A PHARMACOKINETIC INTERACTION BETWEEN PROPRANOLOL AND *Eurycoma longifolia* (TONGKAT ALI)

by

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Praised be to ALLAH

{الحمد لله رب العالمين, الحمد لله ملئ السماوات والأرض, الحمد لله عدد خلقه وقدر عظيم سلطانه, الحمد لله الّذي
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LIST OF ABBREVIATIONS

WHO	World Health Organization
AUC	Area under the curve
T_{max}	Time of maximum plasma concentration
C_{max}	Maximum plasma concentration.
Vd	Volume of distribution
CL	Clearance
Cp	Plasma concentration
k_{el}	Elimination rate constant
$t_{1/2}$	Half-life
t_{eff}	Duration of action
C_{eff}	Effective concentration
P-gp	P-glycoprotein
CYP3A4	Cytochromes P450 enzymes
PPB	Plasma protein binding
CYP	Cytochrome P450
CNS	Central nervous system
GIT	Gastrointestinal tract
BHAT	Beta-Blocker Heart Attack Trial
EMs	Extensive metabolizers
PMs	Poor metabolizers
E. longifolia	Eurycoma longifolia
HPLC	High performance liquid chromatography
PDA	Photodiode array detector
LLOQ	lower limit of quantification
CV	Voefficient of variation
LOD	Limit of deticti on
rpm	round per minute
µm	micrometer
ml	milliliter
µl	microliter
nm	nanometer
ng	nanogram
ng/ml	nanogram/milliliter
mg	milligram
QC	Quality Control

USM	Universiti Saince Malaysia
BMI	Body mass index
ECG	Electrocardiogram
Kg/ m^2	kiliogram/meter square
λZ	Lambda Z range
df	degree of freedom
PRN	propranolol
OXP	oxprenolol
R^2	coefficient of correlation
Min	minute
SD	stander deviation
Sys BP	systolic blood pressure
Dias BP	diastolic blood pressure
PL	placebo
T.Ali	Tongkat Ali
MAX	MXIMUM
MINI	Minimum
hr	hour
In	natural log
p	P-value
SS	sum square
F	Statistical F value
DCA	Drug Control Authority
FDA	Food and drug administration
UV	ultraviolet
PDA	photodiode array
MOH	ministry of health
μT	mean of test treatment
μR	mean of reference treatment

INTERAKSI FARMAKOKINETIK ANTARA PROPRANOLOL DAN *Eurycoma longifolia* (TONGKAT ALI)

ABSTRAK

Objektif kajian ini adalah untuk menilai kemungkinan interaksi farmakokinetik di antara propranolol dan ekstrak herba Tongkat Ali. Kajian rawak bersilang rabun sebelah, melibatkan 14 orang sukarelawan lelaki muda yang sihat dan tidak merokok telah dijalankan. Tablet Propranolol 80 mg diberikan secara oral bersama-sama dengan i) kapsul placebo (200 mg laktos) sebagai kontrol atau ii) 200 mg kapsul Tongkat Ali ekstrak berasaskan air ($0.0272 \pm 0.0026\%$ *eurycomanone*) sebagai rawatan ujian selepas berpuasa semalaman. Sampel darah diambil pada 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8 dan 10 jam. Tekanan darah dan kadar denyutan nadi diukur sebelum setiap pengambilan sampel darah. Kepekatan Propranolol dalam plasma diukur menggunakan kaedah HPLC yang telah disahkan dan parameter farmakokinetik ($AUC_{0-\infty}$, C_{max}, T_{max} dan $t_{1/2}$) dikira menggunakan model bukan kompartmen. Perbezaan dalam parameter farmakokinetik di antara kumpulan ujian dan control, dianalisis secara statistik dengan menggunakan ujian ANOVA untuk $AUC_{0-\infty}$, C_{max} dan $t_{½}$ dan menggunakan ujian Wilcoxon Signed Ranks untuk T_{max} .Perbezaan tekanan darah terendah, kadar denyutan nadi dan waktu ianya berlaku, di antara kumpulan ujian dan kontrol, dianalisa secara statistik dengan menggunakan ANOVA. Keputusan kajian menunjukkan pengurangan yang jelas dalam bioavailabiliti propranolol semasa ia diambil bersama-sama dengan ekstrak Tongkat Ali, nilai $AUC_{0-\infty}$ dan C_{max} Propranolol mengurang secara signifikan sebanyak 29% dan 42%(masing-masing nilai $p < 0.005$ dan $p < 0.002$). apabila ianya diambil bersama Tongkat Ali. Julat keyakinan 90% untuk nisbah min

$AUC_{0-\infty}$ ialah 52% ke 78% dan julat bagi C_{max} ialah 44% ke 64%. Julat-julat ini adalah di luar julat bioequivalen iaitu 80 – 125%. T_{max} menjadi lebih lambat dengan kadar 86% ($p=0.004$). Tiada perbezaan signifikan bagi nilai $t_{1/2}$ di antara kumpulan ujian dan kontrol. Tujuh subjek sudah mencukupi untuk memberikan kuasa kajian 80% bila diambil kira dari C_{max}, walau bagaimanapun 14 subjek hanya dapat menghasilkan 66% kuasa kajian bila diambil kira dari parameter $AUC_{0-\infty}$. Perbezaan antara tekanan darah terendah serta kadar nadi dan masa ianya berlaku, di antara dua kumpulan berkenaan adalah tidak signifikan secara statistik. Berdasarkan kepada keputusan kajian ini, seandainya propranolol diambil bersama Tongkat Ali adalah disarankan agar masa pengambilan kedua-duanya dijarakkan bagi mengelak kesan interaksi farmakokinetik. Oleh kerana kesan ke atas tekanan darah dan kadar denyutan nadi tidak ketara dalam kajian dos tunggal, kajian dos berulang dalam jangka panjang adalah disarankan bagi mendapatkan maklumat yang lebih jelas berkaitan interaksi farmakokinetik dan farmakodinamik antara Propranolol dan ekstrak Tongkat Ali.

A PHARMACOKINETIC INTERACTION BETWEEN PROPRANOLOL AND *Eurycoma longifolia* (TONGKAT ALI)

ABSTRACT

The objective of this study is to evaluate the possible pharmacokinetic interaction between propranolol and Tongkat Ali herbal extract. A randomised single-blinded crossover study for single dose treatments was conducted in 14 healthy non-smoker young males. Propranonol (Inderal®) 80 mg tablet was orally-administered with i) placebo [200 mg Lactose] as control treatment and with ii) 200 mg of water-based extract of Tongkat Ali [0.0272±0.0026% eurycomanone] as test treatment following an overnight fasting. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 hrs. Blood pressure and heart rate were measured before each blood sampling. Plasma concentrations of propranolol were estimated using a validated HPLC method and the pharmacokinetic parameters ($AUC_{0-\infty}$, C_{max}, T_{max} and $t_{½}$) were calculated by non-compartmental model. The differences in the pharmacokinetic parameters between control and test treatments were statistically analyzed using ANOVA for $AUC_{0-\infty}$, C_{max} and $t_{½}$ and using Wilcoxon Signed Ranks Test for T_{max}. The differences in the lowest blood pressure and heart rate and their time of occurrence between test and control treatments were also statistically analysed using ANOVA. The study results demonstrated a decrease in the bioavailability of propranolol when it was administered with Tongkat Ali extract, its $AUC_{0-\infty}$ and C_{max} were significantly decreased by 29% and 42% ($p<0.005$ and $p<0.002$ respectively). The 90% confidence interval of the ratio of the means of $AUC_{0-\infty}$ was 52 to 78% and for the C_{max} the range was 44 to 64%. These ranges were outside the bioequivalence range of 80-125%. T_{max} was significantly delayed by

86% ($p=0.004$). The $t_{½}$ was not significantly different ($p > 0.1$) between the test and control treatments. Seven subjects were sufficient to give 80% power of study when calculated from C_{max}, however fourteen subjects could only give 66% power, when calculated from $AUC_{0-\infty}$ parameter. The difference in the mean lowest blood pressure and heart rate and their time of occurrence between the two study phases was not statistically significant. Based on the results, it is recommended that when the propranolol and Tongkat Ali are consumed together, their administrations should be separated to avoid pharmacokinetic interaction. Since the effect on blood pressure and heart rate were not obvious in single dose study, a long term repeated dose study is recommended for further evaluation of both the pharmacokinetic and pharmacodynamic interactions between propranolol and Tongkat Ali.

Chapter 1

INTRODUCTION

1.1 Overview

Herbal medicine is the traditional or folk medicine practice based on the use of plants or plant extracts. People in all nations have used many kinds of herbs for the treatment of ailments since prehistory until now. In the 19th century the scientists started with extracting the active ingredients form plants to form medicines but over the time and due to the development of chemistry, chemists began making their own version of plant compounds. Therefore a transition from raw herbal compounds to modern pharmaceutical drug usage occurred with subsequent decline in the herbal medicine usage. However recently, people are coming back to the herbal medicines. They believe that herbal medicines are safe because they are natural.

Herbal medicines or as it is called nowadays, herbal supplements, tend to be a commercial product in the form of tablet or capsule manufactured and marketed by the health food or medicinal industries to the general public. Herbal supplement is consumed at the same time with pharmaceutical medicines which is still the dominant way of treatment but usually without medical control. Pharmaceutical medicines can interact with herbal medicines. The likelihood of drug-herb interactions could be higher than drug-drug interactions, if only because drugs usually contain single chemical entities, while almost all herbal medications (even single-herb products) contain mixtures of pharmacologically active constituents such as, essential oils, tannins, coumarins, anthraquinones, saponins, glycosides, anthocyanins, alkaloids and flavonoids, all of which may potentially participate in drug-herb interactions. This interaction occurs in two general ways, pharmacokinetic or pharmacodynamic or both together. The popularity of herbal medicinal products makes it important to understand the potential interactions between herbs and drugs (Cott, 2001; UMMC, 2000).

Tongkat Ali *(Eurycoma longifolia)* is one of the most famous herbs in Malaysia and Southeast Asia. This fame came from the wide spectrum of pharmacological activities associated with the constituents and crude extracts of this plant, especially its aphrodisiac and health-enhancing effect. The roots of this plant are used as folk medicine for the treatment of sexual insufficiency (it is claimed to increase virility and sexual prowess), persistent fever, malaria, aches, dysentery, glandular swelling, and as a health supplement (Jaganath & Teik, 2006).

The consumption of Tongkat Ali is currently done in two ways, the traditional way, by soaking the herb or boiling it in water then drink it like tea, or the modern way, by taking the manufactured products of Tongkat Ali. The manufactured products could be the extract or the ground powder. They are in various types of preparations such as, capsules, soft drinks and tea, coffee or cocoa drink mixed with Tongkat Ali. These products are easily available in the Malaysian markets as herbal supplements or enriched food products.

Many studies have been conducted on this herb to identify its chemical components and to prove its pharmacological activities. To our knowledge, there is only one study concerning the pharmacokinetic interaction between a pharmaceutical drug and Tongkat Ali herb (Zainah *et al.*, 2005). This study was conducted in vitro on rats hepatocytes and indicated that some of the Tongkat Ali products have an effects on the liver drug metabolizing enzymes. However, the pure compounds of Tongkat Ali did not demonstrate this effect.

From the discussion above, it was therefore important to conduct a study on the pharmacokinetic interaction between Tongkat Ali herb and pharmaceutical drugs, especially those drugs which patients may combine Tongkat Ali with them to overcome their side effects, as with β-Blockers.

Beta-Blockers have been frequently associated with sexual dysfunction, particularly impotence and decreased libido. Fogari and Zoppi (2002) mentioned that the majority of reports dealing with sexual dysfunction due to β-blockade relate to propranolol. Propranolol is a lipophilic β-blocker. It can readily cross the blood-brain barrier and increase the tendency toward sedation or depression, which in turn may cause loss of libido (Fogari and Zoppi, 2002). So, we have decided to study the pharmacokinetic interaction which may occur between propranolol and Tongkat Ali when taken concomitantly in vivo.

1.2 Objectives of the Study

1.2.1 General objective

The overall objective of conducting this study is to determine a single dose pharmacokinetic interaction when propranolol is consumed together with the water-base extract of Tongkat Ali in healthy volunteers.

1.2.2 Specific Objectives

i) To establish an analytical method for measuring propranolol concentrations in plasma.

ii) To conduct pharmacokinetic interaction study between propranolol and the water base extract of Tongkat Ali.

iii) To measure the pharmacokinetic parameters of propranolol when taken with placebo and when taken with Tongkat Ali. The pharmacokinetic parameters to be measured are the bioavailability parameters i.e area under the curve (AUC), maximum plasma concentration (C_{max}) and the time to reach maximum concentration (T_{max}) and other pharmacokinetic parameters like terminal half life ($t_{½}$), volume of distribution (Vd) and total body clearance (CL).

iv) To compare some of the pharmacokinetic parameters (AUC & $t_{½}$) calculated using advanced pharmacokinetic program (WinNon Lin) and calculated manually on Excel program.

v) To compare the pharmacokinetic parameters of propranolol when taken with placebo and when taken with Tongkat Ali.

vi) To correlate the pharmacokinetic results with pharmacodynamic changes (Blood Pressure and heart rate) in the volunteers.

Chapter 2

LITERATURE REVIEW

2.1 Analysis Method for Measuring Propranolol Concentrations in Plasma.

To date, various methods have been developed for the determination and quantification of propranolol from the biological matrices (serum, plasma, blood or urine). In the method developed by El-Saharty, (2003), reversed phase high-performance liquid chromatography (RP-HPLC) was used to quantify propranolol extracted from the rabbit's plasma. However, the quantitative determination of propranolol was only valid in the concentration range 5 to 200 μg/ml which was not sensitive enough for clinical pharmacokinetic studies where expected propranolol concentrations were in nanograms (mean peak plasma concentrations of propranolol is between 15 to 180 ng/ml following the usual oral dose of propranolol 80 mg (Katzung, 2001). In another method developed by Wren & Tchelitcheff, (2006), ultra-performance liquid chromatography linked to a mass spectrometer (UPLC/MS) was used to identify propranolol. This article unfortunately did not provide enough information either on the extraction procedures or the quantified concentration of propranolol. Furthermore, the usage of UPLC plus MS instrument is an expensive combination compared to an HPLC system. In another method developed by Kristoffersen *et al.,* (2006), an automated solid phase extraction (SPE) was used to extract propranolol from whole blood samples followed by an RP-HPLC coupled with an MS for the determination and quantification of the drug. The use of whole blood samples however, is not recommended as it may lead to loss of sensitivity of the column due to the formation of an irreversible binding between the macromolecules found in blood (such as proteins) and the surface of the backing material. Moreover, this method is expensive because of the equipment used (SPE, RP-HPLC and MS) with low recovery of propranolol (80%).

In this study, HPLC method of analysis of propranolol from plasma was adopted from Rasool *et al.* (1997) study and Zain-Hamid *et al.* (1999) study. This method can give good recovery (90%), sensitive (upto 9 ng/ml) and the cost of the method is reasonable.

2.1.2 Chemical Analysis Using High Pressure Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is a powerful technique of analysis. It can easily achieve separations and analysis of very small concentrations, up to fractions of ng/ml of the drugs which have been extracted from biological matrix like blood, plasma, serum and urine. This technique of analysis is based on the partition coefficient of the analyte between the solid stationary phases (column) and the liquid mobile phase. The column usually contains many kinds of packings that consist of uniform, porous silica particles with spherical or irregular shape. The mobile phase which usually consists of a mixture of solvents is pumped through the column under high pressure. The function of the mobile phase is to push the injected analyte through the column to be separated. The separated sample will then be detected by many kinds of detectors, for example UV detectors, fluorescence detector or photodiode Array detector (PDA) (Waters, 2007).

Photodiode array detector (PDA) can be used to measure and detect samples over the entire UV to visible (UV-Vis) spectrum. It is a highly beneficial tool in identification and analysis of sample compounds (Jones, 1985).

The separation process of the HPLC instrument can be done by two systems, reversed phase HPLC system and normal phase HPLC system. The reversed-phase system is characterized by strong interaction between the sample molecules and the polar mobile phase, due to dipole interaction or hydrogen bonding of the solvents basicity (proton acceptor) or the solvent acidity (proton donor). On the other hand, weak interaction exists between the sample molecules and the nonpolar stationary phase. Normal-phase system is just the opposite of the reversed-phase system. The interaction is strong between the sample molecules and the nonpolar mobile phase but weak with the polar stationary phase (Snyder *et al.*, 1988).

2.1.3 Internal Standard

Internal standard is a known amount of a compound, different from the analyte that is added to the unknown. Signal from analyte is compared with signal from the internal standard to find out how much analyte is present. Internal standards are especially useful for analyses in which the quantity of sample analysed or the instrument response varies slightly from run to run for reasons that are difficult to control. For example, if signal from the internal standard increases or decreases by 8.4% because of a change in solvent flow rate or sample loss which can occur during sample preparation steps prior to analysis, the signal from the analyte usually increases or decreases by the same quantity (also 8.4%). As long as a known quantity of the internal standard was added to the unknown analyte prior to any manipulations, the ratio of internal standard to unknown analyte remains constant because the same fraction of each is changing in any operation (Garrett, 1999).

2.1.4 Bioanalytical Method Validation

Published methods of analysis are often modified to suit the requirements of the laboratory performing the assay. To use the same methods of analysis in another laboratory with different condition, method of analysis has to be validated first. To ensure the acceptability of the performance of a bioanalytical method, bioanalytical method validation includes all of the procedures that demonstrate the reliability and the reproducibility of this method is conducted. The fundamental parameters for this validation include accuracy, precision, selectivity, sensitivity, reproducibility, and stability (FDA, 2001a).

The accuracy of the measurement refers to how close the measured value is to the true or accepted value. The precision refers to how close together a group of measurements actually are to each other. Accuracy and precision are determined using a minimum of five determinations per concentration level (excluding blank samples). The mean value should be within

±15% of the theoretical value, except at lower limit of quantification (LLOQ), where it should not deviate by more than ±20%. The precision around the mean value should also not exceed 15% of the coefficient of variation (CV), except for lower limit of quantification LLOQ, where it should not exceed 20% of the coefficient of variation CV. Recovery of an analyte in an assay is determined by the ratio of the detector response obtained from an amount of the analyte added to the biological matrix (plasma) and extracted from it, compared to the detector response obtained for the true concentration of the pure authentic standard (FDA, 2001a).

The recovery % = (extracted analyte / unextracted analyte) x 100

The selectivity of the methods describes the ability of this method to quantify the analytes with less interference from each other or with interference with other components. The sensitivity of the methods describes the ability of this method to detect and/or quantify the lowest concentrations of the analyte. The reproducibility of the methods describes the ability of this method to provide the same results if it is applied in different laboratories. The stability of the analytical method describes the drug stability in the biological matrix and in the chemical preparations under different storage conditions and the container systems.

2.2 Pharmacokinetic

Pharmacokinetics is the dynamics of a drug which is concerned with how drug is absorbed, distributed, metabolized and excreted from the body (Goodman and Gilman, 2001). When a drug is administered extravascularly (orally, intramuscularly, or applied to the skin by transdermal patch), the drug molecules has to absorbed first to reach the systemic circulation. When a medication administered orally, the drug dosage form must release drug molecules via dissolution, and the molecules must pass through the vascular layers of gastrointestinal tract, where they enter capillaries. The distribution of the drug occurs after drug molecules entered the vascular system. Drug molecules will pass from the bloodstream into various tissues and organs, such as muscles and heart. The metabolism of the drug is the chemical conversion of the drug molecules; usually by an enzymatically mediated reaction, to another chemical entity referred to as a metabolite. The metabolite may have the same or a different pharmacologic effect as the parent drug or may even cause toxic effect. The excretion of the drug is the irreversible removal of a drug from the body and commonly occurs through the kidney or biliary tract (Bauer, 2001).

2.2.1 Basic Pharmacokinetic Parameters

The pharmacokinetic behavior of most drugs can be summarized by the following parameters.

2.2.1.1 Bioavailability

Bioavailability means the rate and extent to which the active substance or therapeutic moiety is absorbed from a pharmaceutical form and becomes available at the site of action. Bioavailability means the fraction of the dose that reaches the systemic circulation as intact drug (Birkett, 1999).

The rate of bioavailability depends upon pharmaceutical factors (drug formulations) and gastrointestinal absorption factors, metabolism being relatively unimportant. On the other hand the extent of bioavailability depends on both, the extent of absorption and the extent of metabolism (Smith and Aronson, 1984).

Measurement of drug concentrations in blood, plasma, or serum after drug administration is the most direct and objective data to determine systemic drug bioavailability. By appropriate blood sampling, an accurate description of the plasma drug concentration versus time profile of the therapeutic active drug substance can be obtained using a validated drug assay (Bauer, 2001).

The plasma concentration versus time curve contains three features of interest, the peak height (C_{max}) and the time taken to reach the peak (T_{max}) are measures of the rate of availability and the total area under the curve (AUC) is a measure of its extent (Smith and Aronson, 1984).

The peak height C_{max} represents the maximum plasma drug concentration obtained after the oral administration of the drug. C_{max} provides indications that the drug is sufficiently systemically absorbed to provide a therapeutic response. In addition C_{max} provides warning of possible toxic levels of drug. T_{max} represents the time required to reach that maximum drug concentration after the oral administration of the drug. At T_{max}, the rate of drug absorption is exactly equals to the rate of drug elimination. Drug absorption still continues after T_{max} is reached but at a slower rate. When the value of T_{max} becomes smaller that will indicate less time to reach peak plasma concentration but if it is bigger that will indicate more time to reach peak plasma concentration. Area under the curve (AUC) represents the total amount of active drug that reaches the systemic circulation, (Shargel and Yu, 1999).

There are factors at work which reduce the availability of the drug prior to it entering the systemic circulation. Such factors may include, but are not limited to poor absorption from the gastrointestinal tract, hepatic first-pass effect and degradation of the drug prior to reaching systemic circulation (Shargel and Yu, 1999). These factors will be discussed on the sections of pharmacokinetic interactions below on page 20.

2.2.1.2 Apparent Volume of Distribution (Vd)

Apparent volume of distribution is a measure of the extent of distribution of a drug in the body. Volume of Distribution is the fluid volume required to contain the drug in the body at the same concentration as in plasma. For the single oral dose, volume of distribution is determined by the ratio of the total amount of drug in the body (dose) and the plasma drug concentration (AUC) and elimination rate constant (Boomer, 2003).

Vd = Dose/ (AUC X elimination rate constant)

At any given time after a drug has been absorbed from extravascular sites, the serum and the tissue drug concentrations are in equilibrium. The serum concentration of drug is equal to the quantity of the amount of drug in the body tissues. Volume of distribution is inversely proportional to the amount of the drug in blood, volume of distribution can be very small if the drug is primarily contained in the blood (warfarin Vd=5-7 L) or very large if the drug is distributed widely in the body (digoxin Vd=500 L) and is mostly bound to the tissues (Bauer, 2001).

This parameter (Vd) is helpful to measure the dose required to produce a given concentration of the drug in plasma at steady (Beers and Berkow, 2005), Steady state is the condition in which the amount of drug eliminated at each dose interval equals the dose for that interval (Shargel and Yu, 1999). If maintenance dose is just started, it will take some time for the drug to accumulate to steady state. To get close to steady state more quickly, a loading dose is often used and the volume of distribution is the determinant of the size of the loading dose (Birkett, 1999).

Loading dose = Vd x target plasma concentration

The volume of distribution is influenced by some factors like plasma protein binding and by the lipophilicity of the drug, as explained in section 2.1.1.2.1.

2.2.1.2.1 Plasma Protein Binding

Plasma protein binding is the situation when drugs bind to the circulating plasma proteins. Since these proteins are large molecules, drugs that are bound to proteins cannot pass out of vascular space. Thus, plasma protein binding has the effect of restricting the distribution of drugs. When plasma protein binding increases the extent of the drug distributed in the body will decrease. Only unbound drug (free drug) is available for passive diffusion to tissue sites where pharmacologic effects occur. Therefore, the unbound drug concentration may be more closely related to drug concentration at the active site and to drug effects. However, it is important to remember that binding of drugs to plasma proteins is a dynamic equilibrium between the bound and the unbound drug and the rates of drug binding and release are very fast, occurring in millisecond range (Beers and Berkow, 2005).

In lipophilic or basic drugs, the unbound (or free) drug is able to cross the biological membranes easily and the drug may exhibit an extensive volume of distribution, despite of its high degree of protein binding. In this way, a drug with a very low free fraction (i.e., a high degree of plasma protein binding) can exhibit a large volume of distribution. For example, the volumes of distribution of imipramine, nortriptyline and propranolol are 1600 ± 600, 1300 ± 300 and 270 ± 40 liters respectively and their percentages of plasma protein bindings are 90.1 %, 92 % and 90 % (Shargel and Yu, 1999).

2.2.1.3 Clearance (CL)

Clearance is defined as the volume of blood cleared of drug per unit time and the units are liter per hour or milliliter per minute. Drugs are cleared by various organs in the body. Kidneys and liver are the most common organs involved in excretion and metabolism of the drug. For any organ, clearance may be defined as the fraction of blood volume containing drug that flows through the organ and gets eliminated of the drug per unit time. From this definition clearance depends upon the blood flow rate and the ability of the organ to eliminate the drug. Clearance can be referred to as the total body clearance, which is the sum of all the different clearance processes occurring for a given drug by the total body organs. Total body clearance may be defined as the rate of drug elimination divided by the plasma drug concentration (Shargel and Yu, 1999). Total body clearance of a single dose of the drug can be calculated from the administered dose and the plasma drug concentration (AUC) (Birkett, 1999).

Total body Clearance (L / hour) = dose/AUC

Clearance describes the efficiency of irreversible elimination of the drug from the body. When a drug is metabolized, the parent drug will then be cleared or eliminated, even if the metabolites of the drug are still in the body but they are different chemical entities. Also the uptake of the drug into the tissues is not considered clearance if the unchanged drug eventually comes back out of the tissue, however slowly this occurs.

Clearance is an important parameter because it is used for the determination of the maintenance dose rate required to achieve a target plasma concentration (Birkett, 1999).

At steady state: Elimination rate = maintenance dose rate

The extraction rate of a drug from the blood by an eliminating organ, such as the liver, cannot exceed the rate of drug delivery to the organ. Thus, clearance has an upper limit, based on drug delivery and hence on blood flow to the organ. Furthermore, when the eliminating organ is the liver or gut wall and a drug is given orally, part of the dose may be metabolized as it passes through the tissues to the systemic circulation; this process is called first-pass metabolism**.** Thus, if extraction (clearance) of a drug is high in the liver or gut wall, oral bioavailability is low, sometimes precluding oral administration or requiring an oral dose much larger than an equivalent parenteral dose. Drugs with extensive first-pass metabolism include propranolol, hydralazine, isoproterenol, lidocaine, meperidine, morphine, nifedipine, nitroglycerin, alprenolol, testosterone, and verapamil (Beers and Berkow, 2005).

2.2.1.3.1 Elimination Rate Constant (k_{el})

Elimination rate constant is the first order rate constant describing drug elimination from the body. This is an overall elimination rate constant describing removal of the drug by all elimination processes including excretion and metabolism. The elimination rate constant is the proportionality constant relating the rate of elimination of the drug and the amount of drug which remains to be eliminated (Birkett, 1999).

$k_{el} = \ln(C_{p1}) - \ln(C_{p2}) / (t_2 - t_1)$

2.2.1.3.2 Half-Life ($t_{1/2}$)

Half-life is the time taken for the amount of the drug in the body or the plasma concentration to fall by half. Half-life is determined by both volume of distribution and clearance, and is increased by an increase in volume of distribution (Vd) or by a decrease in clearance (CL).

$t_{1/2} = 0.693 \times Vd / CL$

When the ratio of clearance and volume of distribution is constant ($CL/Vd = k_{el}$), half-life is a reciprocal function of the elimination rate constant (Shargel and Yu, 1999).

$t_{1/2} = 0.693 / k_{el}$

Half-life concept is applied to the drugs which are cleared from the body (eliminated and/or metabolized) by a fixed rate. This means that the amount eliminated is proportional to the amount available to be eliminated. Mathematically this is known as a first order kinetic, most drugs are in this category. This is in contrast to zero order kinetic in which a fixed amount of the drug is eliminated per unit time. Thus, the amount cleared is independent of the amount to be cleared. Drugs such as acetyl salicylic acid, ethanol and phenytoin are primary examples in this category (Shargel and Yu, 1999; Bateman and Eddleston, 2006).

2.2.1.3.2.1 Importance of Half-Life

i) The time required to reach steady state

With chronic dosing, the time required to reach steady state can take many half-lives to reach the target plasma concentration. If the drug is given at every half-life, half the first dose is eliminated over the first dosing interval. Therefore, after the second dose there are 1.5 doses in the body and half of this amount is eliminated before the third dose. The drug continues to accumulate with continued dosing until there is double the dose in the body, at which point the equivalent of one dose is eliminated at each dosing interval (half-life). The accumulation of the drug to reach steady state is a mirror image of the elimination when dosing is stopped. It takes approximately 5 half-lives to reach or to eliminate 97% of the drug concentration (Birkett, 1999).

Dosing interval is the interval between maximum concentration and minimum concentration which is just before the next dose. With steady state dosing, the extent to which the plasma concentration fluctuate over dosing interval is determined by the half-life of the drug and the time between the doses. Fluctuations will be greatest when the interval in relation to the half-life of the drug is increased and will be smallest when the dosing interval is decreased. The degree of fluctuation can be expressed as the ratio of the maximum concentration to the minimum concentration (Birkett, 1999).

In addition to the above, during oral dosing, the degree of fluctuation over the dosage interval is also determined by the extent and rate of absorption. The slower the absorption of the oral doses the smoother the plasma concentration profile so that the fluctuation over the dosing interval is less. And the decrease or increase in bioavailability will reflect on the required dose of each interval to reach steady state (Birkett, 1999).

ii) Therapeutic index of drugs

Therapeutic index of drugs is the ratio of the mean toxic to the mean effective dose. Therapeutic index plays an important role in determining its optimal dose regimen. Theoretically, a drug level has to stay above the minimum therapeutic concentrations as long as possible and sufficiently below the toxic dose to avoid an unacceptable frequency of adverse effects. For most drugs, this can be achieved quite simply by giving the dosage once every half-life such that the maximal variation, peak to trough, is less than 2-fold. However, for drugs with high therapeutic indices, it may be more convenient to give a much higher dosage of the drug and less frequently. The rational of this type of dosing system is when the risk of toxicity is remote. A sufficiently high dose can be given to keep drug concentrations above minimum effective levels even at the end of the dosing interval, more than one half-live out from the dose. For example, Penicillin G ($t_{1/2}$ = 0.7 hrs) is given every 4-6 hours. Alternatively, if therapeutic index is low, the drug may have to be given at lower dosages and more frequently than every half-life in order to reduce peak to trough variations (Birkett, 1999).

iii) Drug duration of action after single dose administration

The duration of action (t_{eff}) is a pharmacodynamic parameter which is proportional to half-life. The longer the half-life, the longer the plasma concentration will stay in the effective range (C_{eff}). However, the duration of action is a logarithmic, not a linear function of the dose (D_0) so that increasing the dose is an inefficient way of increasing the duration of action. A simple rule of thumb is that doubling the dose increases the duration of action by one half-life (Shargel and Yu, 1999).

2.3 Pharmacokinetic Interactions

Pharmacokinetic interactions can occur between drug with another drug, with herbs or with food. This interaction result in alterations of drug absorption, distribution, metabolism, or excretion. Pharmacokinetic interactions are often associated with significant changes in a drug's plasma concentrations, area under the curve, C_{max}, T_{max}, half-life or clearance and volume of distribution. These interactions affect the drug by quantitative alterations, either increasing or decreasing the amount of drug available to have an effect. To determine this quantitative alteration, bioavailability of the drug has to be measured (Leucuta and Vlase, 2006).

2.3.1 Absorption

In oral administration, drug absorption occurs predominantly in the small intestine. In humans, the mucosa of the small intestine has a large surface area which is increased greatly by the folds of villi, and microvilli because of the large surface area provided by epithelial folding and the villous structures of the absorptive cells. Drug absorption occurs by either passive or active transport, with most drugs being absorbed by passive transport. This process involves diffusion of a drug from areas with a high drug concentration to regions with lower drug concentration by either transcellular or paracellular transport, or a combination of both. For transcellular transport, drugs are transported into and through the epithelial cells, and then into the blood circulation, whereas for paracellular transport, drugs reach the blood circulation via the tight junctions between epithelial cells. Only those drugs that are absorbed via the transcellular, but not the paracellular, pathway are subjected to intestinal first-pass metabolism. The relative contribution of the transcellular and paracellular pathway to overall absorption is highly dependent on the lipophilicity of the drugs. In vitro studies with Caco-2 cells, a model of cell culture system which is used to study the intestinal permeability in vitro, revealed that the relative contribution of the transcellular pathway was 25%, 45%, 85%, and 99% for chlorothiazide, furosemide, cimetidine, and propranolol, respectively. These values correlated well with the lipophilicity of the compounds (Lin *et al.*, 1999; Oulianova *et.al.*, 2006).

Active transport involves the movement of drugs (i.e., ionized and water-soluble molecules) against a concentration gradient (i.e., from regions of low concentration to areas of high concentration) and therefore requires an energy source. Drug absorption that occurs by active transport is usually more rapid than that which occurs by passive diffusion. The nonionized form of a drug is lipid soluble and readily diffuses across the cell membrane, while the ionized form of the drug is lipid insoluble and nondiffusible (Shargel and Yu, 1999).

2.3.1.1 Factors Which Affect Drug Absorption and Bioavailability

There are many factors that affect the rate and extent of drug absorption and bioavailability, these factors are described below.

2.3.1.1.1 Effect of Food on Gastrointestinal Drug Absorption

The volume of food, type of meals and even the temperature of food and beverages are factors that influence gastrointestinal drug absorption and reduce the gastric empting. The larger starting volume of food, the greater the initial rate of emptying. After this initial time, the larger the volume the slower the rate of emptying. The consumption of fatty meals is the potent inducers of cholecytokinin hormone. This hormone leads to retention of the fatty meal in the stomach. Carbohydrate and amino acids can also delay gastric emptying, as a result of osmotic pressure (Shargel and Yu, 1999; Mason, 2002).

When drugs are taken with food, there is usually a second peak. This corresponds to the delivery of the drug in to the intestine with food, since food is not delivered in one delivery. The height and the duration of this peak depend on gastrointestinal transit time, when drug is taken with water alone there is only one peak in plasma concentration (Mason, 2002).

The presence of food in gastric lumen stimulates the flow of bile. Bile contains bile acids which are surfactants involved in the degradation and solubility of fats and increases the solubility of fat-soluble drugs (lipophilic drugs). On the other hand the presence of food in the stomach stimulates hydrochloric acid secretion, which lowers the pH, causing more rapid dissolution of the drugs (Shargel and Yu, 1999).

2.3.1.1.2 Perfusion of Gastrointestinal Tract

Once the drug is absorbed from the small intestine, it enters the mesenteric circulation. Any decrease in mesenteric blood flow will decrease the rate of drug removal from intestine thereby reducing the rate of bioavailability. There is a role of lymphatic circulation in drug absorption where the drugs are absorbed through the lacteal or lymphatic vessels under the microvilli. This way of absorption will bypass the first-pass metabolism of the liver because the absorption through the hepatic portal vain is avoided. This way may partially be responsible for the absorption of some lipophilic drugs (Shargel and Yu, 1999).

2.3.1.1.3 Affect of P-glycoprotein (P-gp)

P-glycoprotein is an efflux transporter localized in the apical membrane of the intestinal cells, besides other drug eliminating organs. Immunohistological studies with human small intestine indicated that P-gp is located on the apical brush border membrane of the mature epithelium; therefore, P-gp may play a role in limiting the absorption of orally administered drugs by extruding the drugs from the epithelial cells into the intestinal lumen (Lin, 2003).

The cytochromes P450 enzymes (CYP3A4) and P-gp work together to co-ordinate an absorption barrier against xenobiotics. P-gp activity can be induced by drugs such as clotrimazole, erythromycin, phenobarbital and rifampicin, or inhibited by other drugs such as quinidine and verapamil (Adithan, 2005).