyme (Levy, 1995).

The extent to which an inhibitor affects the metabolism of a drug depends upon factors such as the dose and the ability of the inhibitor to bind to the enzyme. For instance, sertraline is considered a mild inhibitor of CYP2D6 at a dose of 50 mg, but if the dose is increased to 200 mg, it becomes a potent inhibitor (Sproule et al., 1997).

Drugs may be intentionally combined to take advantage of CYP450 inhibition. Ritonavir, a protease inhibitor and potent CYP3A4 inhibitor, is added to lopinavir to boost serum levels in patients with human immunodeficiency virus (Cozza, Armstrong and Oesterheld, 2003).

Additionally, a drug can be both metabolized by and inhibit the same enzyme (e.g., erythromycin), or it can be metabolized by one enzyme and inhibit another enzyme (e.g., terbinafine) (Ray et al., 2004).

Inhibitors of the CYPs can be divided into three general categories that differ in their mechanisms which are compounds that bind reversibly, compounds that form quasi-irreversible complexes with the iron of the heme prosthetic group and compounds that bind irreversibly to the prosthetic heme or to the protein, or that cause covalent binding of the heme prosthetic group or its degradation product to the apoprotein. These inhibitors interfere with the catalytic cycle prior to the formation of the activated oxygen intermediate are reversible inhibitors that act as competitive, noncompetitive, uncompetitive, product, or transition-state inhibitors. Those compounds that act during or subsequent to the formation of the activated oxygen intermediate are generally either quasi-irreversible or irreversible inhibitors and, in many cases, have been shown to be mechanism-based inactivators (Ortiz de Montellano and Correia, 1995).
The mechanisms of inhibition should be discussed in the following section.

2.2.1 Irreversible inhibition

Irreversible inhibition is called quasi-irreversible inhibition. The inhibitor in this process undergoes metabolic activation by the CYP enzymes to form inhibitory intermediate metabolites which form stable inactive complexes with the prosthetic heme of CYP (Lin and Lu, 1998).

These complexes are stable in vivo because the CYP enzymes involved are unavailable for drug metabolism, and synthesis of new enzymes is required to overcome the inhibition (Lin and Lu 1998). The inhibitors are suicide substrates called mechanism-based inactivators because metabolic activation is needed (Lin and Lu,1998).

When irreversible inhibition occurs, a metabolic intermediate is formed by the permanent binding of the inhibiting drug with the P450 enzyme at the heme, the protein, or both. Irreversible inhibitors are of particular importance because they can decrease the first pass clearance and the functional catalytic activity of drugs that normally are cleared by CYP3A4 until new enzyme can be manufactured (Zhou et al., 2005).

The metabolic activity of the inactive CYP can be reversed in vitro during incubation with highly lipophilic compounds that displace the metabolic intermediate from the active site, or by irradiation, or by oxidation to the ferric state by the addition of potassium ferricyanide. (Lin and Lu, 1998) .

Certain drugs with functional groups are oxidized by the CYP enzymes to form reactive intermediate metabolites, that covalently bind and irreversibly inactivate CYP as the metabolic intermediates of the macrolide antibiotics erythromycin and clarithromycin. They form

complexes with the iron of the heme of CYP3A4 and thus inactivate it so, erythromycin and clarithromycin are potent inhibitors of CYP3A4 (Ohmori et al., 1993).

2.2.2 Reversible inhibition

Generally, reversible inhibition is thought to be the most common cause of drug–drug interactions. Reversible inhibition of CYPs is transient and the normal metabolic functions of the enzymes will continue following elimination of the inhibitor from the body and can be further classified depending on kinetic as competitive, noncompetitive, and uncompetitive and may involve product inhibition, or inhibition by transition-state analogs or slow, tight binding inhibitors (Guengerich, 1999; Lin et al., 2000 ; Ito, Kusuhara and Sugiyama, 1999).

2.2.2.1 Competitive inhibition

In competitive inhibition, the inhibitor competes with the substrate for the same binding site within a CYP enzyme. It may involve simple competition for binding to a lipophilic domain in the active site or it may involve hydrogen bonding or ionic bonds with specific amino acid residues in the active site (Ito, Kusuhara and Sugiyama, 1999; Ortiz de Montellano and Correia, 1995).

It is believed to be the result of the inhibitor sharing some degree of structural similarity with the substrate(s) of that CYP. For example, when single oral doses of metoprolol (50 mg), a beta-adrenoceptor blocking agent and propafenone (150 mg) were administered in combination to healthy subjects, an approximately two-fold reduction in the oral clearance of metoprolol was observed when propafenone was included (Wagner et al., 1987). Similar drug-drug interactions are seen in the combined administration of thioridazine and propranolol (Markowitz, Wells and Carson, 1995).

2.2.2.2 Noncompetitive

In noncompetitive inhibition, the inhibitor binds to the same enzyme as does the substrate, but the binding site differs (Ito, Kusuhara and Sugiyama, 1999).It was reported that the hydrazino group isoniazid, an antituberculous drug,form an interaction with the haem portion of P450,So it inhibits the metabolism of phenytoin (Walubo and Aboo, 1995) .

The duration of this type of inhibition may be longer if new enzymes are synthesized after the inhibitor is discontinued (Ito, Kusuhara and Sugiyama, 1999).

Cimetidine is bound to P450 and produces a stable cytochrome substrate complex which prevents access of other drugs to the P450 system. It binds to the haem portion of P450 and is, thus, an inhibitor of phase I drug metabolism reactions i.e. hydroxylation, dealkylation(Somogyi and Gugler,1982; Maurice, Pichard and Daujat, 1992).

2.2.2.3 Uncompetitive

In uncompetitive inhibition, the inhibitor binds only to an enzyme that forms a complex with the substrate. it is relatively rare form of inhibition of human CYP450 by therapeutic drugs although NSAID meloxicam capable to uncompetitively inhibition of quinidine in vitro, it is not likely to be a significant clinical interaction. Omeprazole and lansoprazole are uncompetitive inhibitiors for CYP3A4 in vitro(Ito, Kusuhara and Sugiyama, 1999).

2.3 Enzyme induction

Drug interactions involving enzyme induction are not as common as inhibition based drug interactions but equally profound and clinically important. Exposure to environmental pollutants as well as large number of lipophilic drugs can result in induction of CYP enzymes. The most common mechanism is transcriptional activation leading to increased synthesis of more CYP enzyme proteins **(**Dossing et al., 1983).

Rifampicin is possibly associated with plural molecular species of P450 (several isozymes), but mainly, a large increase in the CYP3A content often becomes a problem, while phenobarbital, carbamazepine and phenytoin, antiepileptic drugs, also induce CYP3A (Levy, 1995).

Rifampicin has short half life and induces CYP3A4 and CYP2C. The inducing effect is shown within 24 hours. Phenobarbital, which has a half-life of 3–5 days, requires one week for induction effect (CYP3A4, CYP1A2, CYP2C) to become clear (Levy, 1995).

Induction effect observed with smoking and long term alcohol or drug consumption and can reduce the duration of action of a drug by increasing its metabolic elimination. If a drug induces its own metabolism, it is called autoinduction as is the case with carbamazepine (Levy, 1995).If induction is by other compounds it is called foreign induction. Metabolism of the affected drug is increased leading to decreased intensity and duration of drug effects. If the drug is a prodrug or it is metabolised to an active or toxic metabolite then the effect or toxicity is increased **(**Dossing et al., 1983).

The induction of CYP enzymes can be caused by at least 5 different mechanisms. They are ethanol selectively induces CYP2E1 primarily by stabilizing the enzyme protein, intracellular receptors, namely the aryl hydrocarbon receptor (Ah), constitutive androstane receptor (CAR), pregnane x receptor (PXR), and peroxisome proliferator-activated receptor (PPAR) (Fuhr, 2000).

The Ah receptor is a transcription factor that belongs to the basic-helix-loop-helix PAS family, whereas CAR, PXR, and PPAR are orphan nuclear receptors (Waxman, 1999).

Polycyclic aromatic hydrocarbons, found for instance in tobacco smoke and charcoal grilled meat, bind to the Ah receptor. The inducer-Ah receptor complex, together with the Ah receptor nuclear translocator (Arnt), binds to a deoxyribonucleic acid (DNA) response element and increases protein synthesis (Fuhr, 2000).

The most important enzyme induced through this mechanism is CYP1A2. Other enzymes induced by this mechanism include CYP1A1 and some phase II enzymes such as glutathione S-transferases (GSTs) and UDP glucuronosyltransferases (UGTs) (Fuhr, 2000).

A clear dose dependency is evident between smoking and caffeine clearance (an indicator of CYP1A2 activity) with a 1.22-fold increase in caffeine clearance in subjects who smoke 1 to 5 cigarettes per day and a 1.72-fold increase in subjects who smoke more than 20 per day. Smoking also raises the systemic elimination of other CYP1A2 substrates, such as theophylline, tacrine, and clozapine (Fuhr, 2000; Tantcheva-Poór et al., 1999).

Clinically significant induction results from a more than 50-fold increase in the number of enzyme molecules. This generally occurs through an increase in CYP450 synthesis by either receptor- mediated transcriptional activation or mRNA stabilization (Waxman, 1999).

The mechanism of the induction of protein synthesis by the nuclear receptors CAR, PXR, and PPAR is essentially similar. An inducer binds to CAR, PXR, or PPAR, and the inducer-receptor complex forms a heterodimer with the retinoid X receptor (RXR). This heterodimer binds to a DNA response element and enhances DNA transcription and eventually protein synthesis (Waxman, 1999).

Induction of the CYP1 family by cigarette smoke, charcoal-broiled foods, indoles (found in broccoli, cauliflower, cabbage, brussels sprouts, kale, watercress), and omeprazole occurs primarily by substrate binding to the Ah-receptor (dioxin receptor). This complex subsequently binds with a receptor nuclear translocator, enters the hepatocyte nucleus, and binds with regulatory deoxyribonucleic acid (DNA) sequences to enhance gene transcription and stabilize mRNA (Dogra, Whitelaw and May, 1998).

The CYP2 family is induced by a variety of structurally diverse compounds. Although the mechanism of CYP2 gene induction is not well understood and a specific receptor has not been identified, transcriptional CYP2C gene activation and mRNA stabilization were demonstrated to occur with the azole antifungal agents ketoconazole, clotrimazole and miconazole (Dogra, Whitelaw and May, 1998)

Phenobarbital binds to CAR and affects the expression of approximately 50 genes. Of the CYP enzymes, phenobarbital seems to have the greatest effect on CYP2B6, but clearly also induces CYP1A2, CYP2C8, CYP2C9, and CYP3A4 and also some UGTs (Fuhr, 2000).

Phenobarbital also induces CYP2C19, although to a smaller extent than CYP2C8 or CYP2C9 (Gerbal-Chaloin et al., 2001).

PXR is activated by a number of compounds that are known to induce CYP3A4, such as rifampicin, phenobarbital, dexamethasone, and St John's wort (Lehmann et al., 1998;Moore et al., 2000).

Recent studies suggest that PXR is also involved in the induction of CYP2C8 and CYP2C9 (Gerbal-Chaloin et al., 2001).

Inducers increase CYP450 enzyme activity by increasing enzyme synthesis. Unlike metabolic inhibition, there is usually a delay before enzyme activity increases, depending on the half-life of the inducing drug. A decrease in the concentration of a drug metabolized by CYP2C9 can occur within 24 hours after the initiation of rifampin , an inducer with a short half-life, but can occur up to one week after the initiation of phenobarbital, an inducer with a very long half-life (Lynch and Price, 2007). CYP-isoenzymes and corresponding inhibitors and inducers are given in table 2.5.

Table2.5: Main human hepatic drug-metabolising CYP enzymes (substrates, inhibitors, and inducers, their relative amounts of the total hepatic CYP content (Tiina, 2000) .

CYP enzyme	Substrates	Inhibitors	Inducers
CYP2C19 < 5%	**Omeprazole Mephenytoin Diazepam**	**Fluoxetine Fluvoxamine**	**Rifampicin**
CYP2C8/9 ~ 20%	**Warfarin Phenytoin Cerivastatin**	**Sulphaphenazole Fluconazole**	**Rifampicin**
CYP3A4 ~ 30%	**Simvastatin Midazolam Triazolam Nifedipine Cyclosporin Cisapride Testosterone Ethinylestradiol Cortisol Terfenadine**	**Ketoconazole Itraconazole Diltiazem Verapamil Grapefruit juice Erythromycin Chlarithromycin**	**Rifampicin Phenytoin Carbamazepine**
CYP1A2 ~15%	**Caffeine Theophylline Clozapine**	**Fluvoxamine**	**Smoking Rifampicin**
CYP2E1 < 10%	**Ethanol Halothane Chlorzoxazone**	**Disulfiram**	**Ethanol Isoniazide**
CYP2D6 <5%	**Dextrometorphan Debrisoquine Codeine Metoprolol**	**Quinidine Fluoxetine**	**Not inducible**

2.4 CYP3A subfamily

The CYP3A subfamily, the most abundantly expressed CYP450 gene in the human liver and gastrointestinal tract which consists of at least three isoforms. The three functional genes: CYP3A4, CYP3A5 and CYP3A7 are located on chromosome 7 (Inoue et al.,1992).

The enzymes compromising the CYP3A subfamily share at least 85% amino acid sequence homology. However, they have been shown to differ substantially in substrate specificity and expression (Wrighton and Stevens, 1992; Nelson et al.,1996)

CYP3A appears to be the most important drug-metabolising CYP subfamily,because approximately 40-50% of the drugs used in man are metabolised at least partly by CYP3A-mediatedoxidation (Thummel and Wilkinson, 1998; Wrighton and Thummel, 2000).

2.4.1 CYP3A4

CYP3A4 is present in liver, small intestine, colon, and stomach. CYP3A4 accounted for almost 30% of the total CYP in the liver (Shimada et al., 1994), and is the dominant CYP enzyme in the small intestinal mucosa, with its greatest activity in the proximal small intestine (De Waziers et al 1990 ; Zhang et al., 1999).

Endogenous substrates of CYP3A4 include steroids as testosterone, cortisol, progesterone, androstanediol, dehydroepiandrosterone 3-sulfate and estradiol (Wrighton and Stevens, 1992).

A remarkable feature of CYP3A4 is its extreme promiscuity in substrate specificity and cooperative substrate binding, which often leads to undesirable DDIs and toxic side effects. Owing to its important in drug development and therapy,CYP3A4 has been the most extensively studied mammalian CYP enzymes (Servioukova and Polous, 2013).

The exogenous substrates of CYP3A4 include a large number of therapeutically important drugs such as erythromycin, midazolam, cyclosporin, lidocaine and nifedipine (Leeder and Kearns,1997).

The CYP3A4 enzyme has two substrate binding sites and is allosterically regulated (Shou et al., 1999). The drug interactions caused by inhibition of CYP3A4 exhibit substrate dependency. Induction of CYP3A enzymes is effective usually by rifampin or phenobarbital followed by dexamethasone, phenytoin, and carbamazepine (Thummel and Wilkinson, 1998; Ekroos and Sjögren, 2006).

2.4.2 CYP3A5

CYP3A5 is the second functionally active member of the CYP3A subfamily. It has an amino acid identity of 88% with CYP3A4 (Schuetz, Beach and Guzelian, 1994).

CYP3A5 is present at readily detectable levels in only about 30% of human livers, but it is the most abundant CYP3A isozyme in the human kidney, where it may be important in the hydroxylation of endogenous molecules (Wrighton and Thummel, 2000).

It also exists in other extrahepatic tissues, such as the gastrointestinal tract, lung, and pancreas (Wrighton and Thummel, 2000).

CYP3A5 is inducible in hepatocyte cultures by rifampicin and phenobarbital (Wrighton and Thummel, 2000) and in one human lung adenocarcinoma cell line by glucocorticoids and phenobarbital (Hukkanen et al., 2000).

2.4.3 CYP3A7

CYP3A7 is the major CYP isoenzyme in the fetal liver, accounting for up to 50% of total CYP content (Wrighton, Vanden Branden and Ring, 1996).

It is detected in embryonic, fetal and newborn liver, but is also detected in adult liver, although at much lower levels than CYP3A4 (Yang et al.,1994; Tateishi et al., 1997).

The metabolism of zonisamide by CYP3A7 was approximately 70% of that by CYP3A4 activity (Ohmori et al., 1993). In contrast, the biotransformation of cisapride to either nor-cisapride or its two primary ring-hydroxylated metabolites by CYP3A7 is at least 10-fold less than that observed with CYP3A4 under the same experimental conditions (Gotschall et al., 1999).

2.4.4 Maturation of CYP3A

Developmental changes in CYP3A expression and catalytic activity studies demonstrated a different ontogenic pattern for the individual CYP3A isoforms. Accordingly, the pharmacokinetics of CYP3A substrates may change as a function of developmental changes in CYP3A activity (Leeder and Kearns, 1997; Cresteil, 1998; Lacroix et al., 1997).

In embryonic hepatic tissue (6-12 weeks gestational age) CYP3A4 mRNA could not be detected (Schuetz, Beach and Guzelian, 1994). In contrast, CYP3A4 mRNA was detected in fetal liver microsomes (at between 11 and 30 weeks gestational age) at 10% of adult levels, increasing immediately after birth and reaching approximately 50% of adult levels between 6 and 12 months of age (Lacroix et al., 1997; Greuet et al., 1996).

Testosterone α-hydroxylase activity in human liver microsomes, mainly an activity of CYP3A4, was extremely low in the fetus with an increase after birth reaching 30-40% of adult levels from 3 months to one year of age and 120% of adult levels after the age of one year. Gestational age at birth (25 to 40 weeks) did not have an influence on this ontogenic pattern of CYP3A4 activity (Lacroix et al., 1997).

Hepatic and intestinal CYP3A4 appear to be regulated independently (Lown et al., 1994), and the content of intestinal CYP3A4 varies 11-fold and its catalytic activity 6-fold among individuals (Lown et al., 1994). This finding may contribute to the interindividual variability in disposition of CYP3A4 substrates administered orally. The small intestine is an important place for first-pass metabolism of oral drugs. Interestingly, the intestinal first-pass metabolism of some oral drugs, for example, midazolam and cyclosporine, may even exceed that occurring in the liver (Wu et al., 1995).

In vitro, the expression and catalytic activity of hepatic CYP3A4 can vary as much as 6-fold and 30-fold, respectively (Shimada et al., 1994). Despite this large interindividual variation in CYP3A4 activity, CYP3A4 does not appear to be subject to genetic polymorphism, in contrast to CYP2C9, CYP2C19, and CYP2D6 (Aithal et al., 1999; Gaedigk, 2000). Erythromycin is metabolised about 25% more rapidly by microsomes from female than from male human liver (Hunt and Westerkam and Stave, 1992).

Furthermore, midazolam is cleared 20-40% faster by women than by men . Not all studies, however, have found a sex-related difference in CYP3A4 activity . Neither menstrual-cycle phase, smoking, nor ethanol consumption appear to influence CYP3A4 activity (Harris, Benet and Schwartz, 1995; Kharasch et al., 1999).

2.5 Drug interactions

Drug interactions are often classified as either pharmacodynamic or pharmacokinetic interactions (Bailie et al., 2004).

Such drug interactions can cause serious clinical problems leading to drug withdrwal from the market. The nonsedating antihistamines terfenadine and astemizole, and the gastrointestinal motility agent cisapride, were all withdrawn from the U.S. market because metabolic inhibition by other drugs led to life-threatening arrhythmias (Dresser, Spence and Bailey, 2000).

The calcium channel blocker mibefradil was withdrawn from the U.S. market in 1998 because it was a potent enzyme inhibitor that resulted in toxic levels of other cardiovascular drugs (Mullins et al., 1998).

2.5.1 Pharmacodynamic interactions

Pharmacodynamic interactions between drugs with additive effects may be intentional, for example when combining antihypertensives, or unintentional, for example serotonin syndrome caused by adding tramadol to a selective serotonin reuptake inhibitor (SSRI). Conversely, combining drugs with opposing effects can result in loss of drug effect, for example

reduced bronchodilation by a beta2 agonist prescribed with a non-selective beta blocker (Fallowfield and Marlow, 1996).

Pharmacodynamic interactions include those that result in additive or antagonistic pharmacological effects which can cause changes in the effects of drugs (Bailie et al., 2004).

2.5.2 Pharmacokinetic interactions

Pharmacokinetic interactions involve induction or inhibition of metabolizing enzymes in the liver or elsewhere, displacement of drug from plasma protein binding sites, alterations in gastrointestinal absorption, or competition for active renal secretion. The frequency and prevalence of interactions is dependent upon the number of concomitant medications and the complexity of the regimens. The prevalence is also dependent upon other variables, such as patient adherence, hydration and nutritional status, degree of renal or hepatic impairment, smoking and alcohol use, genetics and drug dosing. Additionally, some patients may exhibit evidence of a particular drug interaction, while others with the same drug combination do not (Bailie et al., 2004).

2.5.2.1 Interactions resulting from alterations in metabolizing enzymes

The liver is the major, though not exclusive, site for drug metabolism. Other sites include the kidney and the lining of the gastrointestinal tract. The two main types of hepatic drug metabolism are phase I and phase II reactions. Phase I oxidative reactions are the initial step in drug biotransformation, and are mediated by the cytochrome P-450 (CYP) system. Phase II reactions occur following Phase I reactions. In this process, drug metabolites are converted into more water-soluble compounds that can be more easily eliminated by the kidneys (Bailie et al., 2004).

Inhibition of cytochrome P450 enzymes is mediated by different mechanisms which were discussed in section (2.3).

Such interaction can be used therapeutically. For example, ritonavir, a strong inhibitor of CYP3A, reduces metabolism of other protease inhibitors thus increasing their effectiveness in treating HIV (so called ‘ritonavir-boosted’ regimens) (Walmsley et al., 2002).

Enzyme induction may result in increased CYP enzyme synthesis, faster drug metabolism, subtherapeutic drug concentrations and the risk for ineffective drug therapy. The rapidity of the enzyme induction is dependent upon the half-life of the inducing drug as well as the rate of synthesis of new enzymes (Bailie et al., 2004).

Examples of inducers and inhibitors of cyp-isoenzymes are given in table (2.5)

2.5.2.2 Interactions resulting from alterations in gastrointestinal absorption

Absorption of a drug is a function of the drug's ability to diffuse from the lumen of the gastrointestinal tract into the systemic circulation. The rate and extent of drug absorption after oral administration may be grossly altered by other agents. Changes in intestinal pH may profoundly affect drug diffusion as well as dissolution of the dosage form. For example, the absorption of ketoconazole is reduced by the co-administration of antacids or H2-blockers (e.g. ranitidine, famotidine) that reduce the extent to which the ketoconazole tablet is dissolved. Formation of insoluble complexes by a process known as chelation is another mechanism by which a drug interaction may lead to reduced oral absorption. For example, fluoroquinolones (e.g. ciprofloxacin) and divalent metal ions (such as calcium and iron) form an insoluble complex that results in reduced absorption of both the antibiotic and the metal ion (Bailie et al., 2004).

2.5.2.3 Interactions resulting from alterations in protein binding

Drugs may exist in plasma either reversibly bound to plasma proteins or in the free (unbound) state. The primary drug-binding plasma proteins are albumin and α1-acid glycoprotein. Free drug that exerts the pharmacological effect. Drugs may compete with each other for plasma protein binding sites, and when this occurs, one drug may displace another that was previously bound to the protein. Displacement of a drug from its binding sites will therefore increase that agent's unbound concentrations, perhaps resulting in toxicity. Some drugs normally exist in a state of high protein binding, often exceeding 90%. Thus, even a small decrease in protein binding could significantly increase the free concentrations. Drugs which are normally highly protein bound, and which might participate in binding interactions, include anticonvulsants and warfarin (Bailie et al., 2004).

2.5.2.4 Interactions resulting from changes in renal excretion

Changes in renal drug clearance may occur due to effects on renal tubular function or urine pH. For example, probenecid reduces the renal clearance of anionic drugs such as methotrexate and penicillin. The majority of renally eliminated drugs are excreted via passive glomerular filtration. Some drugs are eliminated via active tubular secretion, such as penicillins, cephalosporins, and most diuretics. The active secretion may be inhibited by secondary agents, such as cimetidine, nonsteroidal anti-inflammatory agents and probenecid, resulting in elevation of the serum drug concentrations and reduced urinary drug concentrations (Bailie et al., 2004).

2.5.2.5 P-Glycoprotein

P-Glycoprotein (Pgp) was one of the first members of the ATP-binding cassette (ABC) superfamily to be studied. Overexpression of Pgp was linked to multidrug resistance (MDR) in mammalian cell lines and human cancers. Today this fascinating protein, which is proposed to operate as an ATP-powered drug efflux pump, remains one of the most studied membrane transporters (Croop,1993; Lincke et al., 1993).

The tissue localization of Pgp suggests that the protein plays a physiological role in the protection of susceptible organs like the brain, testis and inner ear from toxic xenobiotics, the secretion of metabolites and xenobiotics into bile, urine, and the lumen of the gastrointestinal tract, and possibly the transport of hormones from the adrenal gland and the uterine epithelium (Schinkel, 1998).

Pgp has demonstrated up to 10-fold variability in activity between subjects and has a significant role in oral drug absorption (Lown et al., 1995).

Decreased bioavailability occurs because intact drug molecules are pumped back into the gastrointestinal tract lumen and exposed multiple times to enterocyte metabolism (Willie, Lown and Huszezo, 1997).

Pgp has broad substrate specificity, and inhibiting or inducing the activity of this protein can lead to significant alterations in drug exposure (Willie, Lown and Huszezo, 1997).

However, because many drugs have affinities for both Pgp and CYP3A4/5, it is difficult to determine by which specific mechanism drug interactions occur (Hall et al., 1999; Wacher, Wu and Benet, 1995).

For some compounds, inhibition of both P-glycoprotein function and CYP3A4/5 activity may be required to produce clinically significant interactions.

Many anti-infectives have binding affinity for P-glycoprotein. These include erythromycin, clarithromycin (Wakasugi et al., 1998), ketoconazole, sparfloxacin (Cormet-Boyaka et al.,1998), the nucleoside analog adefovir (Annaert et al., 1998), and the human immunodeficiency virus (HIV)-1 protease inhibitors (Alsenz, Steffen and Alex, 1998; Kim et al., 1998).

In vitro data revealed that grapefruit juice, in addition to inactivating enterocyte CYP3A isozymes, may also increase P-glycoprotein activity (Soldner et al.,1999).

2.5.3 CYP3A4 in drug interaction

The drug-drug interactions mediated through CYP3A4 result either from induction or from inhibition of this enzyme (Lin and Lu, 1998).

Many drugs from different therapeutic areas are substrates for CYP3A4, which is present in high amounts in both the liver and the intestine. Use of a CYP3A4 inhibitor can decrease drug metabolism both during the first-pass and the elimination phases (Shimada et al., 1994, De Waziers et al., 1990).

Many drugs, including the macrolide antibiotics troleandomycin and erythromycin, undergo metabolic activation by CYP3A4 to form inhibitory metabolites which form complexes with the enzyme, resulting in a functionally inactive CYP3A4 enzyme (Danan, Descatoire and Pessayre, 1981; Pessayere et al., 1982).

The risk of rhabdomyolysis appeared to increase with co-administration of a CYP3A4 inhibitor and lovastatin or simvastatin (Neuvonen and Jalava, 1996; Neuvonen, Kantola and Kivisto, 1998).

Quinidine is a substrate for CYP3A4, but is a potent reversible inhibitor of CYP2D6 (Nielsen, Brøsen and Gram, 1990).

Furthermore, concomitant use of drugs such as ketoconazole, itraconazole or verapamil increases the sedative effects of midazolam and triazolam (Olkkola et al., 1993; Backman et al., 1994; Varhe, Olkkola and Neuvonen, 1994) .

Enzyme induction increases the metabolism of drugs that are metabolized by induced enzymes, leading to decreased plasma drug concentrations (Lin and Lu, 1998).

For example, rifampicin can greatly decrease the plasma concentrations of triazolam and abolishes the effect of triazolam (Villikka et al., 1997).

In addition to the hepatic, the intestinal CYP3A4 can be induced by such drugs as rifampicin (Kolars et al., 1992) and inhibited by known CYP3A4 inhibitors such as ketoconazole (Tsunoda et al., 1999).

Components in the natural plant remedy St John's Wort, in particular hyperforin, are potent inducers of CYP3A4, a fact that is of importance for interactions during drug therapy (Moore et al., 2000).

In the enterocyte, the spatial separation of P-gp, located on the apical plasma membrane, and that of CYP3A4, located on the endoplasmic reticulum, supports the idea that P-gp may control the access of drugs to intracellular metabolism by CYP3A4. Drugs absorbed into the intestinal epithelium can interact with P-gp and be actively extruded back into the intestinal lumen. If this process of diffusion and active transport occurred repeatedly, the circulation of the drug from the lumen to the intracellular compartment would potentially prolong the intracellular residence time of the drug, decrease the rate of absorption, and result in increased drug metabolism by CYP3A4 relative to the parent drug crossing the intestine (Ito, Kusuhara and Sugiyama, 1999).

Many drugs metabolised by CYP3A4 are also substrates for P-gp. Accordingly, the coadministration of two CYP3A4 substrates can result in interactions that reflect inhibition of the CYP3A4-mediated metabolism, reduced efflux by P-gp or a combination of both effects (Wacher, Wu and Benet, 1995).

Among the drugs metabolized are sedatives such as midazolam, triazolam and diazepam, the antidepressives amitriptyline and imipramine, the anti-arryhthmics amiodarone, quinidine, propafenone and disopyramide, the antihistamines terfenadine, astemizole and loratidine, calcium channel antagonists such as diltiazem and nifedipine and various antimicrobials and protease inhibitors (Wacher, Wu and Benet, 1995).

Fluoroquinolones antimicrobials and azole antifungals, although not metabolised by CYP3A4 isoenzyme, but cause rapid reversible inhibition of CYP3A4 isoenzyme (Von Moltke et al., 1996).

Examples of commonly used irreversible inhibitors of CYP3A4 are clarithromycin, erythromycin, isoniazid, carbamazepine, irinotecan, tamoxifen, ritonavir, verapamil, nicardipine, 17-α ethynylestradiol, fluoxetine, midazolam, and products in grapefruit juice (bergamottin, 6′7′-dihydroxybergamottin) (Zhou et al., 2005).

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Erythromycin and midazolam both are substrates for CYP 3A4 isoenzyme so, there is competition for enzyme sites and metabolism of midazolam is inhibited **(**Olkkola et al., 1993).

Examples of drug interaction that involve CYP3A4 are listed in table 2.6.

Table 2.6: Drug interactions involving CYP3A4 isoenzymes (Badyal and Dadhich, 2001).

INHIBITORS	Drugs affected (substrates)
Azole antifungals	
Itraconazole	Cisapride, quinidine, astemizole, methylprednisolone, buspirone, felodipine, vincristine, atorvastatin
Ketoconazole	Terfenadine, astemizole, cyclosporine, triazolam, alprazolam
Fluconazole	Terfenadine, triazolam
Macrolide	
Erythromycin	Carbamazepine, triazolam, buspirone, terfenadine, simvastatin
Clarithromycin	Pimozide, cyclosporin, midazolam
Selective serotonin	
Fluoxetine	Diazepam, alprazolam, midazolam, terfenadine
Paroxetine	Alprazolam
Calcium	
Verapamil	Simvastatin, carbamazepine, cyclosporine
Diltiazem	Triazolam, carbamazepine, cyclosporine, quinidine, simvastatin, midazolam, alfentanil
Nifedipine	Midazolam
Protease inhibitors	Terfenadine, astemizole, cisapride, midazolam
Grapefruit juice	Felodipine, nifedipine, nimodipine, nitrendipine, terfenadine, cyclosporin, midazolam,carbamazepine, simvastatin, verapamil, prednisolone, ethinylestradiol, artemether
Ciprofloxacin	Tacrolimus
Cimetidine	Carbamazepin, quinidine, cyclosporine, calcium channel blockers, benzodiazepines
Propofol	Midazolam
Nafimidone	Carbamazepine
INDUCERS	
Rifampicin	Protease inhibitors, diazepam, triazolam, midazolam, estradiol, norgesterol, lidocaine, zopiclone, zolpidem, ondansetron
Rifabutin	Protease inhibitors, estradiol, norgesterol
Carbamazepine	Protease inhibitors, midazolam, itraconazole, vincristine

2.6 Carbamazepine

2.6.1 Introduction

CBZ is an iminodibenzyl derivative, structurally similar to the tricyclic antidepressants. It is a derivative of iminostilbene with a carbamyl group at the 5 position; this moiety is essential for potent anti-seizure activity (Brunton, Chabner and Knollman, 2011) .

CBZ was discovered by the chemist Walter Schindler in Switzerland in 1953. CBZ was first marketed as a drug to treat trigeminal neuralegia in 1962 and has been used as an anticonvulsant and antiepileptic in the UK since 1965, and has been approved in the US since 1974 (Tolou- Ghamari et al., 2013).

2.6.2 Mechanism of Action

The main target of CBZ are voltage-dependent sodium channels. CBZ and carbamazepine-epoxide reduce the frequency of sustained repetitive firing of action potentials in cultured mammalian central neurons. CBZ inhibits high frequency but not low frequency firing (Elliott, 1990; Macdonald and Kelly, 1993). Such voltage- or frequency-dependent block is ascribed to a voltage-dependent inhibitory effect on voltage-gated sodium channels. It has been shown that the inhibition of sodium currents in cultured neuroblastoma cells and in small cells from adult rat dorsal root ganglia is more potent at more depolarized potentials (Rush and Elliott, 1997).

CBZ has also calcium antagonistic properties. A decade ago, Elliot proposed that the efficacy of CBZ in the treatment of seizures could be due to a frequency dependent block of sodium currents and a block of calcium currents (Elliott, 1990). Such calcium antagonistic properties would explain the similarities in the depressant action of CBZ and organic calcium antagonists on epileptic paroxysmal depolarizations (Walden et al., 1993).

Indeed, CBZ reversibly suppresses the calcium-dependent components of action potentials and markedly reduces the calcium currents, presumably L-type, in cultured rat sensory spinal ganglion cells (Schirrmacher et al., 1995)

The anticonvulsant, as well as the therapeutic and prophylactic effects of CBZ in affective psychoses may, in part, be related to the potent interaction of CBZ with adenosine-binding sites in the brain. Indeed, several reports have demonstrated that CBZ acts as an antagonist at adenosine A1 receptors (Van Calker et al., 1991).

CBZ may act postsynaptically by limiting the ability of neurons to sustain high frequency repetitive firing of action potentials through enhancement of sodium channel inactivation; in addition to altering neuronal excitability. CBZ may act presynaptically to block the release of

neurotransmitter by blocking presynaptic sodium channels and the firing of action potentials, which in turn decreases synaptic transmission (Thomson, 2007).

2.6.3 Uses

CBZ became one of the cornerstones of anticonvulsant therapy in the paediatric population, where it is used in partial complex, generalized tonic clonic, mixed seizure disorders and other partial or generalized seizures. Carbamazepine has been used as antiepileptic drug since 1965, and is most effective against partial seizures (Tolou-Ghamari et al., 2013).

CBZ can be used with caution as an alternative treatment option for refractory idiopathic generalized epilepsy IGE, especially in cases in which the main seizure type is generalized tonic-clonic (Kenyon, Mintzer and Nei, 2014).

It is very effective for the severe pain associated with trigeminal neuralgia and in a lesser extent in glosso pharyngeal neuralgia. The exact mechanism unknown and may involve gamma-aminobutyric acid (GABA) receptors, which may be linked to calcium channels (Thomson, 2007).

CBZ may be used under specialist supervision for the prophylaxis of bipolar disorder (manic-depressive disorder) in patients unresponsive to a combination of other prophylactic drugs; it is used in patients with rapid-cycling manic-depressive illness occurring 4 or more affective episodes per year (Brunton, Chabner and Knollman, 2011).

2.6.4 Side effects

Side effects of CBZ referable to the CNS are frequent at concentrations above 9 μg/mL. Adverse neurologic and sensory effects of CBZ include dizziness, vertigo, drowsiness, fatigue, ataxia, disturbances of coordination, confusion, headache, nystagmus, blurred vision, transient diplopia, visual hallucinations, hyperacusis, oculomotor disturbances, speech disturbances, and abnormal involuntary movements (Brunton, Chabner and Knollman, 2011).

CBZ -induced hyponatremia is a moderately well described side effect and may be responsible for some of the more highly reported signs and symptoms associated with CBZ adverse effects. Minimal consensus has been found regarding both dosage/level of CBZ and polypharmacy as a predisposingrisk factor, whereas age is most probably not a predisposing risk factor in CBZ -induced hyponatremia (Gandelman, 1994).

Adverse gastrointestinal effects of CBZ include nausea, vomiting, gastric distress, abdominal pain, diarrhea, constipation, anorexia, dryness of the mouth and pharynx, glossitis, and stomatitis. Severe adverse dermatologic effects of CBZ include pruritic and erythematous rashes, urticaria, photosensitivity reactions, alterations in skin pigmentation, toxic epidermal

necrolysis (Lyell's syndrome), and exfoliative dermatitis (Brunton, Chabner and Knollman, 2011).

2.6.5 Dose and dosage form

It is available for oral administration as chewable tablets of 100 mg, tablets of 200 mg, ER tablets of 100, 200, and 400 mg, and as a suspension of 100 mg/5 mL. The dose is 100-200mg 1-2 times daily, increased slowly to usual dose of 0.08-1.2g daily in divided doses, up to 1year 100-200mg, 1-5 years 200-400mg, 5-10 years 400-600mg, 10-15years 0.06-1g (Ahmed-Jushuf et al., 2011; British Pharmacopeia, 2009).

2.6.6 Pharmacokinetic Profile

The pharmacokinetics of CBZ is complex. CBZ is influenced by its limited aqueous solubility and by the ability of many anti-seizure drugs, including CBZ itself, to increase their conversion to active metabolites by hepatic oxidative enzymes (Brunton, Chabner and Knollman, 2011).

After oral ingestion, CBZ is rapidly absorbed ($\mathbf{t_{max}}$is formulation-dependent) with a bioavailability of 75–85 % (Patsalos, 2013). Most oral formulations of CBZ are well absorbed with high bioavailability and the rate or extent of absorption was not be affected by food (Tolou-Ghamari et al., 2013).

Peak concentrations in plasma usually are observed 4-8 hours after oral ingestion, but may be delayed by as much as 24 hours, especially following the administration of a large dose (Patsalos, 2013).

The systemic availability of CBZ given as an oral suspension to be equal to its availability when given intravenously. CBZ distributes rapidly into all tissues (Brunton, Chabner and Knollman, 2011).

CBZ volume of distribution is about 0.8–2.0 L/kg and plasma protein binding is 75% and may reach 80% of the total plasma concentration (Tolou- Ghamari et al., 2013; patsalos, 2013).

CBZ is distributed into breast milk. Concentrations in breast milk and in the plasma of nursing infants have been reported to reach 60% of the maternal plasma concentration. Therefore, the possibility exists that CBZ may cause adverse effects in the nursing infant (Thomson, 2007).

CBZ initial half-life values range from 25-65 hours, decreasing to 12-17 hours on repeated doses, because of autoinduciton of metabolism. Carbamazepine-10,11-epoxide half life is about 5 to 8 hours. Frequently, its half-life may be further shortened by co-administration of other enzyme inducer (Thomson, 2007).

CBZ is extensively or completely metabolized in the liver, primarily by CYP3A4, to carbamazepine-10, 11-epoxide which is pharmacologically active. CBZ epoxide is as active as the parent compound in various animals, and its concentrations in plasma and brain may reach 50% of those of CBZ (Brunton, chabner and Knollman, 2011).

Carbamazepine-10,11-epoxide which is further metabolized by hydration before excretion. This epoxide-diol pathway is induced during long term treatment with CBZ, and the protein binding of carbamazepine-10,11-epoxide, is 50 % (Patsalos, 2013).

The hydration step of CBZ epoxide is catalyzed by microsomal epoxide hydrolase. CBZ diol is inactive compound and excreted in the urine principally as glucuronide (Brunton, chabner and Knollman, 2011).

CBZ induces its own metabolism, leading to increase its clearance, shortened serum half-life, and progressive decrease in serum levels. Increases in daily dosage are necessary to maintain plasma concentration. This occurs, in chronic treatment, due to autoinduction of the drug metabolism (Tolou-Ghamari et al., 2013).

2.6.7 Drug interactions

CBZ is a potent inducer of a broad spectrum of drug-metabolizing enzymes and drug transporters in the human liver. These effects are mediated at least in part by activation of PXR including multiple CYP genes in the CYP1A, CYP2A, CYP2B, CYP2C, and CYP3A subfamilies, as well as glutathione S-transferase A1, uridine diphosphate glucuronosyltransferase 1As, the drug transporter ABCC2, and the nuclear receptors CAR and PXR, resulting in serious interactions with many commonly prescribed drugs (Oscarson et al., 2006).

CBZ is not a substrate for P-gp. So that, the interaction of CBZ with drugs that modulate both CYP3A4 and P-gp function such as verapamil is probably due to inhibition of CYP3A4 and not P-gp (Owen et al., 2001).

Effect of multiple dose omeprazole on the pharmacokinetics of carbamazepine was studied on 7 patients with duodenal ulcer. An increase in CBZ blood level, significantly prolonged elimination half-life (from 17.2 ± 5.9 hours to 37.3 ± 22.8 hours) and increase in the area under the curve (AUC0-∞) (from 382.3 ± 81.1 μg/ml·h to 668.8 ± 241.6 μg/ml· h) were manifested. The results were explained by inhibition of CBZ oxidative metabolism by omeprazole (Naidu et al., 1994).

The effect of ciprofloxacin on pharmacokinetics of CBZ was studied in healthy adult male volunteers. Ciprofloxacin had significantly increased the plasma concentration Cmax, AUC and $t_{½}$ while it decreased the clearance(CL) and volume of distribution (Vd) of CBZ when was given concurrently. The plasma concentration of orally administered CBZ alone reached its maximum

9.874 ± 0.253 μg mL-1 at 8 h and then declined with passage of time to 2.025 ± 0.243 at 72 h. Similarly, Cmax plasma concentration of CBZ with ciprofloxacin was 16.606 ± 0.212 at 6 h which declined with the passage of time to 3.316 ± 0.162 at 72 h. This Change in pharmacokinetic parameters was probably due to the inhibition of CYP3A4 isoenzyme by ciprofloxacin which is responsible for metabolism of CBZ. Volume of distribution (Vd) was 0.348 ± 0.042 L kg-1 in case of 200 mg dose of carbamazepine administrated to the eight adult subjects. This value was significantly ($P<0.05$) reduced to 0.262 ± 0.022 L kg-1 when carbamazepine and ciprofloxacin were given at a time to the same individuals. Half-life of elimination (t1/2 β) of CBZ (200 mg) with ciprofloxacin (500 mg) was 50.01 ± 3.359 h, which was statistically significant ($P<0.05$) from that of CBZ alone i.e. 38.318 ± 4.691 h. The total body clearance of carbamazepine, 0.00635 ± 0.005065L h^{-1} kg^{-1}, in the eight subjects was significantly reduced to 0.00371 ± 0.000286 L h^{-1} $kg^{-1}$when carbamazepine (200 mg) was administered with ciprofloxacin. Area under the curve (AUC) of carbamazepine (200 mg) after its oral administration was noted as 357.2 ± 12.707 μg h L^{-1}. The AUC of carbamazepine with ciprofloxacin (500 mg) was 548.6 ± 16.698 μg h L^{-1}.Adjustment as well as drug monitoring of carbamazepine was required, when carbamazepine and ciprofloxacin are given concurrently (Shahzadi et al., 2011).

Administration of ketoconazole to eight patients with epilepsy stabilized on CBZ therapy was associated with a significant increase in plasma CBZ concentrations (from 5.6 ± 1.9 to 7.2 ± 2.9 μg/ml) on day 10. This interaction was probably mediated by an inhibiting action of ketoconazole on cytochrome CYP3A4, the main enzyme responsible for CBZ metabolism (Spina et al., 1997).

A study was carried out to evaluate effect of esomeprazole on the pharmacokinetics of carbamazepine in rabbits. There was a significant increase in T_{max} (from 2.83 ± 0.17 to 3 ± 0.40 h) when esomeprazole was given along with carbamazepine. Concomitant use of esomeprazole altered the pharmacokinetics of carbamazepine (Medhi et al., 2011).

A study was conducted to compare the effects of concurrent administration of herbal tea prepared from dried flowers of Cassia auriculata or aerial parts of Cardiospermum halicacabum on carbamazepine by using male Wistar rats as experimental model. Cassia auriculata tea influence the bioavailability of carbamazepine, and hence its therapeutic actions. The results demonstrated that, rats receiving Cassia auriculata tea with CBZ hat significantly enhanced blood level of CBZ (by 47.1%). So concurrent ingestion of carbamazepine with herbal teas containing Cassia auriculata is therefore best avoided by patients under treatment for epilepsy (Thabrew et al., 2004).

2.7 Norfloxacin

2.7.1 Introduction

Norfloxacin (NFX), chemically known as 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid (Figure 2.3), is a fluorized quinolone, inhibits, like the other members of this group, the gyrase of the bacterial DNA. This effect is held responsible for the bactericidal action of norfloxacin (Sharma, Saneja and Jain, 2008).

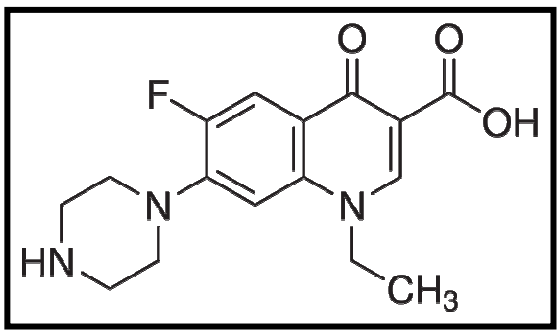

Figure 2.3: Chemical structure of Norfloxacin

NFX is a widely used representative member of flouroquinolone and is the first choice of drug for the treatment of bacterial infections of the urinary, biliary and respiratory tracts (Sharma, Saneja and Jain, 2008).

There are a large number of other flouroquinolones related compounds including ofloxacin, ciprofloxacin, amifloxacin, enoxacin, pefloxacin, difloxacin, gattifloxacin, sparfloxacin, fleroxacin are in clinical practice and others are in vigorous stages of development and clinical investigation (Appelbaum and Hunter, 2000).

2.7.2 Mechanism of action

Norfloxacin inhibits bacterial deoxyribose nucleic acid (DNA) gyrase (topoisomerase II), an enzyme, which converts covalently closed circular DNA into negative supercoils (Brunton, chabner and Knollman, 2011).

DNA gyrase, present in bacteria is the only topoisomerase (Topoisomerase–II) known, which is able to introduce negative superhelical turns into duplex DNA. The introduction of supercoils into DNA is an energy demanding process. This DNA gyrase enzyme is able to couple the energy released from the hydrolysis of ATP to drive the formation of supercoils. Gyrase can also remove positive supercoils in the presence of ATP and relax negatively (but not positively) supercoiled DNA in the absence of ATP (Brunton, chabner and Knollman, 2011; Rang et al., 2003).

It is believed that the drug directly acts on DNA, producing a covalent attachment of DNA gyrase to DNA, which forms a complex that is inaccessible to the action of DNA

polymerase; thus, leading to prevention of DNA synthesis and replication which ultimately results in rapid cell death (Brunton, chabner and Knollman, 2011).

Due to fluorine (F) at the 6^{th} position and piperazine at the 7th position of quinolone carboxylic acid, it has enhanced activity against both; gram positive and gram negative bacteria. Norfloxacin is specifically active against aminoglycoside resistant Pseudomonas aeruginosa and betalactamase producing organisms. It is active against both; gram positive and gram negative bacteria (Brunton, chabner and Knollman, 2011).

2.7.3 Uses

NFX is registered for therapeutic use in acute recurrent urinary tract infections, prostatitis, bacterial gastroenteritis, gonorrhoeic urethritis, proctitis and cervicitis as well for prophylactic use in neutropenic patients (Brunton, chabner and Knollman, 2011) .

NFX has been effective in the treatment of urinary tract infections, Neisseria gonorrhoea urethritis and/or cervicitis. Norfloxacin (400 mg twice or three times daily for 5 days) was as effective as co –trimoxazole or nalidixic acid in the treatment of bacterial gastroenteritis caused by enterotoxigenic E. coli or Shigella species. Effectiveness of norfloxacin oral dosage has been evidenced in treatment of typhoid fever (400 mg three daily for 14 days) (Brunton, chabner and Knollman, 2011) .

In addition, NFX has been used with considerable success to reduce the rate of bacterial peritonitis in patients with hepatic cirrhosis and ascites. It may be used in the treatment of lower respiratory tract infections caused by susceptible bacteria (Brunton, chabner and Knollman, 2011).

Norfloxacin ophthalmic solution was as effective as chloramphenicol in the treatment of bacterial conjunctivitis (Rang et al., 2003).

2.7.4 Side effects

Fluoroquinolones are not of severe consequence when compared to the beneficial features they exhibit. The target tissues are the juvenile cartilage, central nervous system, urinary tract and digestive tract. Some skin eruptions were also observed in man (Rang et al., 2003).

High serum concentrations may produce immediate toxic reactions, possibly due to overwhelming histamine release. These immediate reactions are believed to be principally CNS in nature, and consist of convulsions, defecation, urination, and emesis within 2–3 min of rapid IV injection of norfloxacin solution. These signs subsided within several minutes in the affected dogs, and slower infusion (for 2–3 minutes) did not produce such severe clinical signs (Brunton, chabner and Knollman, 2011; Rang et al., 2003).

The articular cartilage forms vesicles after a single very high dose or after several moderately high doses, which can then progressively rupture and produce cartilaginous erosions. This observation is due to an early phase burst in oxidative metabolism in immature (but not mature) chondrocytes that may precipitate cell death (Brunton, chabner and Knollman, 2011).

2.7.5 Doses and dosage form

It presents in different dosage forms (Tablet and ophthalmic solution) which is produced by different international companies such as MERK and LOMUS.

The dose of NFX are 400mg twice daily for 7-10 days in urinary tract infection (UTI), 400mg twice daily in chronic UTI and 400mg twice daily for 28 days in prostatitis (British Pharmacopeia, 2009).

2.7.6 Pharmacokinetic profile

After an oral dose of 200-400 mg, mean peak serum concentrations of 0.8 ± 0.3 and 1.5 ± 0.6 mg L^{-1}, respectively are achieved within 60 -90 minutes (Delon et al., 1997).

The presence of food/dairy products slightly impairs its absorption. Studies in animals showed that the volume of distribution of NFX is very large, about 50% of the body weight and the bioavailability is 50 – 80% (Delon et al., 1997; Rang et al., 2003).

Approximately 15% of the drug in the serum is bound to plasma proteins . NFX has a very low CNS penetration due to relatively low lipophilicity. NFX is cleared from the body through the kidneys, biliary excretion and metabolism. Its elimination half life is approximately 3 h (Delon et al., 1997).

Elimination of NFX is partly by hepatic metabolism by P450 enzymes and partly by renal excretion (Rang et al., 2003).

2.7.6.1 Drug interactions

Fluoroquinolones can decrease CYP3A- and CYP1A-mediated biotransformation by competitive inhibition and that they have the potential to cause drug interactions with agents metabolized by these enzymes (Mclellan et al., 1996). Ciprofloxacin and NFX significantly depressed the N-demethylation of erythromycin by CYP3A4 in human microsomes and by CYP3A2 in rat microsomes. NFX also inhibited ethoxyresorufin-O-dealkylase (mediated by CYP1A) (Mclellan et al., 1996).

The interaction between NFX and theophylline was studied in eight healthy nonsmoking volunteers. NFX decreased significantly theophylline clearance (14.9%) and increased AUC

(16.6%). Adjustment of the theophylline dosage may be necessary in some patients to minimize the risk of theophylline toxicity (Ho, Tierney and Dales, 1988).

The effect of NFX on theophylline metabolism was investigated in ten healthy, nonsmoking adults. In a randomized crossover sequence, each subject received 6 mg of aminophylline per kg of body weight by intravenous infusion over 30 min on day 4 of taking NFX or while drug free. A small but statistically significant decrease in mean theophylline clearance and an increase in elimination half-life were found when NFX was given in recommended dose for 3 days (Davis et al., 1989).

A randomized, crossover study design with a 2-weeks washout period between treatments was used to investigate the effect of NFX on pharmacokinetics of theophylline in human volunteers. Subjects received oral theophylline (200 mg of aminophylline [theophylline ethylenediamine]) three times daily for 4 days either alone or with 400 mg of NFX (orally) twice daily for the same period. Theophylline concentrations in serum were significantly higher at 0, 3, 4, 10, and 12 h following the final dose in the NFX treatment group than in the group receiving only theophylline. No significant differences was established regarding oral clearance and half-life. It was concluded that NFX had no clinically significant effect on theophylline in most patients (Bowles et al., 1988).

Six healthy volunteers were enrolled in a study to examine the effect of quinolones on caffeine disposition. They received a single caffeine dose after pretreatment with NFX, pipemidic acid, or placebo in a crossover, randomized, single-blind clinical trial. Quinolones altered the pharmacokinetics of caffeine, with a significant increase in the AUCs and a decrease in plasma clearance. In the 24-hour urine samples, the level of caffeine metabolite were reduced indicating that pipemidic acid and, to a lesser degree, NFX inhibited N-demethylation pathway of caffeine (Carbo et al., 1989).

The clearance of cyclosporine was decreased by the concomitant use of NFX as a prophylactic treatment in pediatric patients undergoing renal transplantation. The mean daily dose of cyclosporine needed to maintain trough cyclosporine blood levels of 150 to 400 ng/ml was 4.5 mg/kg/day for the patients who received NFX compared with 7.4 mg/kg/day for patients who did not receive the antibiotic (McLellan et al., 1995).

Ten healthy male subjects were administered a single oral dose of 30 mg warfarin sodium alone or during multiple-dose treatment with NFX 400 mg twice daily in a randomized, crossover fashion. The pharmacokinetic parameters of warfarin were comparable in the absence and presence of NFX, including no significant differences in warfarin's elimination half-life, apparent total clearance, apparent volume of distribution, or peak plasma concentration (Rocci et al., 1990).

2.8 Rabbit as experimental model

The use of animals in biomedical research has been recommended for perfecting and validating existing procedures developing new materials and understanding the various physiological and pathological processes because there are no in vitro models capable of fully mimicking the complexity of the human organism (Mordenti, 1991; Mapra, Thomas and Bhat, 2012)

When research is performed on the appropriate animals, the information obtained approximates what can be expected in humans because the greater the physiological, anatomical and organic similarity. There are some similarities have already been reported regarding bone composition, remodeling and density between animal models and human beings. For example, bone density and resistance to fractures are very similar in rabbits and humans (Harcourt-Brown, 2002).

Rabbit models in terms of their short life spans, short gestation periods, high numbers of progeny, low inter-individual variability, low cost, advanced genomics, proteomics, and broad availability of reagents and the medium-sized model they are readily available and easy to handle and observe. However, rabbits do have many advantages and serve to bridge the gap between these small animal models, which are perhaps best suited for discovery phases of research, and larger animal models often required for pre-clinical, translational research (Harcourt-Brown, 2002; Mapra, Thomas and Bhat, 2012).

Rabbits are relatively inexpensive to purchase, house, and maintain as compared to larger animal models. They are easy to breed and handle and are a well-established model in terms of being recognized by the scientific and regulatory communities. Rabbits are phylogenetically closer to primates than rodents and further offer a more diverse genetic background than inbred and out-bred rodent strains, which makes the model a better overall approximate to humans. Further, rabbit genomics and proteomics are advancing rapidly and several transgenic lines have been created and characterized and are readily available. With more researchers using rabbits in their experiments, industry is catching up to their needs and offering an expanding range of rabbit-specific products and services to support them (Von Staden, 1989).

There are some human conditions that cannot be adequately modeled by invertebrate or rodent species and in some cases, the special characteristics of rabbit anatomy and physiology make it uniquely suitable for the study of particular human diseases (Von Staden, 1989).

Pharmacokinetics of sodium mercaptoundecahydrododecaborate were determined inmice, rat, rabbit and human. The species shown similarities in drug disposition with correlation to their own internal physiological process. The interspecies scaling up of plasma concentration-

time data for the four species using a complex Dedrick plot had similar profiles (Mentha and Lu, 1995).

Some of the fields for which the rabbit often serves as a primary experimental model include atherosclerosis, Alzheimer's disease, eye research, osteoarthritis, and tuberculosis (Bosze and Houdebine, 2006).

Chapter 3

Methodology

3.1 Study design

An in vivo drug-drug interaction study was conducted in healthy male rabbits between Norfloxacin and Carbamazepine. The study was carried out on two periods, period one the CBZ will be administered alone as single daily oral dose and in period two the CBZ will be administered along with NFX with ten days washout period in-between. Pharmacokinetic parameters as C_{max}, t_{max} AUC_{0-t}, $AUC_{0-\infty}$, $t_{½}$ and the constant rate of elimination K_e was determined.

3.2 Animals and Sample size

6 healthy male adult rabbits (weighed 3.2-3.5 kg, aged:8-10 months) should be fasted for 12 hr with free access to water (add libitum) before administration of the drug (Venho and Eriksson, 1986; Mapara, Thomas and Bhat, 2012).

The rabbits were obtained from Asdda for animal production and welfare centre, where follow up care and clinical examination were performed and rabbits' health state was certified (Khanunis, Palestine).

3.3 Inclusion criteria

Only healthy, fasted and male rabbits weighing 3.2-3.5 kg are included.

3.4 Exclusion criteria

Any rabbit was fed, sick, recieved any drug prior or within two weeks before the start of study to avoid any drug-drug interaction effect on the results or female rabbits to avoid risk of pregnancy.

3.5 Instrument and Material

3.5.1 Instruments

- Immulite 1000 analyser: (Siemens Healthcare Diagnostic products LTD, United State of America).
- Centrifuge (type H-103N,Tokyo-Japan).
- Micropipettes (Model 5000DG, Japan).
- Refrigerator (Grand silver line, Israel).

3.5.2 Materials

- NFX tablet (400 mg, Eipico, Egypt) and CBZ suspension (100 mg/5ml, Tegretol, Novartis) were used in the study and purchased from a local pharmacy (Gaza, Palestine).
- The rabbits were purchased from asdda centre for animal production and welfare centre (Khanunis, Palestine).
- Immulite 1000 CBZ detection kit (Siemens Healthcare Diagnostics products LTD, United States of America).

3.5.3 Others

- Glass tubes with stopper (5 ml).
- Rabbit restraining box.
- Shaving machine.
- Cannula 23G.
- Local anesthetic.
- Immulite 1000.
- Syringe 5ml.
- Gloves.
- Roll plaster.
- Normal saline 0.9% W/V.
- Blood collection tubes (Vacutanier tubes).
- Alcohol 70%.
- Medical cotton.
- Oral Gavage.

3.6 Experimental process

- Installation of IV-cannula to the ear marginal vein for each rabbit by a veterinary doctor.
- In the first period CBZ suspension is given as single oral dose (40 mg/kg, equivalent to 7 ml tegretol suspension 2%) by a special oral gavage for 10 days.
- On the tenth day CBZ dose is given followed by collection of 1 ml of blood samples in vacutainer tubes according to the time schedule 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0 and 24.0 hours.
- Centrifugation of blood samples at 3000 rpm for 5 minutes to separate serum.
- Transfer serum in a clean tubes and kept at 2-8 ^{o}C until being analyzed.
- Washout period for ten days.

- In the second period CBZ is given alone as a single oral dose (40 mg/kg) for three consecutive days. On the fourth day a single dose of NFX (11.4 mg/kg) was given orally along with CBZ for the following seven days to each rabbit.
- A suspension was prepared by mixing 400 mg NFX in 10 ml distilled water, 7 ml of this suspension were mixed with 49 ml tegretol 2%. From the final suspension 8 ml were given orally to each rabbit.
- On the tenth day CBZ and NFX are given at the same dose followed by collection of 1ml blood samples in vacutainer tubes according to the time schedule 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0 and 24.0 hours.
- Centrifugation of blood samples at 3000 rpm for 5 minutes to separate serum.
- Transfer serum in a clean tubes and kept at 2-8 ^{o}C until being analyzed.

3.7 Analytical procedure

3.7.1 Theory of assay

Analysis of rabbit serum samples to determine the concentration of CBZ was performed at laboratory of Medical Relief Society-Gaza using CBZ detection kit and IMMULITE 1000 System apparatus and (Siemens healthcare Diagnostics).

The Kit is used for rapid quantitative detection of CBZ concentrations in serum for a large number of samples.

IMMULITE 1000 CBZ is a solid phase, competitive, chemiluminescent enzyme immunoassay.

3.7.2 Validation

According to FDA requirements of analytical method (Swarts and Krull, 1997), the applied analytical method in such study must be validated. Validity was performed by manufacturer Siemens Healthcare Diagnostics company (Siemens healthcare Diagnostics, 2014). The kit has limit of detection: 2 mcg/ml, linearity: 10-200 mcg/ml.

The validity of kit for analysis of rabbits serum was tested (see pilot study).

3.8 Data Analysis

The data obtained experimentally was be treated and analyzed by using the following statistical software:

- SPSS program, version 16.
- WinNonlin 9 program to estimate the different pharmacokinetic parameters of the two treatments.
- Sigma plot 11.

A list of statistical methods was used e.g. Descriptive analysis (Mean, standard deviation, coefficient of variation), Paired samples t-test , Independent samples t-test and One way ANOVA test will be done to compare the significance of differences between the two treatments.

3.9 Pilot study

The complete Procedure was applied using 2 rabbits and one carbamazepine syrup to identify the problem which can be faced by dealing with rabbits, collection of blood sample collection, drug intake and the efficacy of analytical method.

Carbamazepine standard was mixed with volunteer and rabbit serum in order to examine the efficacy of kit to determine carbamazepine in serum at a concentration of 60 μg/ml. Five samples of human and rabbit serum were analyzed per Immulite 1000 kit. The statistical analysis had showed no significant difference between both serum samples.

3.10 Limitation of the study

- Lack of fund and the high cost of materials and equipment.
- Israeli siege on Gaza Strip which leads to delay or unavailability of chemicals and instruments.
- Human volunteers are not available to carry out our project. The data will be obtained from animal model.

3.11 Ethical consideration

An ethical approval from Helsinki Committee is not required for animal study in Gaza.
A permission to conduct the study should be obtained from the committee of pharmacy college-post graduate studies, Al-Azhar University-Gaza. The blood collection will follow the recommended published regulations (Parasuraman, Raveendran and Kesavan, 2010).

Chapter 4

Results

4.1 Pharmacokinetic parameters of CBZ

After oral administration of 140 mg of CBZ (40 mg/kg) for the six utilized rabbits for ten days, the blood samples were collected at different times according to the designed time schedule. The pharmacokinetic parameters were calculated, as the maximum plasma concentration **(C_{max})**, the time to reach maximum concentration **(t_{max}),** the area under the plasma concentration-time curve from zero to last measurable concentration (24 hours), **(AUC_{0-24})** and from zero to infinity **($AUC_{0-\infty}$)** also the constant rate of elimination **K_e** and the **$t_{1/2}$** were determined.

The maximum plasma concentration (C_{max}), and the time to reach maximum concentration (t_{max}) were determined directly from the plasma concentration versus time curves. The area under the curve (AUC_{0-24}) was calculated by the linear trapezoidal rule. The area under the curve ($AUC_{0-\infty}$) was determined by following equitation:

$$\mathbf{AUC_{0-\infty} = AUC_{0-t} + C_t / k_e}$$

AUC_{0-t}: is the area under the plasma concentration-time curve from 0 to last measurable concentration.

Ct: is the last measured plasma concentration at time t.

Ke: is elimination rate constant determined by linear regression from the points describing the elimination phase in a logarithmic linear plot.

The half-life (**$t_{1/2}$**) was calculated by following equation:

$$\mathbf{t_{1/2} = 0.693/k_e}$$

4.1.1 Pharmacokinetic parameters of CBZ in the first period

The results of plasma CBZ concentration versus time for each rabbit are shown in tables 4.1, 4.3, 4.5, 4.7, 4.9 and 4.11. The pharmacokinetic parameters per rabbit are summarized in tables 4.2, 4.4, 4.6, 4.8, 4.10 and 4.12. The plasma concentration–time curves for each rabbit after administration last dose in the first period of CBZ alone are illustrated in figures 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6

Table 4.1: Serum CBZ concentration for rabbit no.1 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Time (h)	Conc. (µg/ml)
0.00	1.52
0.50	8.74
1.00	10.32
1.50	10.91
2.00	11.2
2.50	11.53
3.00	11.62
3.50	11.75
4.00	11.94
5.00	12.26
6.00	10.83
24.0	1.12

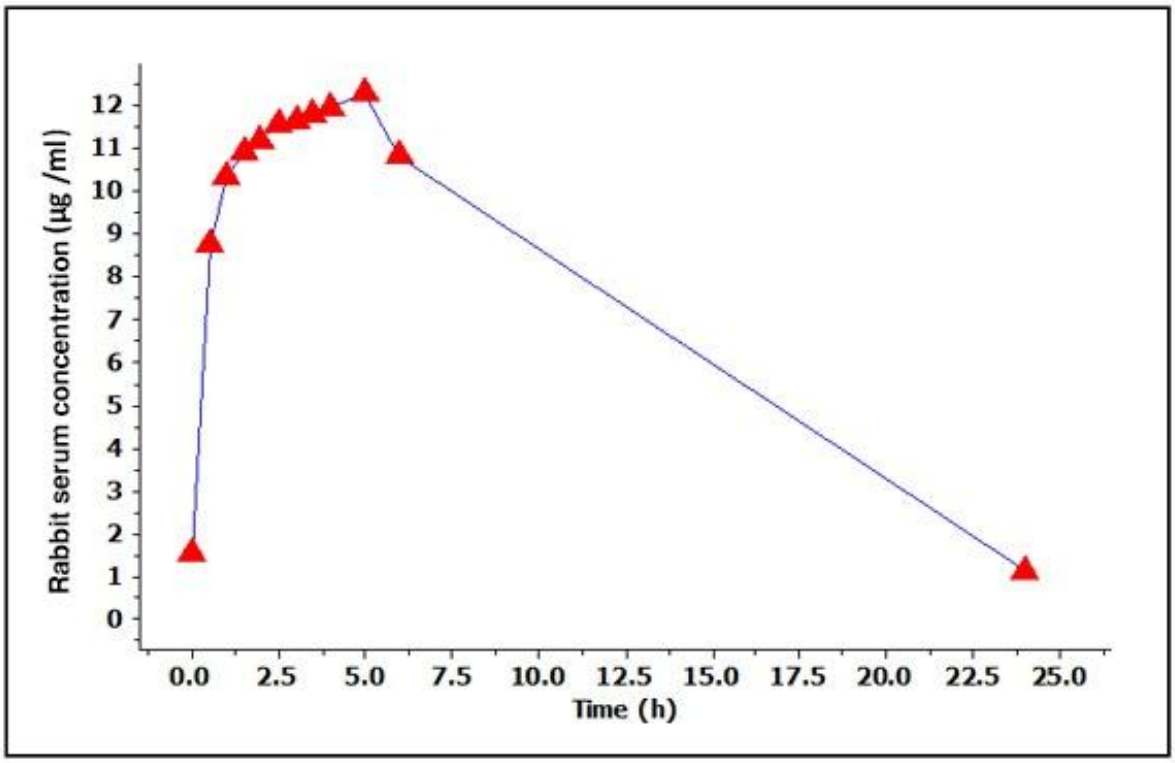

Figure 4.1: Serum concentration-time profile of CBZ for rabbit no.1 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Table 4.2: PK parameters of CBZ for rabbit no.1 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Pharmacokinetic parameters	Value	Units
C_{max}	12.26	µg/ml
t_{max}	5.00	h
Ke	0.0774	h^{-1}
$t_{½}$	8.99	h
AUC_{0-24}	172.59	µg.h/ml
$AUC_{0-\infty}$	187.07	µg.h/ml

Table 4.3: Serum CBZ concentration for rabbit no.2 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Time (h)	Conc. (µg/ml)
0.00	1.31
0.50	5.88
1.00	8.38
1.50	8.64
2.00	8.86
2.50	9.72
3.00	10.41
3.50	10.93
4.00	11.32
5.00	10.88
6.00	10.41
24.0	2.31

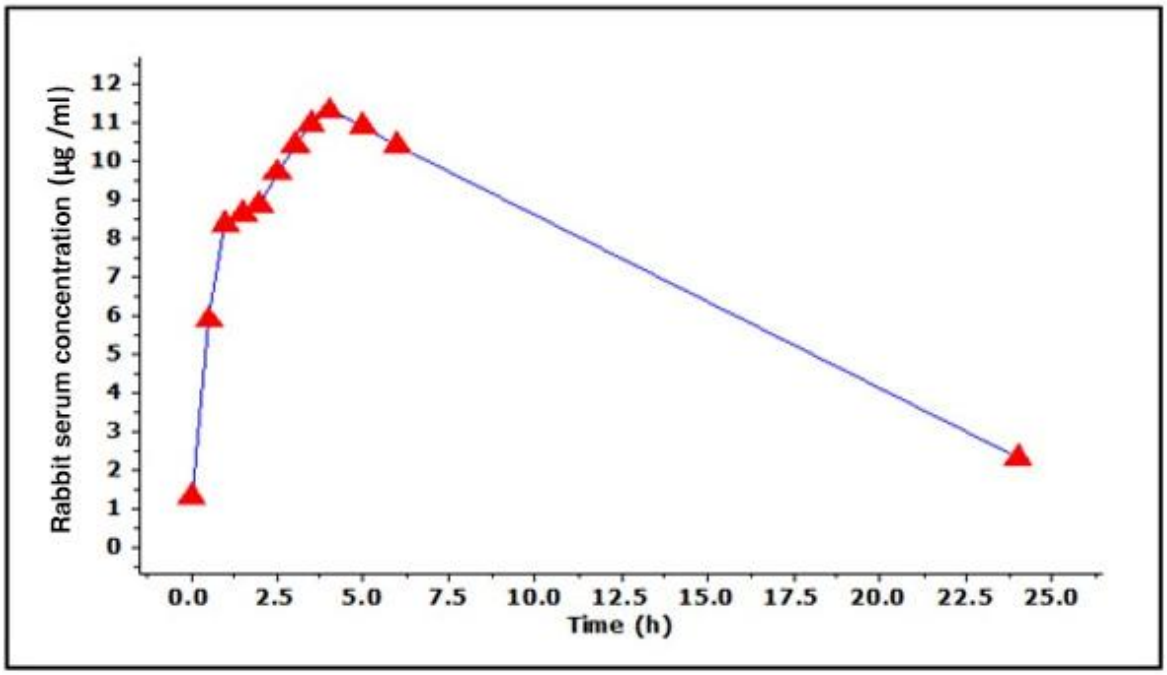

Figure 4.2: Serum concentration-time profile of CBZ for rabbit no.2 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Table 4.4: PK parameters of CBZ for rabbit no.2 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Pharmacokinetic parameters	Value	Units
C_{max}	11.32	µg/ml
t_{max}	4.00	h
Ke	0.036	h^{-1}
$t_{½}$	19.25	h
AUC_{0-24}	170.79	µg.h/ml
$AUC_{0-\infty}$	234.96	µg.h/ml

Table 4.5: Serum CBZ concentration for rabbit no.3 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Time (h)	Conc. (µg/ml)
0.00	1.68
0.50	1.92
1.00	2.43
1.50	3.42
2.00	4.81
2.50	6.54
3.00	7.53
3.50	7.68
4.00	7.94
5.00	8.31
6.00	7.11
24.0	0.98

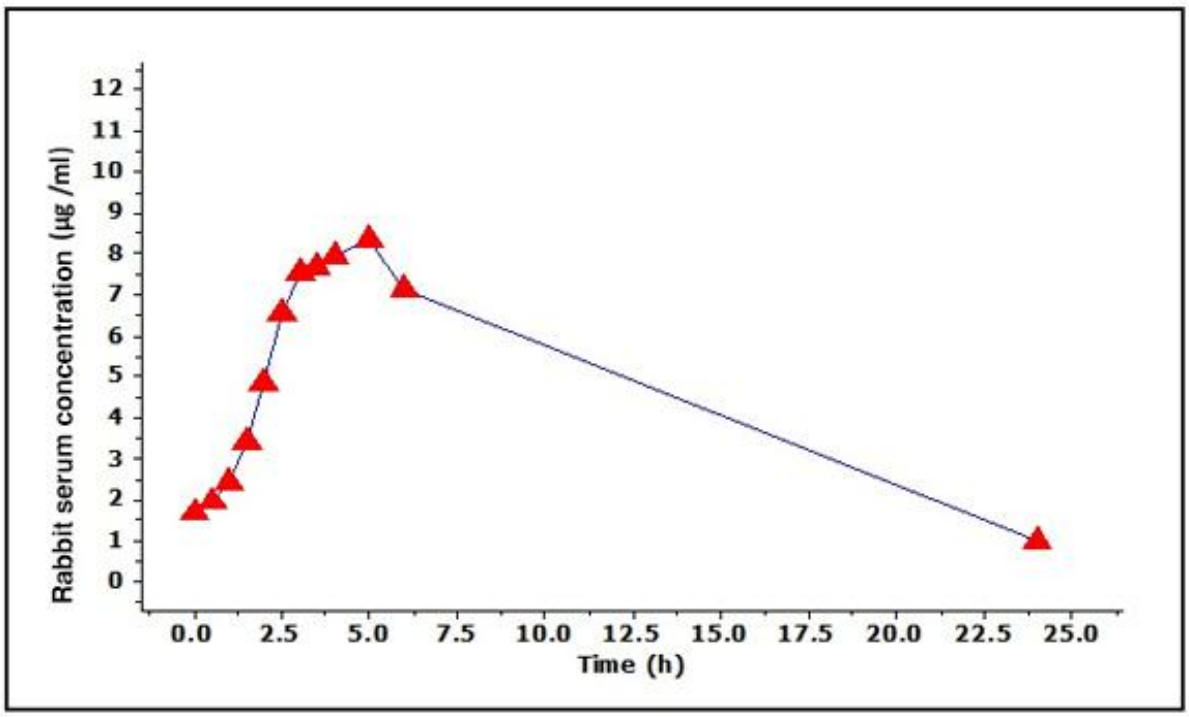

Figure 4.3: Serum concentration-time profile of CBZ for rabbit no.3 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Table 4.6: PK parameters of CBZ for rabbit no.3 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Pharmacokinetic parameters	Value	Units
C_{max}	8.31	µg/ml
t_{max}	5.00	h
Ke	0.045	h^{-1}
$t_{½}$	15.34	h
AUC_{0-24}	108.21	µg.h/ml
$AUC_{0-\infty}$	129.90	µg.h/ml

Table 4.7: Serum CBZ concentration for rabbit no.4 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Time (h)	Conc. (µg/ml)
0.00	3.09
0.50	4.41
1.00	5.92
1.50	7.16
2.00	8.28
2.50	9.71
3.00	10.21
3.50	10.62
4.00	10.97
5.00	9.83
6.00	8.62
24.0	1.03

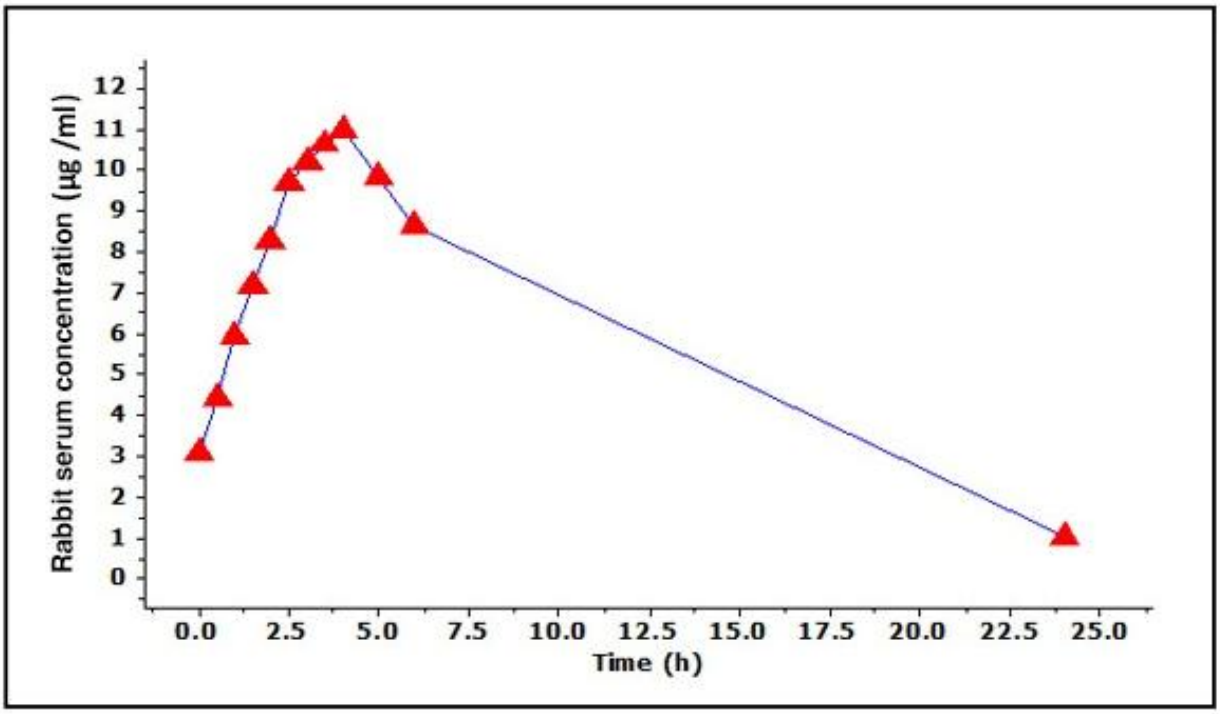

Figure 4.4: Serum concentration-time profile of CBZ for rabbit no.4 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Table 4.8: PK parameters of CBZ for rabbit no.4 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Pharmacokinetic parameters	Value	Units
C_{max}	10.97	µg/ml
t_{max}	4.00	h
Ke	0.073	h^{-1}
$t_{½}$	9.47	h
AUC_{0-24}	138.14	µg.h/ml
$AUC_{0-\infty}$	152.22	µg.h/ml

Table 4.9: Serum CBZ concentration for rabbit no.5 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Time (h)	Conc. (µg/ml)
0.00	0.91
0.50	2.03
1.00	2.54
1.50	2.96
2.00	3.52
2.50	4.31
3.00	4.77
3.50	5.34
4.00	5.83
5.00	4.52
6.00	3.25
24.0	0.72

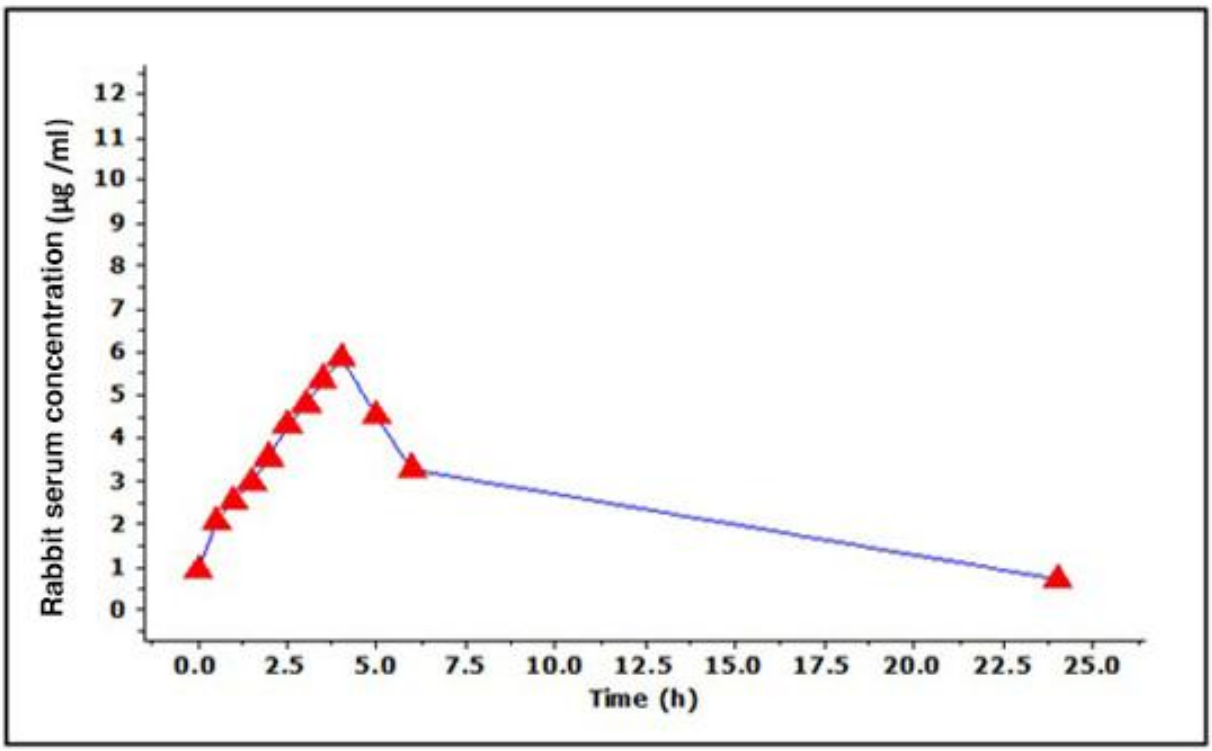

Figure 4.5: Serum concentration-time profile of CBZ for rabbit no.5 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Table 4.10: PK parameters of CBZ for rabbit no.5 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Pharmacokinetic parameters	Value	Units
C_{max}	5.83	µg/ml
t_{max}	4.00	h
Ke	0.0501	h^{-1}
$t_{½}$	13.85	h
AUC_{0-24}	59.21	µg.h/ml
$AUC_{0-\infty}$	73.59	µg.h/ml

Table 4.11: Serum CBZ concentration for rabbit no.6 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Time (h)	Conc. (µg/ml)
0.00	2.25
0.50	3.91
1.00	5.42
1.50	8.63
2.00	8.91
2.50	9.33
3.00	10.42
3.50	10.94
4.00	11.13
5.00	9.22
6.00	8.07
24.0	1.01

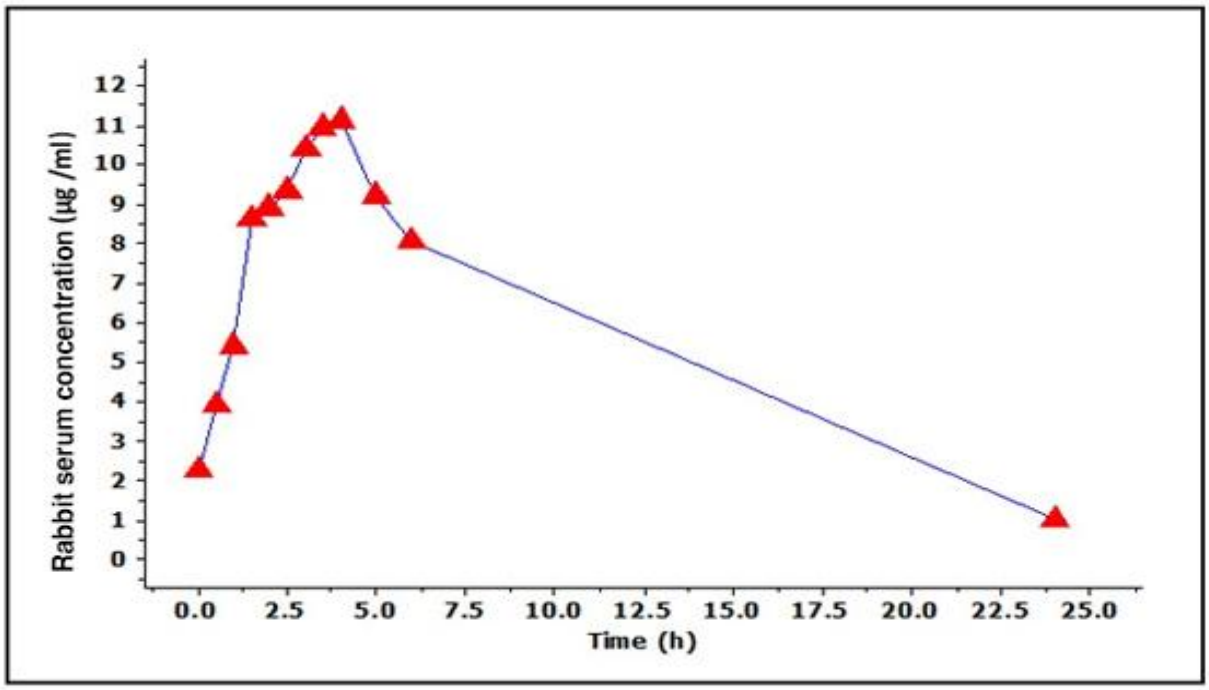

Figure 4.6: Serum concentration-time profile of CBZ for rabbit no.6 in the first period. (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Table 4.12: PK parameters of CBZ for rabbit no.6 in the first period (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Pharmacokinetic parameters	Value	Units
C_{max}	11.13	µg/ml
t_{max}	4.00	h
Ke	0.0701	h^{-1}
$t_{½}$	9.75	h
AUC_{0-24}	132.66	µg.h/ml
$AUC_{0-\infty}$	146.87	µg.h/ml

The mean CBZ concentration in serum for rabbits are listed in table 4.13. In addition further statistical evaluation to determine inter-individual variation are shown as mean, standard deviation (SD), and coefficient of variation (CV).

Furthermore, the mean PK parameters of CBZ in rabbits in the first period are listed in table 4.14.

A plot of the serum concentrations of CBZ versus time for the six rabbits in the first period are illustrated in figure 4.7.

A plot of the mean serum concentration of CBZ versus time in the first period is illustrated in figure 4.8.

Table 4.13: Serum concentration of CBZ versus time and statistical evaluation for rabbits (n=6) in the first period (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Time (h)	CBZ serum concentration (µg/ml)						Statistical parameters		
	R1	R2	R3	R4	R5	R6	Mean	SD	CV
0.00	1.52	1.31	1.68	3.09	0.91	2.25	1.793	0.773	43.13
0.50	8.74	5.88	1.92	4.41	2.03	3.91	4.48	2.57	57.33
1.00	10.32	8.38	2.43	5.92	2.54	5.42	5.84	3.14	53.77
1.50	10.91	8.64	3.42	7.16	2.96	8.63	6.95	3.16	45.38
2.00	11.2	8.86	4.81	8.28	3.52	8.91	7.60	2.87	37.77
2.50	11.53	9.72	6.54	9.71	4.31	9.33	8.52	2.62	30.69
3.00	11.62	10.41	7.53	10.21	4.77	10.42	9.16	2.54	27.72
3.50	11.75	10.93	7.68	10.62	5.34	10.94	9.54	2.49	26.11
4.00	11.94	11.32	7.94	10.97	5.83	11.13	9.85	2.41	24.53
5.00	12.26	10.88	8.31	9.83	4.52	9.22	9.17	2.66	28.96
6.00	10.83	10.41	7.11	8.62	3.25	8.07	8.05	2.74	34.05
24.0	1.12	2.31	0.98	1.03	0.72	1.01	1.19	0.56	47.07

SD: standard deviation CV: coefficient of variation R: Rabbit

Table 4.14: PK parameters of CBZ in the first period and statistical evaluation for rabbits (n=6) (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

Rabbit no.	C_{max}	t_{max}	T ½	Ke	AUC $_{0-24}$	AUC $_{0-\infty}$
1	12.26	5.00	8.99	0.0774	172.59	187.07
2	11.32	4.00	19.25	0.036	170.79	234.96
3	8.31	5.00	15.34	0.0452	108.21	129.90
4	10.97	4.00	9.47	0.0732	138.14	152.22
5	5.83	4.00	13.85	0.0501	59.21	73.59
6	11.13	4.00	9.75	0.0701	132.66	146.87
Mean	9.970	4.33	12.78	0.0586	130.30	154.1
SD	2.42	0.156	4.10	0.0171	42.5	54.3
CV	24.28	11.92	32.10	29.14	32.64	35.24

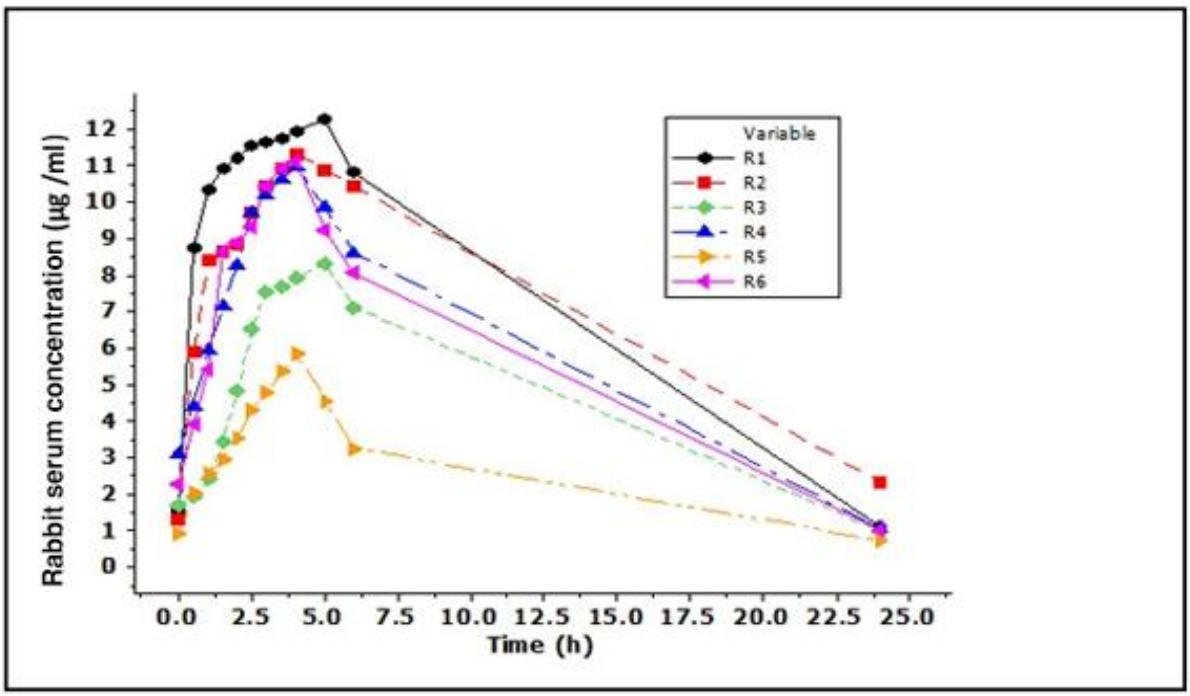

Figure 4.7: A plot of the serum concentrations of CBZ versus time for the six rabbits (n = 6) in the first period (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

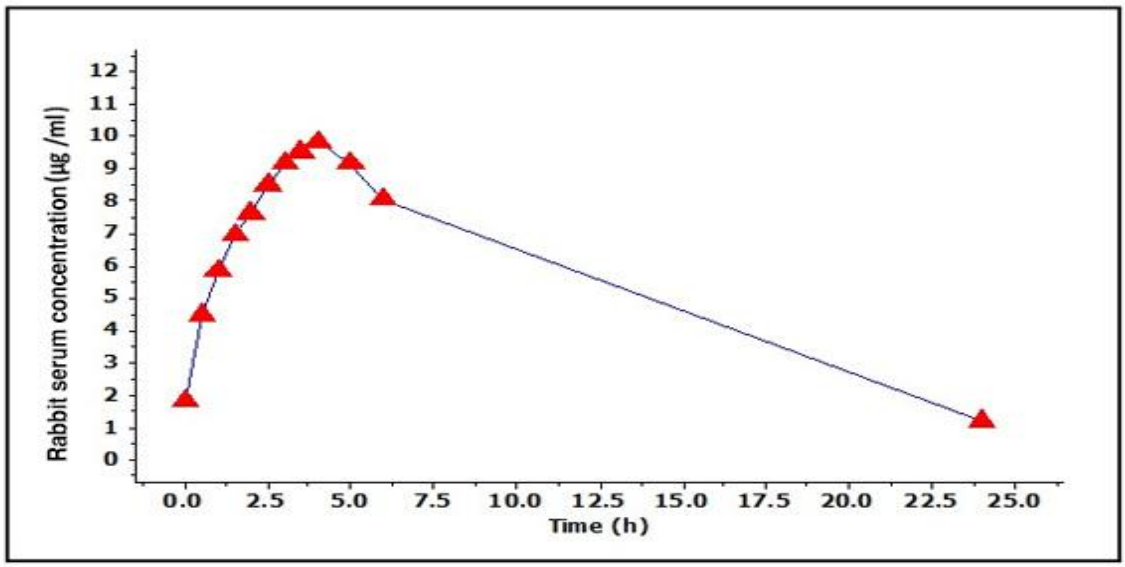

Figure 4.8: A plot of the mean serum concentration of CBZ versus time for the six rabbits (n = 6)in the first period (CBZ given orally 40 mg/kg/day for ten days, blood samples after last dose).

4.1.2 Pharmacokinetic parameters of CBZ in the second period

The results of serum CBZ concentration versus time for each rabbit are shown in tables 4.15, 4.17, 4.19, 4.21, 4.23 and 4.25 and the corresponding pharmacokinetic parameters are listed in tables 4.16, 4.18, 4.20, 4.22, 4.24 and 4.26. The serum concentration–time plot for each rabbit after oral administration of CBZ (40 mg/kg) coadministered with Norfloxacin (11.4 mg/kg) are illustrated in figures 4.9, 4.10, 4.11, 4.12, 4.13 and 4.14.

Table 4.15: Serum CBZ concentration for rabbit no.1 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX11.4 mg/kg/day was administered concurrently with CBZ).

Time (h)	Conc. (µg/ml)
0.00	1.42
0.50	2.31
1.00	3.72
1.50	4.56
2.00	4.74
2.50	4.96
3.00	5.11
3.50	5.31
4.00	5.92
5.00	6.51
6.00	7.28
24.0	2.11

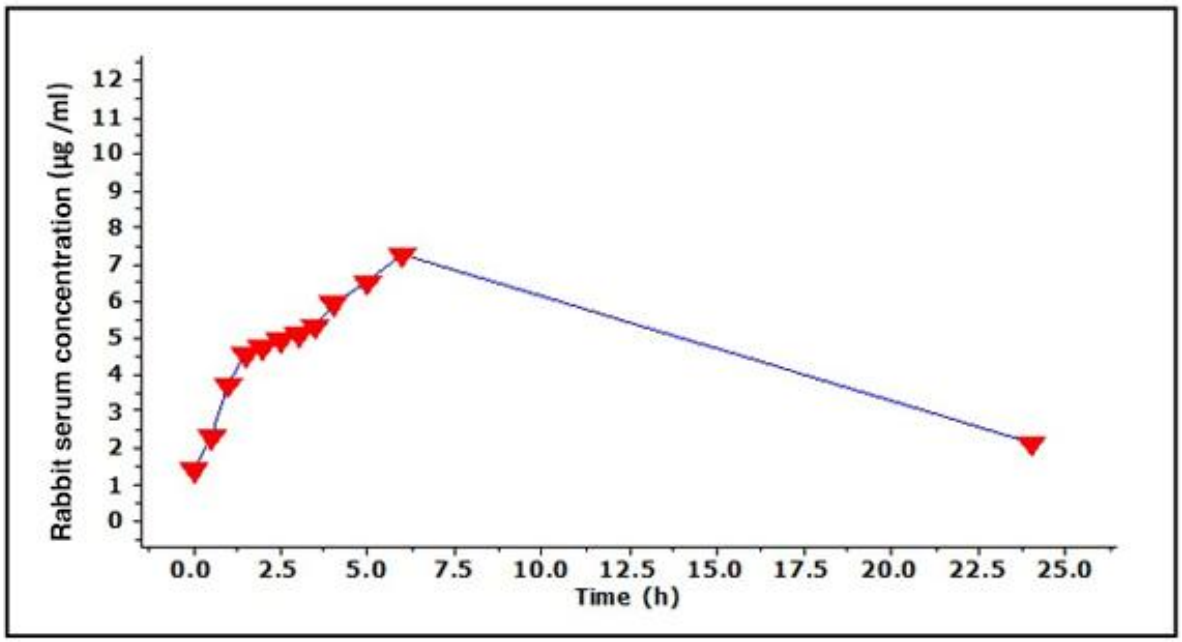

Figure 4.9: Serum concentration-time profile of CBZ concentration for rabbit no.1 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4mg/kg/day was administered concurrently with CBZ).

Table 4.16: PK parameters of CBZ for rabbit no.1 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Pharmacokinetic parameters	Value	Units
C_{max}	7.28	µg/ml
t_{max}	6.0	h
Ke	0.0139	h^{-1}
$t_{½}$	49.97	h
AUC_{0-24}	114.81	µg.h/ml
$AUC_{0-\infty}$	266.94	µg.h/ml

Table 4.17: Serum CBZ concentration for rabbit no.2 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Time (h)	Conc. (µg/ml)
0.00	1.62
0.50	4.21
1.00	11.92
1.50	12.41
2.00	12.83
2.50	12.97
3.00	11.42
3.50	10.63
4.00	9.91
5.00	9.62
6.00	9.15
24.0	2.61

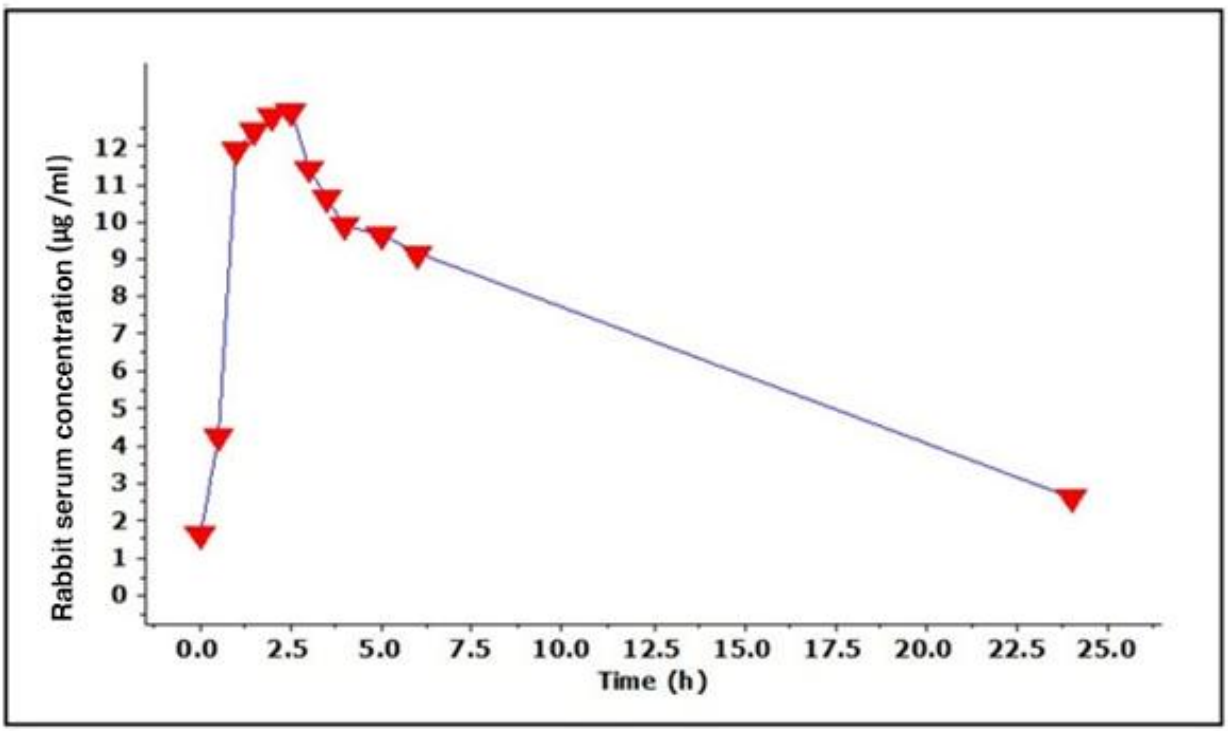

Figure 4.10: Serum concentration-time profile of CBZ concentration for rabbit no.2 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Table 4.18: PK parameters of CBZ for rabbit no.2 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Pharmacokinetic parameters	Value	Units
C_{max}	12.97	µg/ml
t_{max}	2.50	h
Ke	0.038	h^{-1}
$t_{½}$	17.86	h
AUC_{0-24}	166.06	µg.h/ml
$AUC_{0-\infty}$	233.33	µg.h/ml

Table 4.19: Serum CBZ concentration for rabbit no.3 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Time (h)	Conc. (µg/ml)
0.00	1.83
0.50	3.17
1.00	3.46
1.50	3.84
2.00	4.08
2.50	4.29
3.00	4.58
3.50	4.97
4.00	5.33
5.00	5.69
6.00	4.71
24.0	1.06

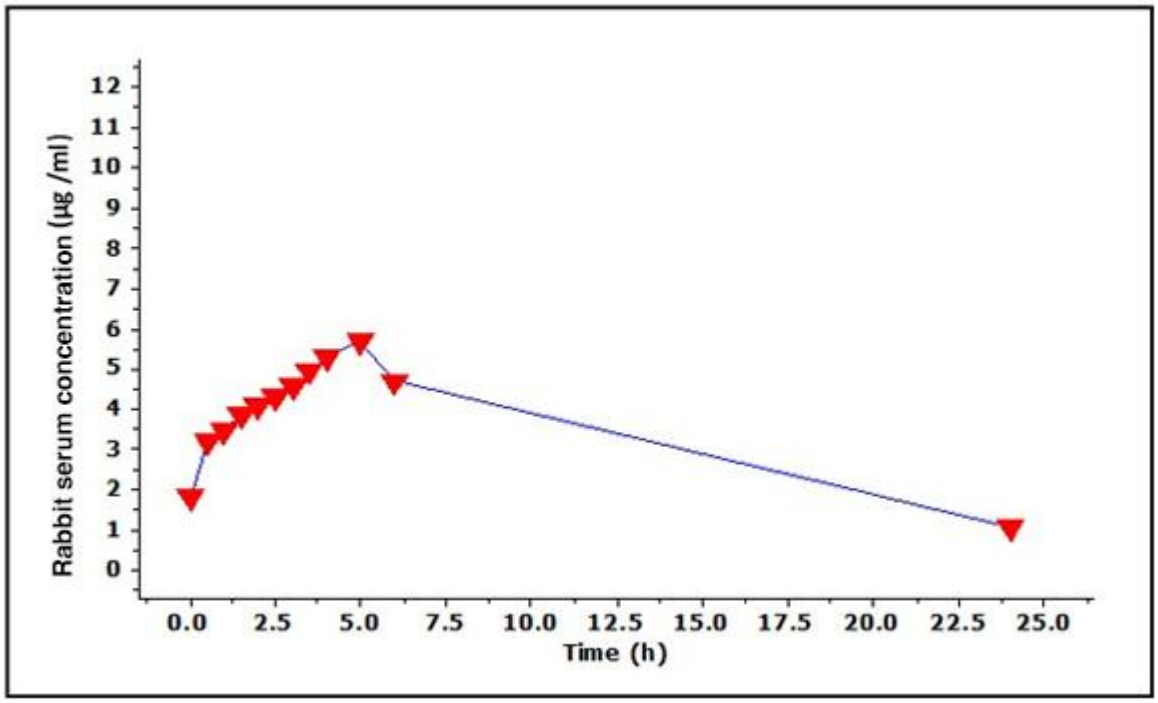

Figure 4.11: Serum concentration-time profile of CBZ for rabbit no.3 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Table 4.20: PK parameters of CBZ for rabbit no.3 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Pharmacokinetic parameters	Value	Units
C_{max}	5.69	µg/ml
t_{max}	5.0	h
Ke	0.0468	h^{-1}
$t_{½}$	14.80	h
AUC_{0-24}	78.62	µg.h/ml
$AUC_{0-\infty}$	101.26	µg.h/ml

Table 4.21: Serum CBZ concentration for rabbit no.4 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Time (h)	Conc. (µg/ml)
0.00	2.21
0.50	4.89
1.00	5.74
1.50	6.56
2.00	7.37
2.50	7.94
3.00	8.23
3.50	8.87
4.00	9.12
5.00	8.62
6.00	6.98
24.0	1.16

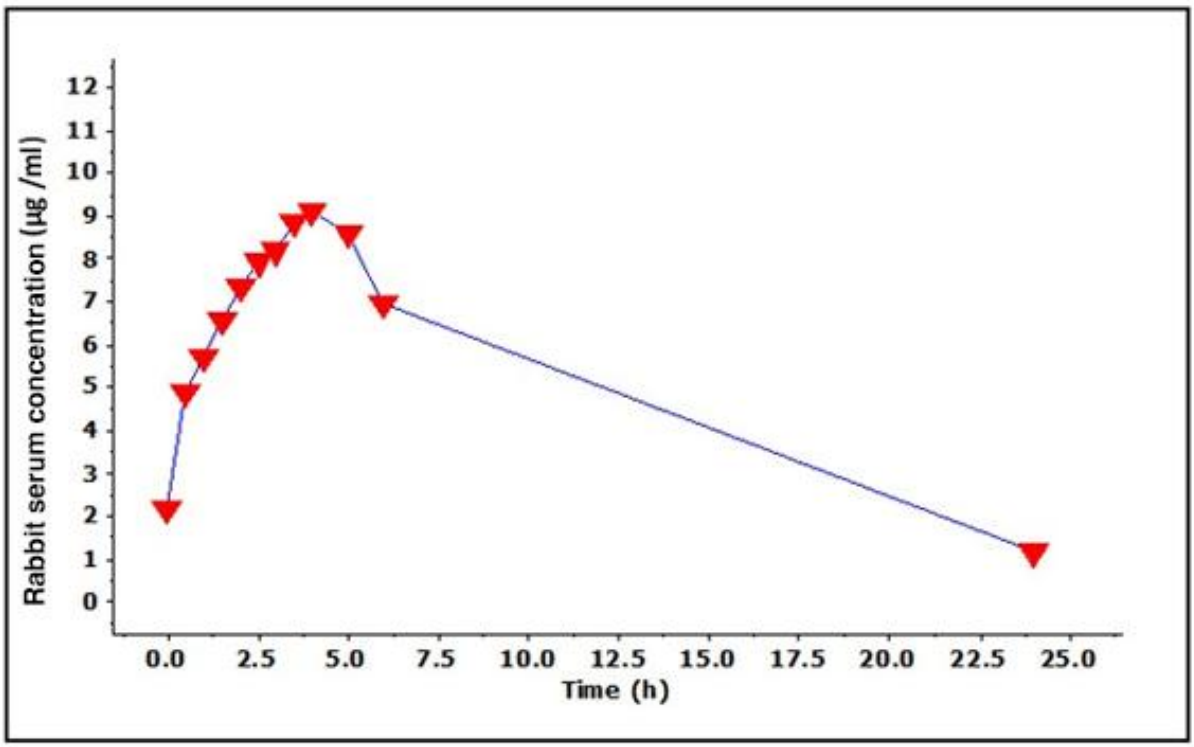

Figure 4.12: Serum concentration-time profile of CBZ for rabbit no.4 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Table 4.22: PK parameters of CBZ for rabbit no.4 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Pharmacokinetic parameters	Value	Units
C_{max}	9.12	µg/ml
t_{max}	4.0	h
Ke	0.0631	h^{-1}
$t_{½}$	10.98	h
AUC_{0-24}	117.56	µg.h/ml
$AUC_{0-\infty}$	135.94	µg.h/ml

Table 4.23: Serum CBZ concentration for rabbit no.5 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Time (h)	Conc. (µg/ml)
0.00	1.96
0.50	3.79
1.00	4.12
1.50	4.66
2.00	4.98
2.50	5.64
3.00	5.97
3.50	6.33
4.00	6.94
5.00	7.41
6.00	6.2
24.0	0.94

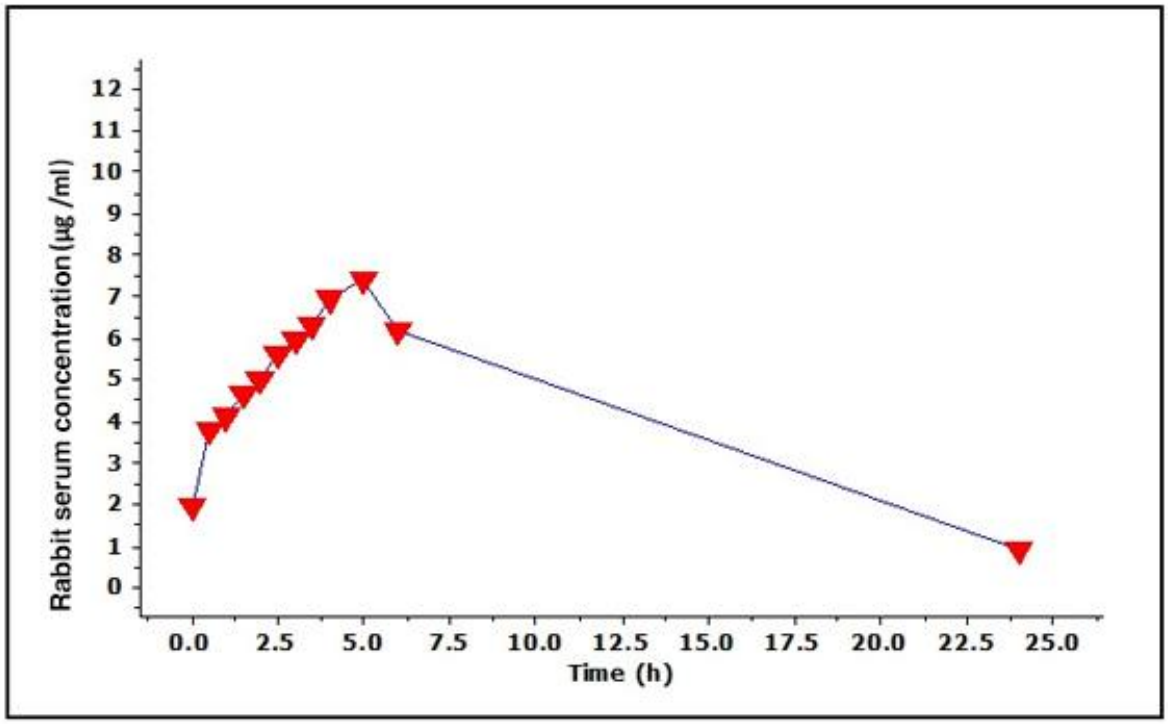

Figure 4.13: Serum concentration-time profile of CBZ for rabbit no.5 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Table 4.24: PK parameters of CBZ for rabbit no.5 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Pharmacokinetic parameters	Value	Units
C_{max}	7.41	µg/ml
t_{max}	5.0	h
Ke	0.0593	h^{-1}
$t_{½}$	11.69	h
AUC_{0-24}	98.21	µg.h/ml
$AUC_{0-\infty}$	114.07	µg.h/ml

Table 4.25: Serum CBZ concentration for rabbit no.6 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Time (h)	Conc. (µg/ml)
0.00	1.34
0.50	2.22
1.00	2.84
1.50	3.36
2.00	4.29
2.50	5.48
3.00	5.97
3.50	6.61
4.00	7.51
5.00	7.93
6.00	6.87
24.0	1.31

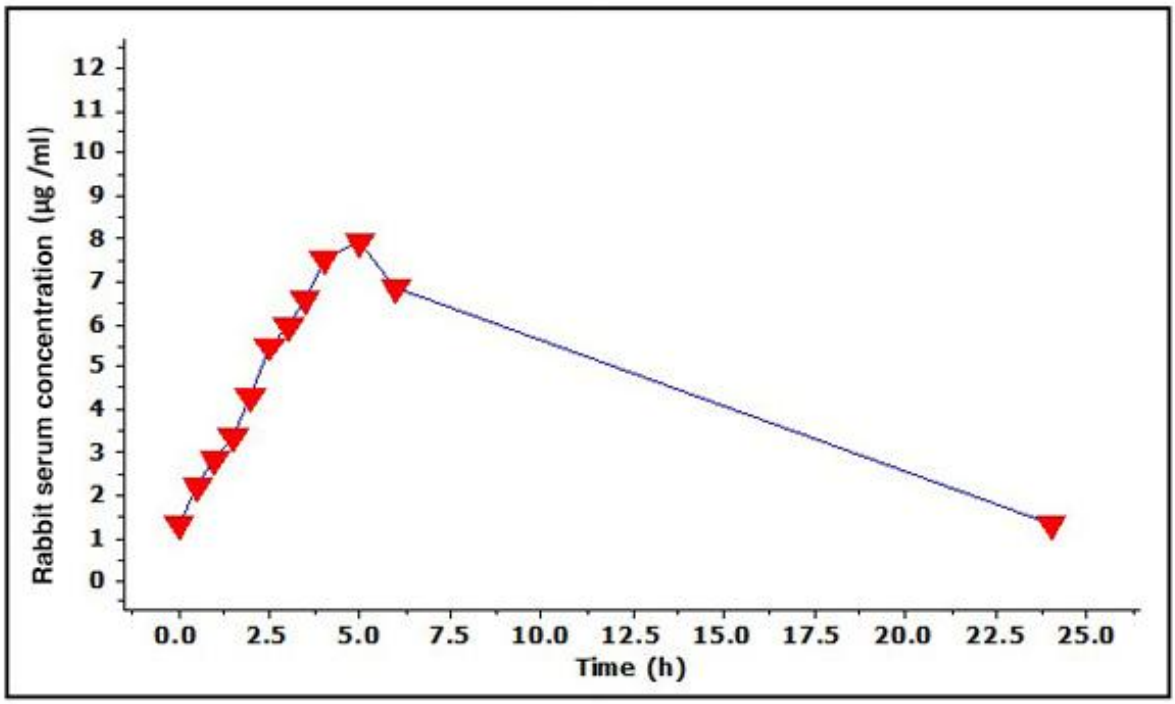

Figure 4.14: Serum concentration-time profile of CBZ for rabbit no.6 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Table 4.26: PK parameters of CBZ for rabbit no.6 in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Pharmacokinetic parameters	Value	Units
C_{max}	7.930	µg/ml
t_{max}	5.000	h
Ke	0.0305	h^{-1}
$t_{½}$	22.71	h
AUC_{0-24}	106.33	µg.h/ml
$AUC_{0-\infty}$	149.26	µg.h/ml

The mean CBZ concentration in serum when administerd concurrently with NFX (11.4 mg/kg) for the six rabbits in the second period are listed in table **4.27**. In addition, further statistical evaluation to determine inter-individual variation are shown e.g. mean, standard deviation (SD), and coefficient of variation (CV).

Furthermore, the mean PK parameters of CBZ in the second period for the six rabbits are listed in table **4.28**

The serum concentrations of CBZ versus time in the second period for the six rabbits are illustrated in figure **4.15**

A plot of the mean serum concentration of CBZ versus time in the second period is illustrated in figure **4.16**

Table 4.27: Serum concentration of CBZ versus time and statistical evaluation in rabbits (n=6) in the second period. (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Time (h)	CBZ serum concentration (µg/ml)						Statistical parameters		
	R1	R2	R3	R4	R5	R6	Mean	SD	CV
0.00	1.42	1.62	1.83	2.21	1.96	1.34	1.73	0.333	19.23
0.50	2.31	4.21	3.17	4.89	3.79	2.22	3.432	1.064	30.99
1.00	3.72	11.92	3.46	5.74	4.12	2.84	5.30	3.39	63.90
1.50	4.56	12.41	3.84	6.56	4.66	3.36	5.90	3.37	57.16
2.00	4.74	12.83	4.08	7.37	4.98	4.29	6.38	3.37	52.86
2.50	4.96	12.97	4.29	7.94	5.64	5.48	6.88	3.23	46.92
3.00	5.11	11.42	4.58	8.23	5.97	5.97	6.88	2.55	37.06
3.50	5.31	10.63	4.97	8.87	6.33	6.61	7.120	2.198	30.88
4.00	5.92	9.91	5.33	9.12	6.94	7.51	7.45	1.78	23.95
5.00	6.51	9.62	5.69	8.62	7.41	7.93	7.630	1.42	18.62
6.00	7.28	9.15	4.71	6.98	6.2	6.87	6.865	1.45	21.10
24.0	2.11	2.61	1.06	1.16	0.94	1.31	1.532	0.672	43.87

SD: Standard deviation CV: Coefficient of variation R: Rabbit

Table 4.28: PK parameters of CBZ in the second period and statistical evaluation for rabbits (n=6). (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

Rabbit no.	C_{max}	t_{max}	$T_{½}$	Ke	AUC_{0-24}	$AUC_{0-\infty}$
1	7.28	6.00	49.97	0.0139	114.81	266.94
2	12.97	2.50	17.86	0.038	166.06	233.33
3	5.69	5.00	14.80	0.0468	78.62	101.26
4	9.12	4.00	10.98	0.0631	117.56	135.94
5	7.41	5.00	11.69	0.0593	98.21	114.07
6	7.93	5.00	22.71	0.0305	106.33	149.26
Mean	8.40	4.58	21.34	0.0419	113.6	166.8
SD	2.50	1.20	14.68	0.0184	29.30	67.50
CV	29.74	26.20	68.78	44.04	25.76	10.48

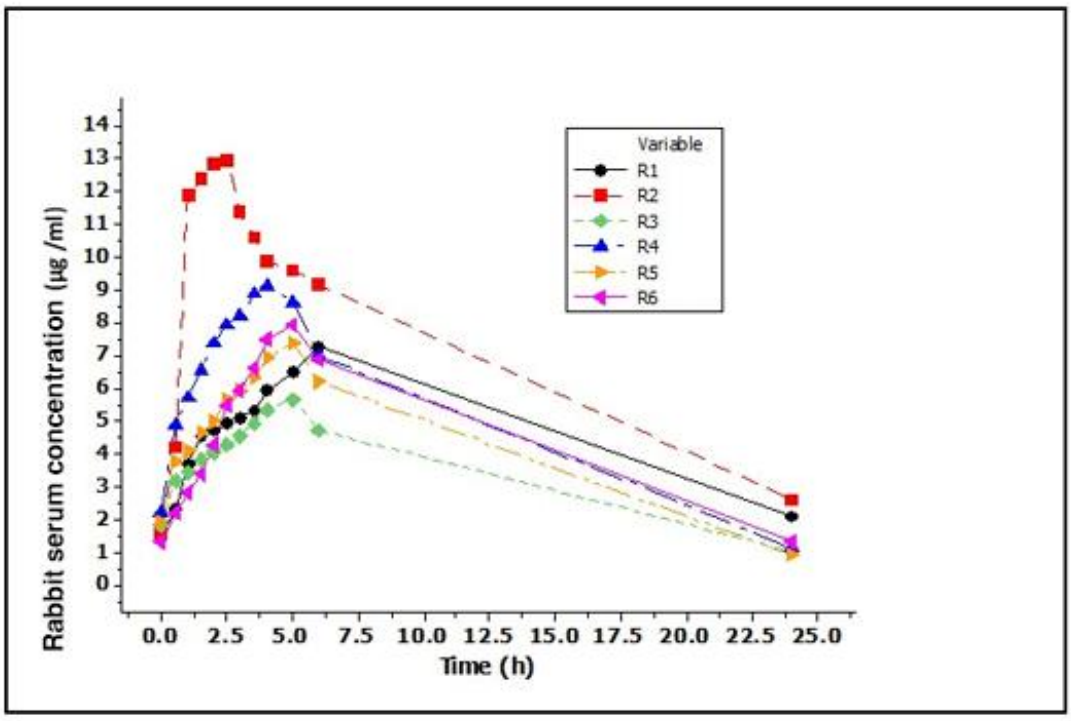

Figure 4.15: A plot of the serum concentrations of CBZ versus time for the six rabbits (n = 6) in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

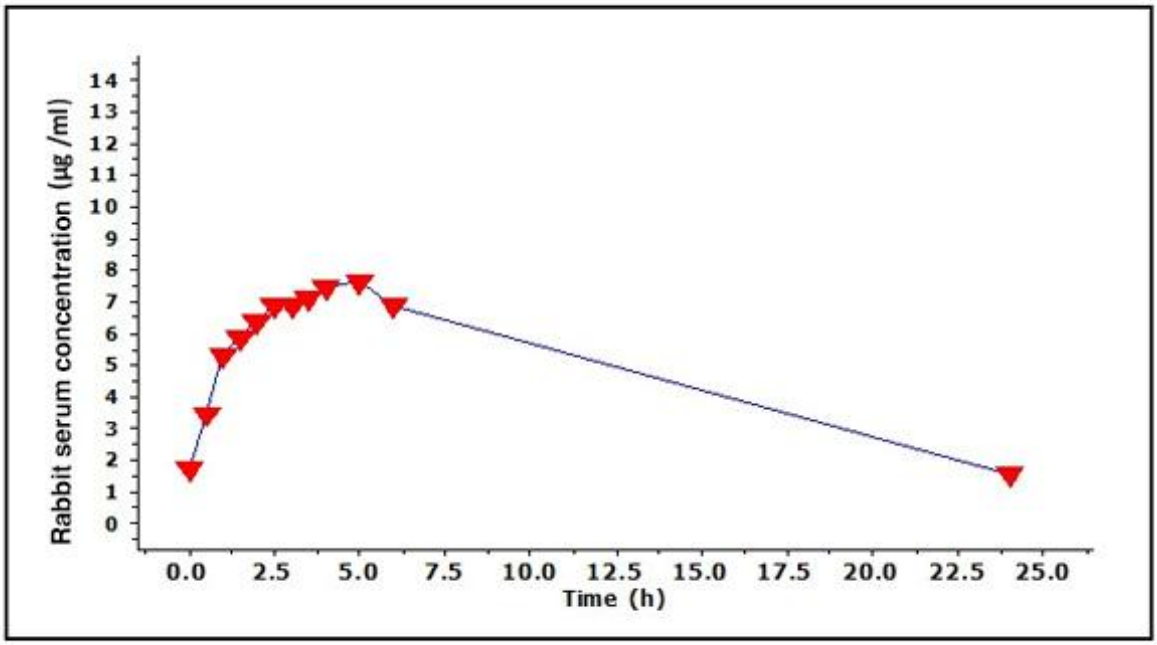

Figure 4.16: A plot of the mean serum concentration of CBZ versus time for the six rabbits (n = 6) in the second period (CBZ given orally 40 mg/kg/day for ten days, on the fourth day NFX 11.4 mg/kg/day was administered concurrently with CBZ).

4.2 Statistical evaluation of data

4.2.1 Comparison between PK of CBZ in the two period

Table 4.29 showed paired test t-test for quality between mean PK parameters (C_{max}, t_{max}, ke, AUC_{0-24}, $AUC_{0-\infty}$) of CBZ in the first and second period in rabbits.

No significant difference in PK parameters of CBZ was found when CBZ was administered in the first period or in the second period ($p>0.05$).

A plot of an overlap mean serum concentration-time profile of CBZ in the first and second periods are illustrated in figure 4.17. The curves obtained in both cases were obviously comparable.

Table 4.29: Paired-samples t test for the equality between the means of the pharmacokinetic parameters of CBZ in both periods.

Parameter	Group	N	Mean	Standard deviation	Difference	T	P-value [a]
C_{max}	**First period** [b]	6	9.970	2.42	5	1.43	**0.210**
	Second period [c]	6	8.400	2.50			
t_{max}	**First period**	6	4.330	0.516	5	-0.620	**0.562**
	Second period	6	4.580	1.20			
$t_{1/2}$	**First period**	6	12.77	4.100	5	-1.246	**0.268**
	Second period	6	21.33	14.67			
Ke	**First period**	6	0.0587	0.0171	5	1.429	**0.212**
	Second period	6	0.0419	0.0184			
AUC_{0-24}	**First period**	6	130.3	42.51	5	1.265	**0.262**
	Second period	6	113.6	29.26			
$AUC_{0-\infty}$	**First period**	6	154.1	54.31	5	-0.771	**0.476**
	Second period	6	166.8	67.51			

a: Significant statistical difference ($p \leq 0.05$), b: first period: CBZ was administerd orally as single dose (40 mg/kg) for ten days, c: second period: CBZ was administered as above, on fourth day NFX (11.4 mg/kg) was given concurrently.

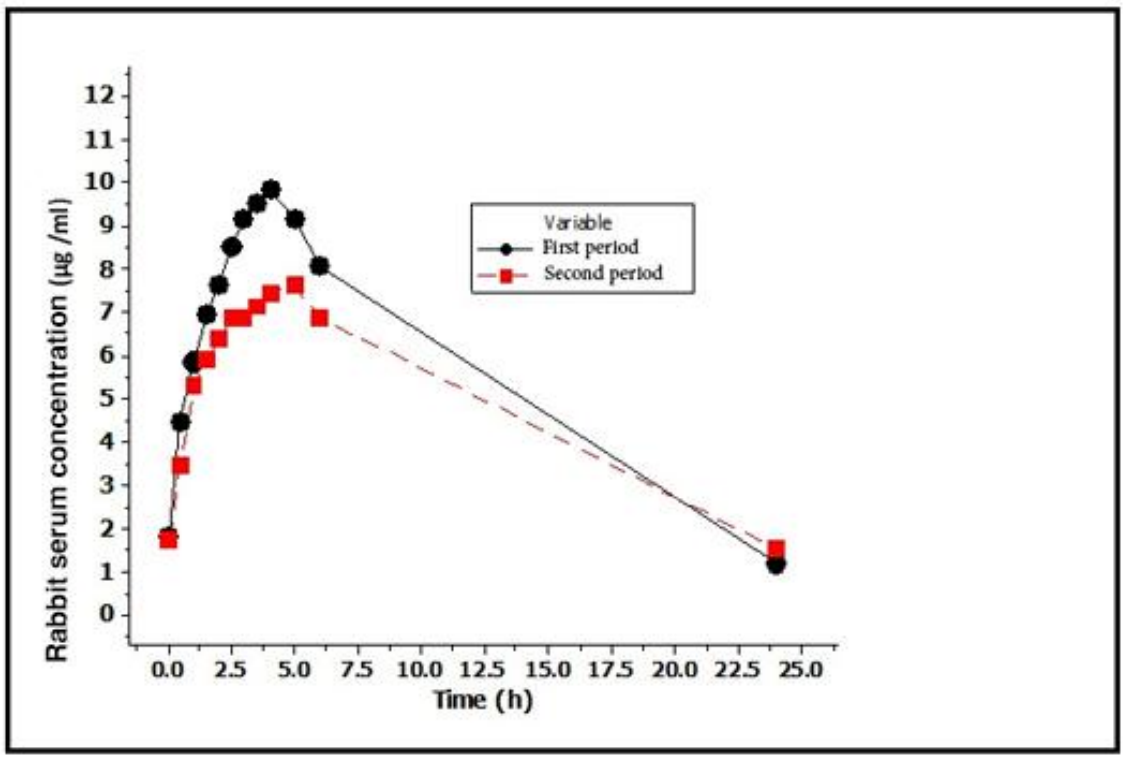

Figure 4.17: Mean serum concentration-time profile of CBZ in the first period and second period in rabbits (n=6).

Chapter 5

Discussions

CBZ as anti-epileptic drug is used lifelong. It is a substrate of CYP3A4. CBZ has narrow therapeutic index. Inhibition of CYP3A4 can result in the accumulation of parent drug that can put the patient at increased risk for side effects or possible toxicity.

Rabbits were ideal animals for studying the pharmacokinetic parameters and drug-drug interaction between CBZ and NFX. They were easy to handle for giving multiple oral doses of drugs and collecting blood samples. In rabbits it has been seen that the isoenzyme CYP3A6 correspond to the CYP3A4 activity in human hepatocytes (Mesdjian et al., 2001). Hence, drugs like CBZ that are metabolized by CYP3A4 in human will be biotransformed by CYP3A6 in rabbits. Any drug interaction occurring due to an effect on this particular cytochrome i.e. CYP3A6 in rabbits will correlate to an interaction at CYP3A4 levels in humans.

Statistical analysis of PK of CBZ showed no significance difference between the two periods by using paired sample t-test (**Table 4.29**)

From statistical treatment, insignificant difference in C_{max} was observed. The mean C_{max} was decreased from 9.970 μg/ml in the first period to 8.400 μg/ml in the second period (P-value=0.210).

The decrease in the C_{max} of CBZ in the second period because NFX may interfer with the absorption of CBZ when used concurrently at the same time.

A slight decrease in the elimination phase was observed (ke= 0.0587 and 0.0419h^{-1} for the first and second period, respectively). This effect was statistically insignificant(p= 0.212).

Similar results were found by studying the effect of NFX on PK of caffeine (Carbo et al., 1989) and theophylline at steady state (Bowles et al., 1988).

The elimination half life $t_{1/2}$ was increased ($t_{1/2}$ =12.77 h and 21.33h for the first and second period, respectively). This is due to inhibitory effect of NFX on CBZ metabolism which was little and remained statistically insignificant (p=0.268).

These results are similar to those recorded in drug interaction studies, applied to investigate the effect of NFX on PK of warfarin (Rocci et al., 1990) and theophylline (Bowles et al., 1988).

In contrast, a fluoroquinolone antibiotic " ciprofloxacine " had significantly increased C_{max}, AUC and t ½ of CBZ, while CL and Vd of CBZ were decreased significantly when CBZ was administered concurrently with ciprofloxacin (single dose 500 mg) by adult volunteers (shahadi et al., 2011).

CBZ is one of the drug after multiple doses, can stimulate the synthesis of enzymes that catalyze its own metabolism by a process known as auto-induction (Patsalos, 2013; Punyawudho et al., 2009). An autoinduction effect can be observed after 2 weeks of treatment (Parihar et al., 2006). This can explain the insignificant effect of NFX on CBZ. CBZ may increase its metabolism during the ten days of treatment which may abolish the effect of NFX on the PK of CBZ. Autoinduction of CBZ metabolism appeared to be complete within 1 week of starting CBZ therapy or dose change, and its degree was linearly related to CBZ daily dose (Kudriakova et al., 1992).

In this study CBZ was given for ten days during which metabolism of CBZ can be induced. This can abolish the expected inhibitory effect of NFX on metabolizing enzymes.

In addition, flouroquinolone antibiotics e.g. Norfloxacin and Ofloxacin have been shown to be a subject to active efflux (Cao et al., 1995; Rabbaa et al., 1996). An inhibitory effect of NFX is dose dependent. NFX may be effluxed back to intestine.

A crossover study design is recommended to minimize the subject variability between the two group of treatments (Chow and Liu, 2008).

CLEIA technique which was used to estimate the serum levels of CBZ at different times after administration was very convenient and time efficient. A rapid quantitative analysis for serum samples (n= 132) were achieved by this method.

During the course of antiepileptic therapy, the drug should maintain a certain therapeutic level in the plasma or serum. The therapeutic concentration of carbamazepine has been reported as, 5- 12 μg mL^{-1} (Alexander, 2007).

Chapter 6
Conclusions

- In our study conditions NFX had statistically insignificant effect on PK of CBZ (C_{max}, t_{max}, $t_{1/2}$, k_e, AUC_{0-t} and $AUC_{0-\infty}$) at therapeutic doses in rabbits.
- According to the conducted experimental conditions CBZ and NFX can be used safely with precautions or dose monitering.
- NFX can be used as preferable alternative antibiotic to ciprofloxacin (which significantly increase plasma concentration of CBZ) in patient under CBZ treatment.
- Rabbits were very convenient choice for the pharmacokinetic drug-drug interaction studies.
- CBZ and NFX were well tolerated by rabbits at the given dose . No adverse events were reported during the study.
- A small inter-individual variation recorded among the utilized male rabbits
- The analytical method (CLEIA) was simple but cost effective.

Chapter 7

Recommendations

- Conduction of drug-drug interaction studies on human to confirm the obtained results.
- Development regulations by corresponding authorities in Palestine e.g. Ministry of Health, Medical Faculties and Pharmaceutical Industry for use of animals in studies of drug-drug interaction.
- Planning of strategies and programs to conduct drug drug interaction studies in human.
- It is very important to establish scientific center for drug-drug and food-drug interactions to ensure safety and efficacy of drugs used in Palestine.
- Introducing of pharmacokinetic studies of drugs in hospitals to facilitate drug monitoring as an important step for starting of clinical pharmacy program, and as step for development of pharmaceutical medical profession.

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