CHAPTER - 5

PHARMACOLOGICAL EVALUATION
BIOLOGICAL ACTIVITY

Infectious diseases are one of the main causes of high morbidity and mortality in human beings around the world, especially in developing countries [1]. Infections such as food poisoning, rheumatic, salmonellosis and diarrhea caused by multidrug-resistant gram-positive and gram-negative pathogens such as Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhimurium and Escherichia coli. These pathogens are responsible for significant morbidity and mortality in both the hospital [2] and community settings [3-5]. Millions of people in the subtropical regions of the world are infected and 20,000 deaths occur every year due to these parasitic bacterial infections. Amoxicillin, norfloxacin, ciprofloxacin are the principal drugs of the choice in the treatment of bacterial infection since they are effective against intestinal infection [6]. The leading drugs have been shown to have both mutagenic effects in bacteria and carcinogenic effect in rodents [7]. These drugs also show severe side effects (nausea, metallic taste, dizziness, hypertension, etc.) as well as resistance to these drugs has been reported [8]. The ideal treatment for these diseases does not exist and therefore, new agents are required.

ACUTE TOXICITY STUDY

In screening drugs, determination of the LD{sub}50 (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations and is one of the initial screening experiments performed with all compounds.

Data from the acute study may: (a) Serve as the basis for classification and labeling; (b) Provide initial information on the mode of toxic action of a substance; (c) To help in arriving at a dose of a new compound; (d) Help in dose determination in animal studies; (e) Help to
determine LD$_{50}$ values that provide many indices of potential types of drug activity.

**ANALGESIC, ANTIPYRETIC AND ANTI-INFLAMMATORY ACTIVITIES**

The drugs are heterogeneous compounds, often chemically distinct, but nevertheless share certain therapeutic actions and side effects. In most of the textbook, these compounds are referred as aspirin-like drugs [9]. More frequently they are recognized as non-steroidal anti-inflammatory drugs (NSAIDs). All NSAIDs are antipyretic, analgesic, and anti-inflammatory, but there might be some differences in their individual activities.

There is no definite classification of analgesic/antipyretics drugs. Most of the textbooks classify them depending on their efficacy. These are divided into two groups; Non-narcotic analgesics (for the mild to moderate pain, some of which may also have antipyretic actions) [10], and narcotic/opioid analgesic (which are principally used in the relief of severe pain). Many analgesics also have marked anti-inflammatory actions and therefore are used for the treatment of arthritis and other inflammatory conditions. Most of them exhibit their effect, at least in part, by the inhibition of prostaglandin synthesis.

At the primary healthcare level, non-narcotic analgesics are of major concern because of their wide use. Analgesics drugs are used to relieve pain. Pain is one of the most common symptoms, and one of the most frequent reasons why people seek medical care. Antipyretic activity results in lowering the temperature (approximate near normal body temperature), and is considered to involve the hypothalamus. Normal body temperature varies according to the individual’s age, sex, level of physical and emotional stress, the environmental temperature, time of the day, and the anatomical site at which the temperature is measured. Body temperature may be measured at rectal, axillary, oral, or tympanic (ear canal) sites. The method used to measure the temperature should...
be indicated on the reported patient’s temperature. Paracetamol, aspirin, and ibuprofen have similar antipyretic activity. Product selection should be based primarily on patient acceptance, the side effects of each agent, concurrent diseases that may prohibit the use of each agent, convenience of administration, and cost of therapy.

Anti-inflammatory agents are drugs that alleviate symptoms of inflammation, but do not necessarily deal with the cause. NSAIDs are one kind of therapeutics, widely used in the world because of their high efficacy in reducing pain and inhibiting inflammation [11, 12]. NSAIDs drugs can inhibit the enzyme cyclooxygenase (COX-1 and COX-2) [13, 14], which catalyze the biotransformation of arachidonic acid to prostaglandins (PGs) and to Thromboxane A\textsubscript{2} \cite{15-19}. These are the mediators of pain, inflammation, fever, stimulates platelet aggregation and leading to the formation of blood clots \cite{20-22}. NSAIDs have been shown to be as effective as aspirin, but not superior than it.

**PHARMACOKINETICS STUDY**

Pharmacokinetics is the study of the movement of drugs into, within, and out of the body. It involves the factors affecting that or, more simply, what the body does to the drug. Knowledge of pharmacokinetics enables drugs to be used rationally and doses tailored to the individual object.

**Principles of First – Order Kinetics**

Pharmacokinetics may be defined as the quantitation of the course of time of a drug and its metabolites in the body or body fluids, and the development of appropriate models to describe observations and to predict the outcomes in other situations \cite{23}. The science of kinetics deals with the mathematical description of rate processes or reactions. Typical examples of naturally occurring processes of pharmaceutical interest which confirm to first-order kinetics are radioactive decay of materials and the absorption, distribution, metabolism, and excretion.
[ADME], of drugs in the body. The Pharmacokinetic rate constants are dependent on the concentration or amount of only one component of the system. The kinetics follows first-order or pseudo first-order processes due to the fact that all other components of the system or model except the drug concentration are constant. In vivo drug processes, the [ADME] follow pseudo first-order or first order processes [24].

**Pharmacokinetics Working Equations**

In mathematical terms, the rate law for a first-order process can be expressed in terms of an infinitesimal small change in concentration (dC) over an infinitesimal small time interval (dt) as;

\[
\text{Rate} = \frac{dC}{dt} = -kC \quad \text{[5.1]}
\]

where, \( k \) is the first - order rate constant. This is the differential rate expression of a first – order process. Upon integration, this yields,

\[
\ln C = \ln C_0 - kt \quad \text{[5.2]}
\]

But \( \ln X = 2.303 \log X \),

\[
\log C = \log C_0 - kt \cdot 2.303
\]

Equation 5.2 is the integrated form of the first – order rate law which is linear.

The exponential form of the rate equation for a first-order process is expressed as;

\[
C = C_0 e^{-kt} \quad \text{[5.3]}
\]

Taking the natural logarithms on both sides of Equation [5.3] yields;

\[
\ln C = \ln C_0 - kt
\]

This is the same as Equation [5.2]. Multiplying both sides of Equation [5.3] by \( V \), the total volume of distribution;

\[
VC = V C_0 e^{-kt}
\]

\[
A = \text{Dose} e^{-kt} \quad \text{[5.4]}
\]

Rearranging this equation yields;

\[
\frac{A}{\text{Dose}} = e^{-kt}
\]
The above equation is the fraction of the dose remaining at time \( t \). where \( A \) is the amount of drug in the body at time \( t \), \( V \) is the total volume of distribution, \( C \) is the plasma concentration at time \( t \) and \( C_0 \) is the initial plasma concentration at time \( t_0 \).

**Half-life \((t_{1/2})\)**

The time required for the plasma concentration \((C)\), to fall to half the original plasma concentration, \((C/2)\), is called the half – life \((t_{1/2})\). For a first – order process, this parameter is constant. Theoretically, a first order process never reaches completion since even the lowest concentration would only fall to half its value in one half – life. For most practical purposes, a first order process may be deemed “complete” if it is 95% or more complete. It has been established that to attain this level of completion at least five half – lives must elapse \([25]\). In urinary analysis, total urine collection is effected or deemed complete after at least five half – lives of collection period. The relationship between half – life \((t_{1/2})\) and rate constant \( k \), is also a very useful working pharmacokinetics equation and is expressed as;

\[
t_{1/2} = \frac{0.693}{k}
\]

**Volume of distribution \((V)\)**

The volume of plasma into which a drug distributes in the body at equilibrium is called the total volume of distribution, \( V \). However, the apparent volume into which a drug distributes in the body at equilibrium is referred to as the apparent volume of distribution, \( V_d \). Thus, the concentration \((C)\) of drug in plasma is achieved after distribution at equilibrium. The total distribution is a function of the amount of drug in the body, \( A \) (or dose) and the extent of distribution of drug into the tissues, \( V \). Mathematically, this is expressed as;

\[
V = \frac{A}{C}
\]

\[
V_d = \frac{\text{Dose}}{C_0}
\]
where, $C_0$ is the initial plasma concentration at zero time ($t_0$).

The total volume of distribution $V$, may also be defined as the proportionality constant between the plasma concentration $C$, and the amount of drug in the body, $A$.

### Clearance (CL)

Clearance is the proportionality factor or conversion factor which relates the plasma concentration, $C$, to the rate of drug elimination, $dA/dt$. Thus, Rate of elimination, $dA /dt = CL \cdot C$

Mathematically, total clearance is expressed as:

$$CL_T = k \cdot V$$

Plasma CL is usually determined from the area under the Cp versus time curve (AUC), after IV administration. The AUC is determined by using the ‘Trapezoidal rule’.

After IV dosing $CL = \frac{Dose}{AUC}$,

After oral dosing $CL/F = \frac{Dose}{AUC}$, where $F =$ oral availability

### Bioavailability

Bioavailability refers to the rate and extent of absorption of a drug from its dosage form into the systemic circulation. It is usually assessed by the maximum drug plasma concentration ($C_{max}$), time to reach $C_{max}$ ($T_{max}$), and the area under the plasma concentration-time curve (AUC). Absolute bioavailability compares the AUC of a drug following non-intravenous administration with the AUC of the same drug following intravenous administration. Relative bioavailability compares the AUCs of a drug when administered via different routes or formulations or standard and test. Absolute bioavailability is usually less than one, but relative bioavailability can be larger than one. The calculation for bioavailability can be corrected for dose and clearance.

Absolute bioavailability ($F$) = $\frac{AUC_{Oral}}{AUC_{intravenous}}$

Relative bioavailability ($F$) = $\frac{AUC_{Test}}{AUC_{Reference}}$

~~~~~~~~~~~~~~~~~~~~~~~~~~~
PHARMACOKINETICS MODELS

Drug processes which often occur simultaneously within the body are in a dynamic state. In order to describe such a complex biological system, a hypothesis or model which is based on simple assumptions is conceived using mathematical terms. These are a concise means of expressing the quantitative relationship concerning the movement or concentrations of drugs in the body. Various mathematical models can be devised to simulate the rate processes of drug absorption, distribution, and elimination. Meanwhile, it is possible to develop equations to describe drug concentrations in the body as a function of time [25].

Pharmacokinetics models may be classified into two main categories, namely, compartmental/non-compartmental on one hand and physiologic or physiologically-based pharmacokinetic [PB-PK] models on the other hand.

Compartmental Models

Compartmental models are based on assumptions of using linear differential equations. A compartmental model provides a simple way of grouping all the tissues, (that have similar blood flow and drug affinity), into one or two compartments where drugs move to and from the central or plasma compartment. The compartmental models are particularly useful when there is little information about the tissues.

One – Compartment Open Model

After intravascular administration (intravenous (i.v.) bolus), a drug may distribute into all the accessible regions instantly. Instant distribution of drugs in the body may lead to the consideration of the body as a homogeneous container for the drug and the disposition kinetics may be described as a one compartment open model. The time course of a drug which follows a one – compartment open model depends upon the initial concentration administered into the body Co
and the elimination rate constant, $K_e$. It must be recalled that $e^{-K_e t}$ is the fraction of the dose remaining in the body of time $t$.

$$C = C_0 \cdot e^{-K_e t}$$

Where, $C$ is the concentration of the drug in the plasma at time $t$. Taking natural log on both sides of the above expression yields [Equation 5.2].

$$\ln C = \ln C_0 - K_e t$$

This is a linear equation, and on a semi-log scale the rate constant $K_e$ is estimated as the slope of the straight line that is obtained after a plot of $\ln C$ against time $t$.

![Figure 5.1: ln Cp versus Time profile for one-compartment model.](image)

Other pharmacokinetic parameters assessable from such plots following both intravascular doses, such as i.v. bolus, and extravascular doses such as oral administration are expressed as follows;

$$t_{1/2} = 0.693/K_e$$

$$V = \text{Dose}/C_0$$

$$CL_T = V \cdot K_e = \text{Dose}/\text{AUC}$$

**Multi-compartment Models**

In practice, very seldom will a drug follow a true one-compartment open model. Upon administration, drugs usually distribute...
into the vascular space and some readily accessible peripheral spaces in a much faster rate than into deeper tissues. In a multi-compartment model, besides elimination, there are distribution processes that are also involved in removing the drug out of the vascular spaces. Consequently, \(-dC/dt\) depends upon more than one single first-order processes. On a semi-log scale, the sum of more than one straight line will be linear curve. The equation describing a multi-compartment open model will have many exponential phases. For example, a two-compartment model has two exponential phases in its equation; one for distribution, \((A_o \cdot e^{-\alpha.t})\), and another for elimination, \((B_o \cdot e^{-\beta.t})\). Hence the overall equation for the amount of drug \(C\), in the body at time \(t\) will be:

\[
C = A_o \cdot e^{-\alpha.t} + B_o \cdot e^{-\beta.t} \quad \cdots [5.5]
\]

Under these conditions, \(\alpha\) and \(\beta\) are rate or hybrid constants controlling the rates of distribution and elimination respectively. \(A_o\) and \(B_o\) are hybrid values representing the respective initial concentration of drug in plasma at the initial time \(t\) during the distribution (\(\alpha\)) and elimination (\(\beta\)) phases.

![Graph](image)

Figure 5.2: Plasma concentration (C) versus time profile for two compartment model.
After time \( t \), the concentration \( C \) will become equal to \( B_0e^{-\beta t} \). The extrapolated the residual distribution of elimination phase is \( A_0e^{-\alpha t} \) and it is reduced to zero. Indeed, depending upon the magnitude of \( \alpha \) relative to \( \beta \), (always \( \alpha \gg\gg \beta \)), \( A_0e^{-\alpha t} \) (the residual distribution phase) reduces progressively until it reaches zero. When time \( t \) becomes so large and consequently the exponent \( e^{-\alpha t} \) becomes negligible. Then the equation will be reduced to; \( C = B_0e^{-\beta t} \). At this time, the concentrations of the drug between the vascular and extravascular spaces have reached a pseudo equilibrium phase.

From the plot, \( \ln C \) versus \( t \), the relationship will be described by a straight line (\( B_0e^{-\beta t} \)). This concept is the basis of “curve stripping” also referred as a method of residuals, which is the common method for identification of compartmental models. After administration of a drug which follows a multi-compartment model, a plot of \( \ln C \) against time \( t \), would result in a curve. Thus the kinetics of such a drug cannot be accurately described by a one-compartment open model. The following sequence describes the method of identification of the number of compartments involved in a multi-compartment model (e.g. a two-compartment model).

i. Make sure the pseudo equilibrium phase has been attained; i.e. the terminal phase is linear. Extrapolate the terminal (linear) portion of the curve \( C \), to the Y-axis. This is the “elimination” line \( B_0e^{-\beta t} \).

ii. Choose sufficient number of corresponding points on elimination line \( B \) and overall concentration curve \( C \). Subtract corresponding \( B \) from \( C \) to get \( A \), and plot \( A \) values against corresponding time \( t \). If the plotted points can be joined by a straight line, then line \( A \), is the “distribution” line, \( A = A_0e^{-\alpha t} \) and the model is a two-compartment model. On the other hand, if \( A \), turned to be curvilinear, then there are more than two compartments and have to continue stripping until a straight line is achieved. Intuitively, each straight line represents one exponent or one compartment.
Non – Compartmental Models

Non – compartmental models offer a fast and easy way to compute graph and analyze the most commonly used pharmacokinetics parameters associated with blood (plasma and serum) concentration – time data. Routes of administration may be oral, rectal, epidermal, or intravenous. Non-compartmental models are also applicable in the urinary data analysis.

The equations involved in these analyses are referred as non – compartmental because they do not require curve-fitting or make any assumptions concerning compartmental models. In non – compartmental modeling, the calculation of pharmacokinetics parameters are based on the analyses of two standard methods;
a. Curve – stripping, or feathering, or method of residuals, to derive the exponential terms that describe the blood level curve, and;
b. Area under the blood level – time curve (AUC), calculations; [the linear and trapezoidal methods] [26].

Physiologic /Physiologically – based Pharmacokinetics (PB-PK) Models

These are models which are based on known anatomic and physiological data. The drug concentrations in tissue and drug binding in tissue are known in physiological pharmacokinetics models. These models are based on actual tissues and blood flow, describe the data more realistically.

Physiologically based - pharmacokinetics (PB – PK) models are frequently used in describing drug distribution in animals, because tissue samples are readily and easily available for assay.

In physiological models, the size or mass of each tissue compartment is determined physiologically rather than by mathematical estimation. The concentration of drug in the tissue is determined by the ability of the tissue to accumulate drug as well as by the rate of blood perfusion to the tissue [25].
ESTIMATION OF PHARMACOKINETICS PARAMETERS USING URINE DATA

Sometimes it may not be possible to collect blood (plasma) samples but one may be able to estimate the amount of drug excreted unchanged into the urine. While in others, the apparent volume of distribution may be so large that plasma concentrations are too low to be evaluated. Furthermore, lack of sufficiently sensitive analytical techniques has often prevented measurement of the concentration of many drugs in plasma [27]. Under these conditions, urinary excretion data become more appropriate for pharmacokinetics studies.

The usefulness of urinary excretion data in pharmacokinetic studies of drugs may further be more appropriate where non-invasive methods are desirable.

The Scheme for the Model

It may be possible to obtain valuable pharmacokinetic information from the amount of unchanged drug excreted in urine data. In this study, when a one-compartment model analysis is applied to the urinary excretion data, it has two parallel pathways of the overall elimination process. The elimination of the fraction of the administered dose excreted in the unmetabolized or in unchanged form in urine is defined by an elimination rate constant $k_e$. The fraction of administered dose which is eliminated in the metabolized form is characterized by an elimination rate constant $k_m$. There are other possible routes of elimination such as air, sweat, and bile metabolism. These are generally considered as shadow of metabolism [27].

Under these conditions the overall elimination rate constant, $K_e$, is related to $k_e$ and $k_m$ by the expression:

$$K_e = k_e + k_m$$

Furthermore, $K_e$ is related to $f_e$, the fraction of the administered dose excreted in the unchanged form and it is expressed by equation:

$$f_e = k_e/K_e$$
The Rate of Excretion of Unchanged Drug Eliminated in Urine (\( \text{du/dt} \))

The cumulative amount of unmetabolized drug excreted into urine is \( U \). The rate of excretion of an infinitesimal amount of unchanged drug is \( \text{du} \), over an infinitesimal time \( \text{dt} \), \( \frac{\text{du}}{\text{dt}} \) may be expressed in terms of \( k_e \) or \( \text{CL}_R \), as:

\[
\frac{\text{du}}{\text{dt}} = k_e \cdot V \cdot \text{Cp}
\]

From the equation \( \text{CL}_R = k_e \cdot V \)

\[
\frac{\text{du}}{\text{dt}} = \text{CL}_R \cdot \text{Cp}
\]

Where, \( k_e \) is the excretion rate constant for the fraction of administered dose that is eliminated in unmetabolized/unchanged form in urine. Substituting for \( \text{Cp} = \text{Cp}_0 \cdot e^{-k_e \cdot t} \) in the above equation;

\[
\frac{\text{du}}{\text{dt}} = k_e \cdot V \cdot \text{Cp}_0 \cdot e^{-k_e \cdot t}
\]

From the equation, \( V = \text{Dose} / \text{Cp}_0 \)

\[
\frac{\text{du}}{\text{dt}} = k_e \cdot \text{Dose} \cdot e^{-k_e \cdot t} \quad \text{[5.6]}
\]

Taking natural logs on both sides of this equation yields;

\[
\ln (\frac{\text{du}}{\text{dt}}) = \ln k_e \cdot \text{Dose} - k_e \cdot t \quad \text{[5.7]}
\]

This is the rate of excretion equation of unchanged drug eliminated in urine.

Cumulative Amount Excreted as Unchanged Drug (\( U \))

The rate of excretion equation-5.6 is expressed as;

\[
\frac{\text{du}}{\text{dt}} = k_e \cdot \text{Dose} \cdot e^{-k_e \cdot t}
\]

\[
\text{du} = k_e \cdot \text{Dose} \cdot e^{-k_e \cdot t} \quad \text{dt}
\]

Integrating this equation between the time limits zero and \( t \);

\[
U = \frac{k_e}{k_e \cdot \text{Dose}} \cdot [e^{-k_e \cdot t}]_0 - k_e \cdot \text{Dose} \cdot [e^{-k_e \cdot t}]_t
\]

\[
U = \frac{k_e}{k_e \cdot \text{Dose}} \cdot [1 - e^{-k_e \cdot t}]
\]

But \( k_e / k_e = \text{fe} \); hence substituting yields;

\[
U = \text{fe} \cdot \text{Dose} \cdot [1 - e^{-k_e \cdot t}] \quad \text{[5.8]}
\]

This is the cumulative excretion equation in the urinary data analysis.
The Amount Remaining to be Excreted (A.R.E.) Concept

Another aspect of the model which can be applied in the current study is the A.R.E concept. The equation describing this plot is expressed as follows. From equation 5.8;

\[ U = fe \cdot \text{Dose} \left[ 1 - e^{-Kel \cdot t} \right] \]

Substituting, \( U_∞ = fe \cdot \text{Dose} \)

\[ U = U_∞ \cdot \left[ 1 - e^{-Kel \cdot t} \right] \]
\[ U = U_∞ - U_∞ \cdot e^{-Kel \cdot t} \]
\[ U_∞ - U = U_∞ \cdot e^{-Kel \cdot t} , \]

Taking natural logs on both sides;

\[ \ln (U_∞ - U) = \ln U_∞ - Kel \cdot t \]

Substituting \( U_∞ = fe \cdot \text{Dose} \),

\[ \ln (U_∞ - U) = \ln fe \cdot \text{Dose} - Kel \cdot t \quad \text{[5.9]} \]

This is the A.R.E. equation and the term \((U_∞ - U)\) is a measure of the amount of drug remaining to be excreted (A.R.E) at time \( t \) \[27\].

The Pharmacokinetics Parameters (fe)

The pharmacokinetic parameter \( fe \) is the fraction of administered dose that is eliminated in the unmetabolized or unchanged form in the urine.

This parameter is an important and has wider applications in the urinary data analysis. These are expressed by following terms. From equation 5.8;

\[ U = (ke/Kel) \cdot \text{Dose} \left[ 1 - e^{-Kel \cdot t} \right] \]

As time approaches infinity, \( U \) turns to \( U_∞ \) and the term \( e^{-Kel \cdot t} \) approaches zero. \( U_∞ \) is the total cumulative amount of unchanged drug excreted at infinity time \( t_∞ \). Thus,

\[ U_∞ = (ke/Kel) \cdot \text{Dose}, \]

This on rearranging, results;

\[ fe = (ke/Kel) = U_∞/\text{Dose} \quad \text{[5.10]} \]
Thus, the parameter $fe$, can be readily estimated from the urinary excretion data.

**Urinary Excretion - Time Plots**

Following a fit and subsequent analysis of a one – compartment model to the urinary excretion data, three main analytical plots can be obtained. The plots are the cumulative excretion, the rate of excretion, and the amount remaining to be excreted, (A.R.E.) \(^{[27]}\). After administration of the drug, urine is collected over finite time intervals and assayed for drug content. Data collected include the volume of urine voided, a time interval of collection and the amount of unchanged drug excreted. The data are treated to calculate the following variables; the cumulative amount excreted $U$, the amount remaining to be excreted (A.R.E), and the rate of excretion $du/dt$. Variables so obtained are used to complete the urinary data table which is subjected to further analyses to derive useful pharmacokinetic information.

**The Cumulative Excretion Plot (U versus T Plot)**

One convenient way of representing the urine data is by a plot of $U$ versus time $t$. It is called the cumulative excretion plot. The equation for this plot, \([equation-5.8]\) is expressed as;

$$U = \text{Dose} \cdot fe \left[1 - e^{-Ke\cdot t}\right]$$

The cumulative excretion-time plot is a mirror image of the amount of the drug lost from the body. As the drug gets eliminated from the body, it will appear in the urine. U versus t plot is fairly qualitative and often difficult to get quantitative results directly.

As the cumulative excretion time approaches infinity $\infty$, the cumulative amount excreted value levels off to $U_\infty$, which is equal to the product of the dose and $fe$; $(fe \cdot \text{Dose})$. Generally, the plot shows $U$ rapidly increasing at initially and then approaches a plateau which is $U_\infty$. It must be ensured that total urine is collected. Urine collection must be made for a sufficient period of time to gain an accurate or good
estimation of the total cumulative amount of unchanged drug excreted $U_\infty$. The period of urine collection must be at least five to six times of the half-life. Drugs with long half-life values, it is difficult to be analyzed with this approach. A major disadvantage of this plot is that it only leads to a qualitative measurement of the parameters.

**The Rate of Excretion Plot (R/E – Plot)**

A second method of urine data analysis, following a fit of one-compartment model to the data, is via the rate of excretion versus time plot, (R/E – plot). From equation-5.7, the rate change of the amount of drug excreted into urine $\frac{du}{dt}$ is expressed as:

$$\ln \left(\frac{du}{dt}\right) = \ln ke\cdot\text{Dose} - Kel\cdot t$$

A plot of $\ln \left(\frac{du}{dt}\right)$ versus time $t$, on a semi-log scale yields a straight line with a slope of $-Kel$, and an ordinate intercept of $\ln ke\cdot\text{Dose}$. This approach involves a plot of the average excretion rate against the mid-point of the collection time interval on a semi-log scale [23]. From the urinary excretion data one can calculate the average rate of excretion during each collection time interval. However, the time point of the plot is the mid-point time within the collection interval.

The measured urinary excretion rate reflects the average plasma concentration during the collection interval. The plasma concentration keeps changing continuously within this collection interval. Shortening the collection period reduces the change in plasma concentration, but increases the uncertainty in the estimation of the excretion rate due to incomplete emptying of the urinary bladder. The urine collection interval, denoted by $\Delta t$, is composed of many such very small increments of time. Similarly, the amount of drug excreted in a collection interval is the sum of the amounts $\Delta u$, excreted in each of these small increments of time. The average rate of excretion is directly proportional to the average plasma concentration. Meanwhile, this average plasma concentration is neither the value at the beginning nor at the end of the collection time but at some intermediate point. Assume that the plasma
concentration changes linearly with time and the appropriate concentration is obtained at the mid-point of the collection interval. Since the plasma concentration of the drug changes exponentially with time, this assumption of linear change is reasonable only when loss during the interval is small. Practically, this interval should be less than the elimination half-life of the drug [23]. A major disadvantage of the procedure is difficult to collect urine sample at accurate time. The difficulty in collection of urine samples is pronounced, especially when the elimination half-life is small. Incomplete emptying of the urinary bladder within the collection time interval is another source of limitation. Furthermore, the error present in “real” data can obscure the straight line and lead to results which lack precision in the rate analysis.

The Amount Remaining to be Excreted Plot (A.R.E. - Plot)

A third analysis of the urinary excretion data which involves a fit of one-compartment model is the amount remaining to be excreted (A.R.E.) plot. The equation, [equation-5.9] for this plot is expressed as;

\[
\ln (U_\infty - U) = \ln fe.Dose - Kel.t
\]

The A.R.E. equation is linear; hence a plot of \(\ln (U_\infty - U)\) against time \(t\), on a semi log-scale results in a straight line of slope, \(-Kel\), and an ordinate intercept of \(\ln fe.Dose\). The term \((U_\infty - U)\) is the amount remaining drug to be excreted at time \(t\). If one subtracts \(U\) from \(U_\infty\) at each time point, one would be calculating A.R.E at that time.

A major disadvantage of this method of urinary excretion data analysis is that the total urine collection is a necessity. Thereby difficulty is encountered in analysis of drugs with long half-lives by this method. Another disadvantage of this approach is that the errors are cumulative, with each collection interval. Hence the total error is incorporated into the \(U_\infty\) value and therefore into each A.R.E value. Moreover, one missed or lost sample means errors in all calculated results.

For this reason, absorption kinetics are difficult to estimate using urine samples, especially when the absorption half-life is relatively low.
In such a case, absorption would have been completed even before the very first urine sample is voided.

**Non–Compartmental Model Analysis of Excretion Rate-Time Data**

Occasionally, it may not be possible to adequately analyze a urinary excretion rate data with a fit of one – compartment model. Under these circumstances a non – compartment model analysis is employed to calculate the required parameters. According to food and drug administration (FDA) non – compartmental analysis of urinary rate data are Rmax, and Tmax. Rmax is the maximal rate of urinary excretion, and Tmax is the time of maximal urinary excretion. These parameters are readily obtainable from excretion rate plots [27].

Assuming that renal clearance is constant, and then the urinary excretion rate is proportional to the plasma concentration. Hence a plot of average urinary excretion rate against the mid - point time simulates a plot of plasma concentration against time. The urinary excretion rate reflects the average plasma concentration during the collection interval. The excretion rate data can therefore be treated in a manner analogous to that of plasma data and estimation of pharmacokinetic parameters can be conveniently calculated from it [23]. If the excretion rate time course gives some clue about the absorption rate then one can describe the drug absorption process. If a first order input (e.g. oral) is simulated, one can estimate the absorption rate constant ka [28]. The absorption rate constant ka may be estimated by the method of residual approach. The overall or terminal elimination rate constant Kel may also be obtained by log-linear regression of the terminal phase of the curve.
Chapter-5 Pharmacological Evaluation

RENEAL ELIMINATION KINETICS (URINARY ANALYSIS)

Physiological Basis of Renal Excretion

The major organ for excretion of drugs is the kidney and the basic or fundamental unit of the kidney is nephron. Three major eliminating processes within the nephron are the glomerular filtration (which occurs in the Bowman’s capsule), tubular secretion (which occurs primarily in the proximal section), and tubular reabsorption, which occurs all along the nephron. Active reabsorption if present usually occurs in the proximal section while passive reabsorption is restricted to the distal portion. The net process from the combined three eliminating processes determines the total renal excretion of the drug by the kidney [23].

Renal Clearance (CL<sub>R</sub>)

One of the methods of quantitatively describing the renal excretion of drugs is by means of the renal clearance value, CL<sub>R</sub> for the drug. Renal clearance can be estimated as part of the total body clearance of a particular drug and can also be used to investigate the mechanism of drug excretion. If the drug is exclusively filtered, but not secreted nor re-absorbed, then the renal clearance will be about, 120 mL min<sup>-1</sup> in normal subjects. This is the creatinine clearance value and indication of the glomerular filtration rate (GFR). If the renal clearance value is less than 120 mL min<sup>-1</sup>, then one can assume that at least two processes are in operation; glomerular filtration and tubular reabsorption. However, if the renal clearance is greater than 120 mL min<sup>-1</sup>, then tubular secretion must be contributing to the overall excretion process. It is also possible that all the three eliminating processes are occurring simultaneously [29].

In mathematical terms,

\[
\text{Excretion rate} = CL_R \cdot Cp
\]

Where, Cp is the plasma concentration at time t.

This implies that, \(CL_R = \frac{\text{Excretion rate}}{Cp}\) \[5.11\]
Analogous to the above series of processes within the kidney (nephron), where the net renal excretion rate is determined by the combined three eliminating processes:
\[
CL_R = \frac{(\text{Filtration rate} + \text{Secretion rate} + \text{Re-absorption rate})}{C_p}
\]

For most of the drugs which are excreted in the unchanged/unmetabolized form, it has been established that there is a good correlation between creatinine clearance and the drug’s clearance or its observed elimination rate constant, \( K_e \) [30].

Renal clearance can be estimated by various methods depending on the available resources and conditions. Some of these methods are briefly enumerated below.

a. Renal clearance may be calculated using the pharmacokinetics parameters \( k_e \) and \( V \) as:
\[
CL_R = k_e V \quad \text{[5.12]}
\]

b. Renal clearance can also be calculated by measuring the total amount of drug excreted \( du \), over time interval \( dt \). Dividing the excretion rate, \( (du/dt) \), by the plasma concentration \( C_p \), measured at the mid-point of the time of collection interval results in \( CL_R \) value (i.e. Equation 5.11). This is particularly useful in urine sampling/data analysis. Thus,
\[
\text{Renal clearance} = \frac{\text{Rate of excretion}}{\text{Plasma concentration, } C_p} = \frac{(du/dt)}{C_p} = \frac{R}{C_p}
\]

c. Renal clearance can also be estimated as the product of the extraction ratio \( E \) and the plasma or blood flow rate \( Q \) to the eliminating organ.
\[
CL_R = E Q \quad \text{[5.13]}
\]

d. Clearance can also be calculated as the fraction of the total dose administered to the total AUC. This data is only for those systems, which are non-model dependent. Thus;
\[
CL_R = \frac{\text{Dose}}{\text{AUC}} \quad \text{[5.14]}
\]
EXPERIMENTAL

BIOLOGICAL ACTIVITY

Generally there are two methods of testing for MIC

(i) Broth dilution method

(ii) Agar dilution method

In the present work Minimum Inhibition Concentration (MIC) was determined by Broth dilution method [31].

Determination of Minimum Inhibition Concentration (MIC) by Broth Dilution Method

All synthesized compounds were evaluated for antimicrobial test procedure. All the necessary controls like drug control, vehicle control, agar control, organism control and known antibacterial drug control were used. Sterile graduated pipettes of 10 mL, 5 mL, 2 mL and 1 mL, sterile capped 7.5 × 1.3 cm tubes, small screw-capped bottles, Pasteur pipettes, over night broth culture of test and control organisms were used for antimicrobial study. All MTCC cultures were tested against synthesized compounds and reference drugs. Mueller Hinton Broth was used as nutrient medium to grow and dilute the drug suspension for the test bacterial. Sabourand Dextrose Broth was used for fungal nutrition. Inoculum size for test strain was adjusted to $10^8$ CFU (Colony Forming Unit) per milliliter by comparing the turbidity. Serial dilutions of synthesized compounds were prepared in primary and secondary screening.

Following common standard strains were used for screening of antibacterial and antifungal activities. The strains were procured from Institute of Microbial Technology, Chandigarh.

- *Staphylococcus aureus* MTCC 96 (Gram positive),
- *Bacillus subtilis* MTCC 441 (Gram positive),
- *Escherichia coli* MTCC 443 (Gram negative)
- *Enterobacter aerogenes* MTCC 111 (Gram negative)
- *Penicillium chrysogenum* MTCC 5108 (Fungus)
Aspergillus niger MTCC 282 (Fungus)
Candida albicans MTCC 227 (Fungus).

DMSO was used as diluents / vehicle to get desired concentration of synthesized compounds and reference drugs to test against standard microbial strains.

**Minimum Inhibitory Concentration (MIC)**

1. Serial dilutions were prepared in primary and secondary screening. Each synthesized compounds was diluted to obtain 2000 µg mL\(^{-1}\) concentration, as a stock solution.

**Primary screen:** In primary screening, 500 µg mL\(^{-1}\), 250 µg mL\(^{-1}\) and 150 µg mL\(^{-1}\) concentrations of the synthesized compounds were taken. The active synthesized compounds founds in this primary screening were further tested in a second set of dilution against all microorganisms.

**Secondary screen:** The synthesized compounds found active in primary screening were similarly, diluted to obtain 100 µg mL\(^{-1}\), 50 µg mL\(^{-1}\), 40 µg mL\(^{-1}\), 30 µg mL\(^{-1}\), 10 µg mL\(^{-1}\), 5 µg mL\(^{-1}\) and 1 µg mL\(^{-1}\) concentrations.

2. Mueller Hinton Broth was used as nutrient medium for bacteria and Sabourand Dextrose Broth for fungal to grow. Inoculum size for test strains was adjusted to 10\(^8\) CFU per mL by comparing the turbidity with McFarland standards.

3. Prepared stock solution of antibiotics of concentrations 2000 mg L\(^{-1}\), as required. Arrange micro well plate 8 × 12 well of sterile well in the rack.

4. In a sterile 30 mL universal screw capped bottle, prepared 8.0 mL of broth containing the concentration of antibiotic required for the first tube in each raw from the appropriate stock solution already made. Mix the contents of the universal bottle using a micropipette and transfer 80 µL to the first well in each row. Using a fresh Micropipette, add 20 µL of broth to the remaining 20 µL to the second well in each row. Continue preparing dilutions in this way.
5. Incubate at 37 °C for 24 hrs for bacteria and 22 °C for 74 hrs for fungal.

6. MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. Thus, the lowest concentration inhibiting growth of the organism is recorded as the MIC.

7. The amount of growth from the control tube before incubation which represents the original inoculum is compared.

**Determination of zone of inhibition:**

**Agar Cup method (Kirby-Bauer Technique) to determine zone of inhibition**

The antibacterial and antifungal activities of synthesized compounds (DPMK, APSA, DPAAPA, PPQC) were determined by Agar Cup method [32]. The synthesized compounds DPMK, APSA, DPAAPA and PPQC were dissolved in dimethyl sulphoxide (DMSO) in the concentration of 500, 150, 30, 100 µg mL\(^{-1}\) respectively. DMSO is use as control. The primary literature survey revealed that DMSO does not inhibit the growth of bacteria [33].

**Preparation of plate and microbial assay:**

In vitro antibacterial activity was carried out against 24 hr old cultures of bacterial strains. In the present work, *Staphylococcus aureus MTCC 96 (Sa)*, *Bacillus subtilis MTCC 441 (Bs)*, *Escherichia coli MTCC 443 (Ec)* and *Enterobacter aerogenes MTCC 111 (Ea)* were used to investigate the antibacterial activity. 20 mL of sterilized agar media was poured into each pre-sterilized petri dish. Excess of suspension was decanted and plates were dried in an incubator at 37 °C for an hour. About 60 µL of 24 hr old culture suspension was poured and neatly swabbed with the pre-sterilized cotton swabs. 6 mm diameter sterile cork borer was used to punch carefully in well and 30 µL of test solutions of each compound (DPMK, APSA, DPAAPA, PPQC) was added into each labeled well. The plates were incubated for 24 hr at 37 °C. Each inhibition zone that
appeared after 24 hr, around the well in each plate was measured as a zone of inhibition. An experiment was carried out in triplicate.

Antifungal activity of synthesized compounds DPMK, APSA, DPAAPA and PPQC were carried out against *Penicillium chrysogenum MTCC 5108 (Pc)*, *Aspergillus niger MTCC 1344 (An)* and *Candida albicans MTCC 227 (Ca)*. Sabourands agar media were prepared by dissolving peptone (10.0 g), D-glucose (40.0 g) and agar (20.0 g) in double distilled water (1000 mL) and pH was adjusted to 5.7. Normal saline (0.9% w/v NaCl) was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 mL of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 hr. A sterile cork borer (6 mm diameter) was used to punch carefully in well. The test solution (30 µL) of compounds DPMK, APSA, DPAAPA and PPQC were added in each labeled well. DMSO was used as a control. The petri dishes were prepared in triplicate and maintained at 22 °C for 74 hr. Antifungal activity was determined by measuring the diameter of inhibition zone.

Antibacterial and antifungal activity of each compound was compared with streptomycin and fluconazole as a standard drug respectively [34]. Zones of inhibition and percentage of relative inhibition zone diameter (% RIZD) were determined for compounds DPMK, APSA, DPAAPA and PPQC [35]. The percentage of relative inhibition zone diameter (% RIZD) was calculated in term of inhibition zone obtained for control as compared to zone of inhibition obtained from the standard at the same concentration. The antimicrobial activity was calculated by applying the following formula,

\[
% \text{RIZD} = \left( \frac{\text{IZD sample} - \text{IZD control}}{\text{IZD standard}} \right) \times 100\% 
\]

where, RIZD is the percentage of relative inhibition zone diameter. IZD is the inhibition zone diameter (mm).
PHARMACOLOGICAL ACTIVITY

Animals Used

Adult Wistar albino rats weighing between 150–200 g were used for the pharmacological studies of synthesized compounds. The animals were maintained under normal laboratory condition and kept in standard polypropylene cages at 30 ± 2 °C temperature and 60 to 65% relative humidity. These rats were provided standard diet and water *ad libitum*. The set of rules followed for animal experiment were approved by the Institutional Animal Ethical Committee (VBT/IAEC/10/12/40).

Acute Oral Toxicity Study

Acute oral toxicity [36] of each synthesized compound (DPMK, APSA, DPAAPA, and PPQC) was performed by the OECD guideline 423 using Wistar albino rat animals. Different drug doses (50-6000 mg kg\(^{-1}\) as per body weight of the animal) were prepared in aqueous suspensions of acacia gum and administered orally. The dose at which 50% animals were dying, that dose was selected as the lethal dose (LD\(_{50}\)). 1/10\(^{th}\) part of lethal dose (LD\(_{50}\)) was selected as an effective dose (ED\(_{50}\)). The screenings of pharmacological activities of the synthesized compounds were carried out at ED\(_{50}\) dose.

Analgesic Activity

Analgesic activity [37] was carried out by hot plate and tail immersion methods. In both methods, rats were taken in six groups. Each group consists of six rats. All the animals were fasted for 18 hrs before the beginning of the experiment and water given *ad libitum*. The animals of group I were treated with 2.0% acacia suspension prepared in distilled water served as a control. The animals of group II were given paracetamol (562 mg kg\(^{-1}\), orally) served as a reference standard [38]. The animals of group III, IV, V and VI were orally administered with the synthesized compounds such as, DPMK (500 mg kg\(^{-1}\)), APSA (600 mg kg\(^{-1}\)), DPAAPA (100 mg kg\(^{-1}\)) and PPQC (600 mg kg\(^{-1}\)) respectively.
**Hot plate and tail immersion method**

In case of hot plate method, rat was placed on the hot plate of Analgesiometer maintained at a temperature of 55.0 ± 0.5 °C. The latency to flick the paw or lick or jump from the hot plate was noted as the reaction time. The reaction time was noted in triplicate at the time interval of 0, 15, 30, 45, 60, 90 and 120 min. The cut off time was considered as 30 second for each measurement.

In case of tail immersion method, the distal 2-3 cm portion of rat tail was immersed in hot water maintained at 55.0 ± 1 °C. The time taken by the rat to withdraw the tail from hot water bath was noted as reaction time. This experiment was repeated three times at a time interval of 0, 15, 30, 45, 60, 90 and 120 minute. The percent analgesic activity (PAA) was calculated by the following formula,

$$PAA = \left[ \frac{T_2 - T_1}{T_2} \right] \times 100$$

Where, $T_1$ is the reaction time (second) before treatment, $T_2$ is the reaction time (second) after treatment.

**Antipyretic Activity**

An antipyretic activity [39, 40] was screened by the Yeast induced pyrexia method. Animals were fasted for 24 hr before inducing pyrexia. Pyrexia was induced by administration of 15.0% w/v aqueous suspension of Brewer’s yeast subcutaneously below the nape of the neck at the dose of 20 mL kg$^{-1}$ of body weight. Immediately after yeast administration, food was withdrawn. The compounds DPMK, APSA, DPAAPA, PPQC and standard drug were dissolved in aqueous suspension of 2.0% gum acacia. After 18 hr of yeast injection, the dose of paracetamol as a standard drug (562 mg kg$^{-1}$ body weight) and the doses of synthesized compounds; DPMK (500 mg kg$^{-1}$), APSA (600 mg kg$^{-1}$), DPAAPA (100 mg kg$^{-1}$) and PPQC (600 mg kg$^{-1}$) were given orally. The control group received only an aqueous suspension of 2.0% gum acacia at the dose of 100 mg kg$^{-1}$ body weight. Rectal temperature was
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determined by introducing a clinical thermometer 1 inch into the rectum of rat and keeping it inside for 1 minute before and 18 hrs after Brewer’s yeast injection at a time interval of 30, 60, 90, 120 and 180 minutes. Percentage reduction in rectal temperature was calculated by the following formula,

\[
\% \text{ reduction} = \frac{\text{Temp. 18 hrs after yeast} - \text{Temp. after drug at different hrs} \times 100}{\text{Temp.18 hrs after yeast} - \text{normal rectal temp. prior to yeast administration}}
\]

---

**Anti-inflammatory Activity**

Anti-inflammatory activity [41] of synthesized compounds DPMK, APSA, DPAAPA and PPQC was evaluated by carrageenan induced rat hind paw edema method. Animals were fasted overnight with free access to water before the experiment. In control, test and standard groups of animals, acute inflammation were produced by sub-planter injection of 0.1 mL of freshly prepared 1.0% suspension of Carrageenan (in normal saline) in the right hind paw of the rats. The solutions of synthesized compounds DPMK (500 mg kg\(^{-1}\)), APSA (600 mg kg\(^{-1}\)), DPAAPA (100 mg kg\(^{-1}\)) and PPQC (600 mg kg\(^{-1}\)) and standard drug diclofenac sodium (100 mg kg\(^{-1}\)) were prepared in 2.0% aqueous suspension of acacia gum and administered orally, 1 hr before carrageenan injection. The control group received only vehicle (2 mL kg\(^{-1}\)). Paw volume was measured plethysmometrically between 0 to 4 hr after carrageenan injection. Percentage inhibition of paw volume was calculated by following formula,

\[
\% \text{ inhibition of edema} = \left(1 - \frac{V_t}{V_c}\right) \times 100
\]

Where, \(V_t\) = mean paw volume of the test group, \(V_c\) = mean paw volume of the control group.

All the data for the pharmacological activities were expressed as Mean ± SEM (n=6). Statistical analysis was performed by using one way (ANOVA) followed by Student’s t-test. (*) for \(P < 0.05\), (**) for \(P < 0.01\) and (***) for \(P < 0.001\) were considered as significant relative to control values.

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PHARMACOKINETICS STUDY

The present study was conducted to evaluate pharmacokinetics parameters, bioavailability, urinary excretion, and renal clearance of synthesized compounds DPMK, APSA, DPAAPA and PPQC following oral administration in Wistar albino male rats. The project was approved by Institutional Animal Ethical Committee (VBT/IAEC/10/12/40).

Animals Used

A crossover study with respect to the synthesized compounds and route of administration was conducted on four rats. Adult Wistar albino rats weighing between 250–320 g were used for the pharmacokinetics studies. The animals were considered to be healthy on the basis of preliminary physical examination and maintained under similar environmental and managemental conditions. The animals were weighed before the day of drug administration to determine the requirement of dose. They had received no medications before two weeks and during washout period. The animals were kept off feed 18 hrs before the administration of synthesized compounds and have accessed to drinking water ad libitum.

Administration of Synthesized Compounds

The animals were divided into five groups and each group consists of four rats. The dose of each synthesized compound and paracetamol were prepared in 2.0% aqueous suspension of acacia gum. The animals of the group I was given paracetamol (500 mg kg$^{-1}$) served as control as well as reference standard. The animals of groups II-V were orally administered synthesized compounds DPMK (500 mg kg$^{-1}$), APSA (600 mg kg$^{-1}$), DPAAPA (100 mg kg$^{-1}$) and PPQC (600 mg kg$^{-1}$) respectively.

Collection of Blood Samples

Blood samples (approximately 1.0 mL) were collected in Eppendorf test tubes from the tail vein of Wistar albino rat [42]. A mixture of
methanol and ethyl acetate (1:2) was previously added in an Eppendorf test tube. The synthesized compounds DPMK (500 mg kg\(^{-1}\)), APSA (600 mg kg\(^{-1}\)), DPAAPA (100 mg kg\(^{-1}\)) and PPQC (600 mg kg\(^{-1}\)) were administered orally to the rat and blood samples were withdrawn at the time intervals of 0.0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 10, 12, and 24 hr. Immediately, a blood sample and the mixture of methanol and ethyl acetate (1:2) was shaken for 5 minute by hand until the contents were properly mixed. Then after, the tubes were shaken for 5 minute on a vertex mixture and centrifuged at 2000 rpm for 5 minute at room temperature. Blood supernatants were separated and after necessary labeling stored at −20 °C until assayed.

**Collection of Urine Samples**

In all experiments, a blank urine sample was collected before administration of the drug. In this study, urine samples (1.0 mL) were collected at the time intervals of 0, 6, 12, 24, 36, 48, 60, 72 and 84 hrs and the volume was measured. 1.0 mL urine was diluted up to 100 mL with double distilled water. Diluted urine samples were stored at -20°C until the analysis.

**UV-Visible Spectrophotometric Method**

**Assay Method for Paracetamol**

Blood supernatants and diluted urine samples of paracetamol were measured by spectrophotometrically [43]. After treatment, 0.2 mL of blood supernatant or 1.0 mL of diluted urine sample was mixed with 1.0 mL of 1.0 M hydrochloric acid and 2.0 mL of 1.0 mM ferric sulphate. The resulting solution was heated at 100 °C in water bath for 10 min. Then after adding 2.0 mL of 1.0 mM potassium ferricyanide and was diluted up to 10 mL with distilled water. The resulting samples were analyzed by spectrophotometrically at \(\lambda_{max} 700\) nm, after 24 min. A calibration curve was constructed (concentration range between 0.2-2.0 µg mL\(^{-1}\)) by spiking drug-free rat blood and urine in duplicate with a standard solution of paracetamol (100.0 µg mL\(^{-1}\)).
Assay Method for the Synthesized Compounds

0.2 mL of blood supernatant and 1.0 ml of diluted urine samples were used for the pharmacokinetic study to determine the concentration of synthesized compounds by assay method. The assay method for synthesized compounds DPMK, APSA, DPAAPA and PPQC are described in chapter 6. The calibration curves of spiked synthesized compounds in blood and urine samples were constructed and are illustrated in chapter 6. The regression equation and calibration curves are used to determine the concentration of synthesized compounds from blood and urine sample which were collected from the Wistar albino rats.

CALCULATIONS

Pharmacokinetics

Pharmacokinetic parameters were calculated by non-compartmental analysis, according to the standard method with the use of MS-Excel. Maximum drug concentration (Cmax) and corresponding time (Tmax) were measured directly from the drug-concentration vs. time plot. The definition and formula of kinetic parameters are given in Table 5.1.

Table 5.1: Definitions and formula of kinetic parameters [44-47].

<table>
<thead>
<tr>
<th>Kinetic Parameter</th>
<th>Unit</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax</td>
<td>µg mL$^{-1}$</td>
<td>The peak blood concentration of a drug after oral administration.</td>
</tr>
<tr>
<td>Tmax</td>
<td>hr</td>
<td>Time to reach $C_{\text{max}}$ (for blood sample) or $R_{\text{max}}$ (for urine sample)</td>
</tr>
<tr>
<td>$AUC_{0-t}$</td>
<td>µg hr mL$^{-1}$</td>
<td>Area under curve calculate using Trapezoidal rule from 0 to the last drug concentration</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$</td>
<td>µg hr mL$^{-1}$</td>
<td>AUC from 0 to infinity time drug concentration</td>
</tr>
</tbody>
</table>

where, $n$= numbers of data points

$$AUC_{0-t} = \sum_{i=0}^{n-1} \frac{(t_{i+1} - t_i)}{2} (C_i + C_{i+1})$$

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_{\text{p last}}}{K_t}$$
Where, $K'$ is terminal slope of curve $C_p$ vs. $t$

**AUMC**

Area under the first moment curve from 0 to the last drug concentration

$$AUMC_{0-t} = \sum_{i=0}^{n-1} \frac{(t_{i+1} - t_i)}{2} \left( C_i t_i + C_{i+1} t_{i+1} \right)$$

**AUMC_{t-\infty}**

Area under the first moment curve from $t$ to the infinity time drug concentration

$$AUMC_{t-\infty} = \frac{C_{p_{last}} t_{last}}{K'} + \frac{C_{p_{last}}}{K'^2}$$

**AUMC_{0-\infty}**

Area under the first moment curve from 0 to the infinity time drug concentration

$$AUMC_{0-\infty} = AUMC_{0-t} + AUMC_{t-\infty}$$

**Kel**

The first order elimination rate at which drugs are removed from the body. In case of blood sample, elimination rate constant was determined from the slope of the elimination part of the drug-concentration time plot.

In case of urine sample, first order elimination rate constant was calculated from the curve of amount of drug remaining to be excreted at a time ($t$) versus endpoint time.

**$t_{1/2}$**

Elimination of half life means time required for the concentration of the drug to reach half of its original value.

$$t_{1/2} = 0.693 / Kel$$

**MRT**

Mean residence time is the average time a molecule stays in the body.

$$MRT = AUMC_{0-\infty} / AUC_{0-\infty}$$

**CL/F**

Total body clearance is the volume of blood or plasma that is totally cleared of its content of drug per unit time and body weight.

$$CL/F = \text{Dose} / AUC_{0-\infty}$$, where F= Oral availability of drug

**Vss/F**

The volume into which a drug appears to be distributed with a concentration equal to that of plasma. The apparent volume distribution at equilibrium,

$$Vss/F = \text{Dose} \times AUC_{0-\infty} / AUMC_{0-\infty}$$, where, F= Oral availability of drug

**du/dt = R**

The renal excretion rate for each interval
### Chapter-5 Pharmacological Evaluation

\[
R = \frac{CV}{\Delta t}
\]

Where, \(\Delta t\) = initiate and end time of each urine collection interval, \(C\) = concentration of drug in urine, \(V\) = urine volumes from the midpoint of each collection interval

\[
\frac{du}{dt}\text{max} = \text{or } R_{\text{max}} \text{ mg hr}^{-1}
\]

The maximal renal excretion rate was observed and the midpoint of the respective collection interval associated with the maximal observed excretion rate (Tmax) was also determined by visual inspection of the urinary excretion rate versus time profile curve.

- **AURC\(_{0-t}\) mg**: Area under the rate of drug excretion versus time curve for \(t\) time
- **AURC\(_{0-\infty}\) mg**: Area under the rate of drug excretion versus time curve for infinite, \(\text{AURC}_{0-\infty} = \text{AURC}_{0-t} + \frac{R_t}{K_e}\)
- **fe %**: The percentage fraction of drug excreted, \(fe = \left[\frac{U_{\infty}}{\text{Dose}}\right] \times 100\)
  Where, \(U_{\infty}\) = cumulative amount of drug at infinite time.

**F** Bioavailability refers to the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. The extent of absorption (F) involves comparison of the AUC of test compound \([\text{AUC}_{0-\infty}]_{\text{test}}\) and AUC of \([\text{AUC}_{0-\infty}]_{\text{Ref.}}\) after oral administration of drug. The relative bioavailability,

\[
F = \frac{[\text{AUC}_{0-\infty}]_{\text{test}}}{[\text{AUC}_{0-\infty}]_{\text{Ref.}}} \quad \text{(for blood sample)}
\]
\[
F = \frac{[\text{AURC}_{0-\infty}]_{\text{test}}}{[\text{AURC}_{0-\infty}]_{\text{Ref.}}} \quad \text{(for urine sample)}
\]

- **\(Ae\) \(_{(0-t)}\) mg**: Amount of (cumulative) drug excreted in urine at \(t\) time

---

**Statistical Analysis**

The pharmacokinetic parameters were calculated as a mean value ± standard deviation (SD) \((n = 4)\). Statistical analysis was performed by student t-test and one way ANOVA. A value of \(P < 0.05\) was considered to be statistically significant.
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