UNIVERSITY OF JOS

TOLLGATES TO EFFECTIVENESS AND SAFETY OF MEDICINES IN DRUG THERAPY

INAUGURAL LECTURE

By

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Ladies and Gentlemen

Introduction

I humbly stand before you to deliver my lecture entitled "Tollgates to Effectiveness and Safety of Medicines in Drug Therapy".

Why Tollgates?

A Tollgate is defined as a gate barring passage to a road, tunnel or bridge until a toll is collected. Vehicles pay some amount of money (toll) at such designated points before entrance is allowed. Where such toll is not paid, entering into the road or bridge is denied. The amount also varied for vehicle size, shape, make, and weight. A vehicle may also be denied access to such roads/bridges if their size, shape or weights do not conform to the build of the road/bridge in spite of having the required toll to pay. Generally, tolls take a toll on the giver. Tollgates are not check points or road blocks. However, in our setting you can almost be certain that, where there is a tollgate, there will be a check point and/or roadblock before or after, and for various open or closed reasons. Chair, ladies and gentlemen, there are a lot of factors (tollgates) that do attempt to limit (collect toll from) drug molecules (the 'Vehicle') from reaching their target sites and effectively execute their potentials thereby delivering their arsenals. The drug molecule therefore pays toll at different gates before reaching the target sites

and subsequently elicit activity. Attempts are made at different levels of drug design, formulation, manufacture, distribution, storage, drug administration and patient care to circumvent or overcome these obstacles. This is technically termed **"OPTIMIZING DRUG THERAPY".**

The Pharmacy Profession

Pharmacy is the health profession that links the health sciences with the chemical sciences and it is charged with ensuring the safe and effective use of drugs and medicines. The scope of pharmacy practice includes the traditional roles of compounding and dispensing medications, and services related to health care, including clinical services, reviewing medications for safety and efficacy, providing drug information and manufacture, including, quality control, production, marketing, research and development. The Pharmacist's specialized education and training enable him to perform various roles to ensure optimal health outcomes for their patients through proper medication use. His knowledge of the chemicals (called drugs), such as its physical, chemical and biological characteristics, help him to synthesize, formulate and understand the mode/mechanism of action of the drug for a **particular 'disease condition',** and also its biotransformation. This in-depth knowledge base helps him play his role in optimizing drug treatment for an individual. They also advise their patients, physicians, nurses, medical laboratory scientists and other health practitioners on the selection, dosages, interactions, and side effects of medications, as well as monitor the health and progress of those patients to ensure that they are using their medications safely and effectively. Some pharmacists specialize in specific drug therapy areas, such as intravenous nutrition support, oncology (cancer), nuclear pharmacy (used for chemotherapy), geriatric pharmacy, and psychiatric pharmacy (the use of drugs to treat mental disorders).

Chemistry is the defining science of Pharmacy. That is why pharmacists are often referred to as chemists or pharmaceutical chemists. **Everything about drugs; its synthesis, extraction from natural sources, identification, structure elucidation, purity determination, formulation into medicines, the administered dose, the route of administration, its interaction at receptor site, metabolism, its absorption, distribution in the body, and elimination from the body requires a**

comprehensive knowledge of the chemistry of the drug. Pharmaceutical Chemistry encompasses to a large extent, Medicinal Chemistry, Biopharmacy and Pharmaceutical analysis. Medicinal Chemistry is an interdisciplinary science, involving the study of molecules with potential therapeutic effects in living organisms. This is achieved through fundamental principles of organic chemistry, natural product chemistry, analytical chemistry and molecular pharmacology. Pharmaceutical analysis deals with quality assurance and control, analysis of drugs and metabolites while biopharmacy deals with the inter-phase science of chemistry and biological effects of xenobiotics.

Pharmaceutical analysis

The detection and measurement of very small concentrations of drugs in matrices such as in formulated product or biological fluids is the challenge faced by the analyst from time to time. The advent of newer instrumental methods had enhanced efficiency and accuracy and reduction in analysis time. Accessibility however, to this cutting edge technological advancement in analysis is almost a mirage in our setting. However, a blend of old/new analytical methods based on sound understanding of scientific principles and standardized specifications/parameters has aided our analytical research in sub-Saharan Africa. Chromatographic techniques (Gas chromatography (GC) and High Performance Liquid Chromatography (HPLC) has facilitated the separation of drug metabolites/impurities from the parent compound, thereby enhancing measurements in formulated products and in biological fluids.

The principles highlighted above have governed our development of relatively simple, sensitive, selective and rapid analytical methods for the determination of drugs in pharmaceutical formulations and in biological fluids. The methods, including HPLC and UV-Visible methods have been used for drugs like anti-malarials (quinine, proguanil, mefloquine) anti infective agents/antibiotic (metronidazole, ampicillin).

What are drugs?

Drugs are chemical substances used in the treatment, cure, prevention, or diagnosis of disease or used to enhance physical or mental well-being in humans or animals. A drug, generally, is any substance that, when absorbed into the body of a living

organism, alters normal biological function. There is no single, precise definition, in view of the different meanings in drug control law, government regulations, medicine, and colloquial usage.

A medication or medicines (as referred to by WHO and defined in Public Health sectors) is a drug taken to cure and/or ameliorate any symptom(s) of an illness or medical condition, or may be used as preventive medicine that has future benefits but does not treat any existing or pre-existing diseases or symptoms. Recently a new word- pharmaceutical drug - was coined to distinguish legal use of chemical substances. A pharmaceutical drug refers to medicines, medication or medicaments, as any chemical substance intended for use in the medical diagnosis, cure, treatment, or prevention of disease.

Figure 1: Typical chemical substances formulated into medicines for convenience of administration.

Are all "drugs", drugs? No!!!

Counterfeit and Fake Drugs: The Nigerian Scene

A counterfeit medication or a counterfeit drug is a medication or pharmaceutical product which is produced and sold with the intent to deceptively represent its origin, authenticity or effectiveness. A counterfeit drug may contain inappropriate quantities of active ingredients, or none, may be improperly processed within the body (e.g., absorption by the body), may contain ingredients that are not on the label (which may or may not be harmful), or may be supplied with inaccurate or fake packaging and

labeling. Medicines which are deliberately mislabeled to deceive consumers—including mislabeled but otherwise genuine generic drugs - are counterfeit. Counterfeit drugs are related to pharmaceutical fraud. Drug manufacturers and distributors are increasingly investing in countermeasures, such as traceability and authentication technologies, to try to minimize the impact of counterfeit drugs (Davison, 2011).

Since counterfeiting is difficult to detect, investigate, quantify, or stopped, the quantity of counterfeit medication is difficult to determine. Counterfeiting occurs throughout the world, although there are claims that it is more common in developing countries with weak regulatory or enforcement regimens. It is estimated that more than 10% of drugs worldwide are counterfeit, and in some countries, more than 50% of the drug supply is counterfeit. In 2003, the World Health Organization cited estimates that the annual earnings of counterfeit drugs were over US$32 billion (WHO | Substandard and counterfeit medicines).

According to the incident database of the Pharmaceutical Security Institute, countries in Asia report the largest share of counterfeits detected globally (See Figure 2). This is likely not only due to lax enforcement, but also to the scale of production of counterfeits emanating from China and countries of South and Southeast Asia. Other major producers include Nigeria, Russia, Mexico, Brazil and Latin America.

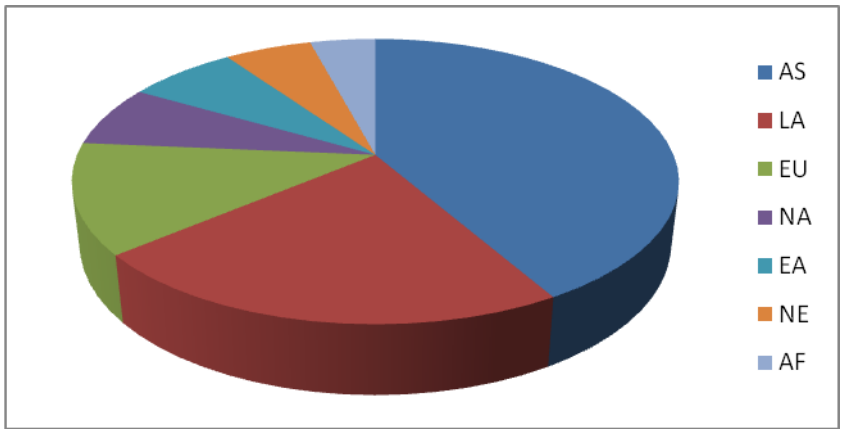

Figure 2: Geographic Distribution of Counterfeit Incidents, 2009. (AS- Asia, LA - Latin America, E - Europe, NA - North America, EA - Eurasia, NE - Near East, AF - Africa)

Source: Pharmaceutical Security Institute, accessed online at http://www.psi-inc.org/geographicdistributions.cfm

The story of fake and counterfeit drugs in Nigeria is best captured by this statement credited to the former Head of NAFDAC, Prof Dora Akunyilli, and posted on WHO site, **"drug counterfeiting was first reported in Nigeria as early as 1968, so people have been dying in this country from the effect of fake drugs since the early 1970s. In 1995, Nigeria reportedly donated 88,000 doses of meningitis vaccine to its neighbor, Niger, before the authorities realized that these vaccines were fake, about 60,000 people had been "inoculated". Akunyili said that when she took office in 2001, fake drugs were openly circulating in her country".** (http://www.who.int/bulletin/volumes/84/9/06-020906/en/). News reports credit Dr Akunyili and the agency for the reduction in the level of fake drugs in Nigeria to 35 per cent, down from around 70 per cent in 2001. The situation today is UNKNOWN!

Why is counterfeiting attractive? Pharmaceuticals sales are a large business with very huge income worldwide (Figure 3). (NOTE: The process of discovery, research and development, before final product manufacture is also extremely expensive. Therefore innovator products are expensive).

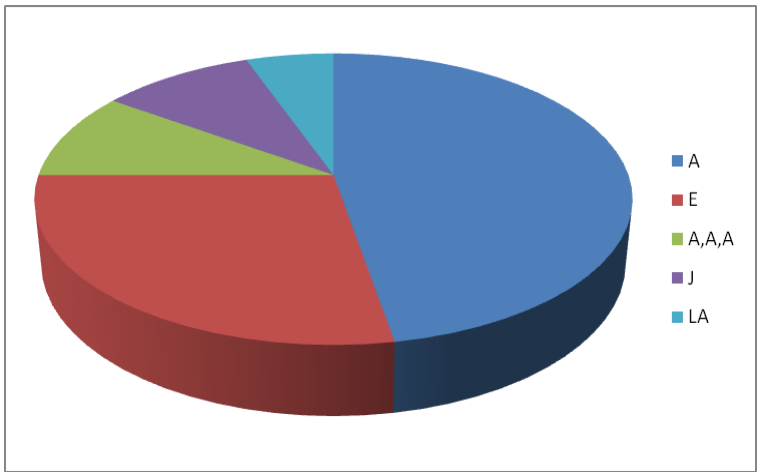

Figure 3: Geographic distribution of Global Pharmaceutical Sales, 2007 US dollars (billions). (A – North America, E – Europe, A, A, A, - Asia, Africa, Austrialia, J-Japan, LA – Latin America)

(Source: UN Office on Drug and crime: Globalization of Crime: A Transnational Organized Crime Threat Assessment: 2010 , p 185)

What Are the Risks of Taking Counterfeit Drugs?

If you use a counterfeit drug you may be at risk of serious health problems, including unexpected side effects, allergic reactions, or a worsening of your health condition. These can occur because a counterfeit drug may:

- be contaminated with harmful substances
- contain the wrong active ingredient, which may not treat your condition or may cause unwanted side effects
- have too little or none of the active ingredient, which will be insufficient to treat your condition
- have too much of the active ingredient, which can cause unwanted and potentially dangerous side effects

Typical example: "After his first in-center dialysis in January 2008, Randy Hubley of Toledo, Ohio suffered severe abdominal pain, diarrhea, and shortness of breath. Two days later, Randy collapsed and did not regain consciousness. Investigations attributed his death to heparin, an anticoagulant that treats blood clotting during kidney dialysis. According to reports, the heparin used before his treatment was counterfeit—the drug was contaminated with oversulfated chrondroitin sulfate, a compound that is structurally similar to heparin, rendering detection of the false substance extremely difficult. Counterfeit heparin induces severe allergic reactions; in 2008, the FDA documented 81 deaths and about 600 allergic reactions linked to the tainted drug. Its origin was traced back to a production plant in Changzhou, China, which also exports pharmaceuticals to Germany, Canada, France, Italy, and other countries", (Brian Finlay 2011).

What drugs are likely to be counterfeited? Any and all drugs are candidates of possible faking. The baseline is to make money. The WHO reported a distribution in 1999 as below;

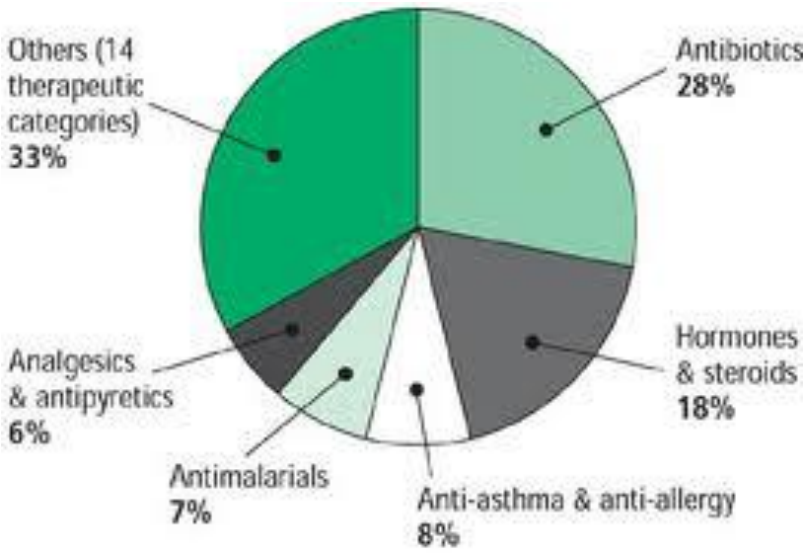

Figure 4: General tread as reported by WHO, 1999.

What Do Counterfeit Drugs Look Like?

A counterfeit drug may look like the genuine version of the medication. Unfortunately, the only sure way to know if it is counterfeit is by performing a chemical analysis in a laboratory. However, there are some signs that may indicate your medication is counterfeit. For example, counterfeit pills may:

- have a strange smell, taste or color
- break apart very easily or be cracked or chipped
- be in poor quality packages with misspelled labels, or labels that have directions that seem incorrect
- may cost very little, especially compared with the normal price of that particular drug

Spot the difference! Difficult?

Figure 5. Sample of Authentic and Fake Serostim injection

Courtesy: FDA Backgrounder: New FDA Initiative to Combat Counterfeit Drugs

Therapy

Therapy is the attempted remediation of a health problem, usually following a diagnosis. In the medical field, it is synonymous with the word "treatment". The process of therapy is to correct a wrong and/or retain acceptable status quo. Preventive therapy or prophylactic therapy is a treatment that is intended to prevent a medical condition from occurring. For example, many vaccines prevent infectious diseases. An abortive therapy is a treatment that is intended to stop a medical condition from progressing any further. A medication taken at the earliest signs of a disease, such as at the very symptoms of a migraine headache, is an abortive therapy. A supportive therapy is one that does not treat or improve the underlying condition, but instead increases the patient's comfort. Supportive treatment may be used in palliative care. **Drug therapy is a dynamic process involving the right molecule (safe and efficacious drug molecule) in the right environment (devoid of possible adverse interactions) at the right time (chronobiology).** Drugs generally act at sites remote to the site of administration. And to produce the expected therapeutic effect, the drug must get to and be present at its site[s] of action in a concentration sufficient to initiate and elicit acceptable response. Therefore when a drug product is administered, absorption, distribution, metabolism, and excretion (ADME) of the drug and its metabolites proceeds continuously at various rates. The relative

rates of these "ADME" processes determine the time course of the drug in the body , most importantly at the particular receptor site responsible for the desired pharmacological action of the drug. The body is constantly trying to eliminate xenobiotics (here the administered drug) and, therefore, it is necessary to balance absorption against elimination so as to maintain the desired concentration. Often the receptor sites are locked away in a specific organ or tissue of the body, such as the central nervous system, and it is necessary to depend upon the blood supply to distribute the drug from the site of administration, such as the gastrointestinal tract, to the site of action. The human body is a very complex system of physiological, biochemical and anatomical compositions, where the drug can lodge or act. These compartments, at first might appear to make any effort to try to describe the time course of the drug at the receptor sites in any mathematically rigorous way hopeless. The picture is further complicated by the fact that, for many drugs , the locations of receptor sites are unknown. Fortunately, the body compartments are networked by the blood system and distribution of drugs among the compartments usually occurs much more rapidly than absorption or elimination of the drug. The net result is that the body behaves as a single homogeneous compartment with respect to many drugs, (particularly, Biopharmaceutical Classification System (BSC) Class 1 drugs) and the concentration of the drug in the blood directly reflects the proportion available to all organs and tissues. Since it may be almost impossible to isolate a receptor site and determine the concentration of drug around it, the concentration at the receptor site usually can safely be assumed to be in equilibrium with the biological fluid perfusing the system/organ.

Pharmacodynamics

Pharmacodynamics; is the study of how a drug acts on a living organism, including the pharmacologic response, the duration and magnitude of response observed relative to the concentration of the drug at an active site in the organism. (Mosby's Medical Dictionary, 8th edition. © 2009, Elsevier).

What is Pharmacokinetics?

Pharmacokinetics, a combination of the Greek Word 'Pharmakon' meaning drugs, and kinetics (motion), is a discipline that is concerned with the study of the rates of movement of a drug or its metabolites into the body, around its many compartments and out of the body. It can be defined simply as, 'what the body does to the drug'. Specifically, it addresses the process and time for drug absorption, distribution, metabolism and elimination (ADME). The observed time course of the concentration of a drug is the net result of the four processes, all of which take place at the same time, at rates that are continuously changing. The application of these principles "in patients", (or disease conditions) to the optimization of drug therapy is called Clinical Pharmacokinetics.

The objective of pharmacokinetics is to describe the time course of a drug concentration in blood in mathematical terms so that (a) the performance of pharmaceutical dosage forms can be evaluated in terms of the rate and amount of drug they deliver to the blood, and (b) the dosage regimen of a drug can be adjusted to produce and maintain therapeutically effective blood concentrations with little or no toxicity (Figure 6).

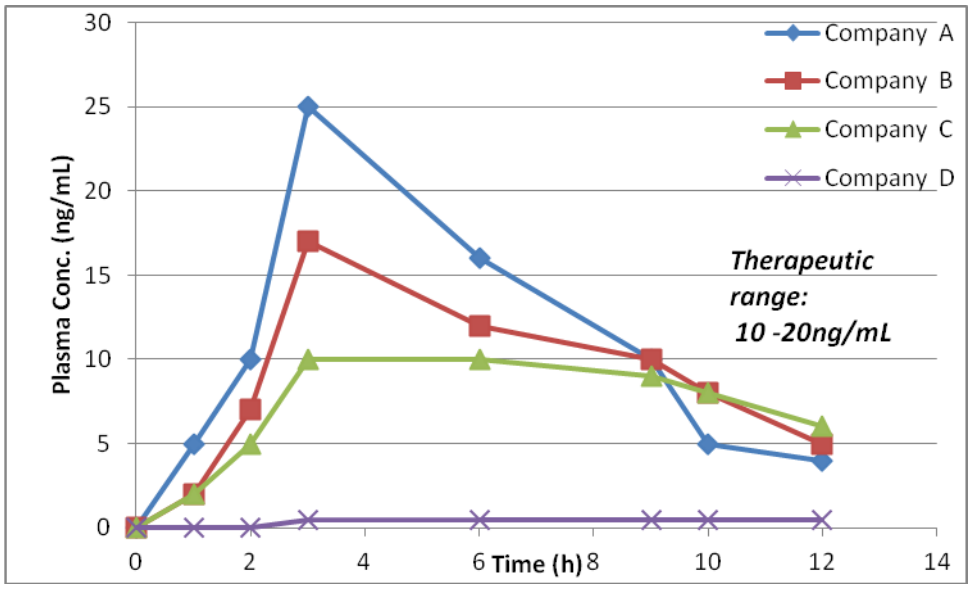

Figure.6. Plasma concentration–Time profile of a drug from four different companies, illustrating their relationship with the therapeutic range of 10 - 20ng/mL. A is above, B is within, C is below and D contain nothing.

Knowledge of pharmacokinetics is therefore crucial for drug development, at the preclinical testing in whole animal pharmacological assay and to decide on an appropriate dosing regimen for pivotal phase III studies of efficacy and at clinical trials. Understanding the general principles of pharmacokinetics is also important for clinicians, who need to understand how dosage recommendations in the product information provided with licensed drugs have been arrived at if they are to use the drug optimally and understand its limitations. In particular, clinicians dealing with a severely ill patient often need to individualize the dose regimen depending on how rapidly a therapeutic plasma concentration is required, and whether the clearance of the drug is impaired because of renal or liver disease.

Pharmacokinetics, addresses the process and time for drug absorption, distribution, metabolism and elimination (ADME).

Absorption

Absorption is the transfer of the drug from its site of administration into the blood stream. Regardless of the route of administration, it is dependent on the aqueous solubility of the drug. Thus, drugs given as aqueous solutions are more rapidly absorbed then suspensions, oils or solid dosage form e.g. tablet, because they mix more readily. Drug absorption from the gastrointestinal tract mostly occurs **via passive diffusion processes** and depends on both the physiochemical properties of the drug (molecular weight, pH/pka relationship, solubility, charges etc) and a number of patient factors (age, sex, weight, etc).

Active Transport: the energy-dependent movement of compounds across membranes, most often against their concentration gradient, is referred to as active transport. This transport involves the reversible binding of the molecule to be transferred to a membrane component (a carrier) of complementary configuration. Several mechanisms of active transport have been postulated. One transport model proposes that the drug molecule combines with a specific mobile carrier probably a protein, on one side of the membrane. The complex formed diffuses across the membrane to the opposite side, where the complex dissociates, thus releasing the drug into the aqueous compartment bordering the opposite membrane surface. The carrier protein can then return to its initial side to bind more drugs. Another model involves a

chainlike arrangement of sites in transport channels to which the drug molecules can bind. The drug would be transferred from one site to another until it had traversed the membrane. Active transport of a particular substance occurs in one direction only. The number of molecules transported per unit of time will reach a maximum (Tm) once the binding capacity of the carrier becomes saturated.

Distribution

Following absorption of a drug into the bloodstream, it is transported to the organs and other body fluids. Drugs can be distributed to every organ (depending on affinity of the organ) but there are marked differences in the extent and rate of distribution, depending on the nature (physicochemical characteristics of the compound) of the drug. The process is dependent on the same principle as those controlling the absorption process. Several kinetic models are often used to explain and understand distribution. The compartmental and non-compartmental analysis are often used.

Metabolism

Metabolism involves the biotransformation of drugs or foreign compounds (xenobiotics), ingested or absorbed by the body, to metabolites that are more water soluble and thus more easily excreted from the body. The characteristic effect of a drug will disappear when the drug is removed from the body and consequently from its site action, either in an unchanged form or after biotransformation of the drug has taken place giving metabolites, which are removed by the process of excretion. If a drug molecule has a suitable 'handle' (e.g. a hydroxyl, thiol or amino group), either in the parent molecule or in a product resulting from phase I metabolism, it is susceptible to conjugation, i.e. attachment of a substituent group. This synthetic step is called a phase II reaction. The resulting conjugate is almost always pharmacologically inactive and less lipid-soluble than its precursor, and is excreted in urine or bile. The groups most often involved are glucuronyl, sulfate, methyl, acetyl and glycyl. The tripeptide glutathione can conjugate drug metabolites via its sulfhydryl group, as in the detoxification of paracetamol. Glucuronide formation involves the formation of a high-energy phosphate compound, uridine diphosphate (UDP) glucuronic acid (UDPGA), from which glucuronic acid is transferred to an electron-rich atom (N, O or S) on the

substrate, forming an amide, ester or thiol bond. UDP glucuronyl transferase, which catalyses these reactions, has very broad substrate specificity embracing many drugs and other foreign molecules. Several important endogenous substances, including bilirubin and adrenal corticosteroids, are conjugated by the same system.

Metabolism of xenobiotics principally occurs in the liver, though other sites like the kidneys, lungs, gut, skin and placenta may also be involved. Phase 1 metabolism generally involves the hepatic cytochrome P450 enzyme system (which include CYP 1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A7, 4A9, 4A11, etc) of microsomal mixed function oxidases. (Ma, et al 2002). Generally CYP 450 has been implicated in drug pharmacokinetic interactions with drugs, food and drinks.

These enzymes, some of which are polymorphic in nature, act on same substrate differently in different individuals and are also subject to influence of its activities by other xenobiotics.

Pharmacokinetic Parameters

For ease of assessment of the processes above, that describes what happens in pharmacokinetics, these processes are transformed into mathematical descriptives based on general principles of thermodynamics, "kinetics".

$C = Ae^{-kt}$ Monophasic intravenous
$C = Ae^{-\alpha t} + Be^{-\beta t}$ Biphasic intravenous
$C = Ae^{-kt} - De^{-kat}$ Monophasic Oral
$C = Ae^{-\alpha t} + Be^{-\beta t} - De^{-kat}$ Biphasic oral dosage form

C is concentration at Time t
Ka is absorption rate constant
K is elimination rate constant
A, B, D, Concentration constants for each exponential

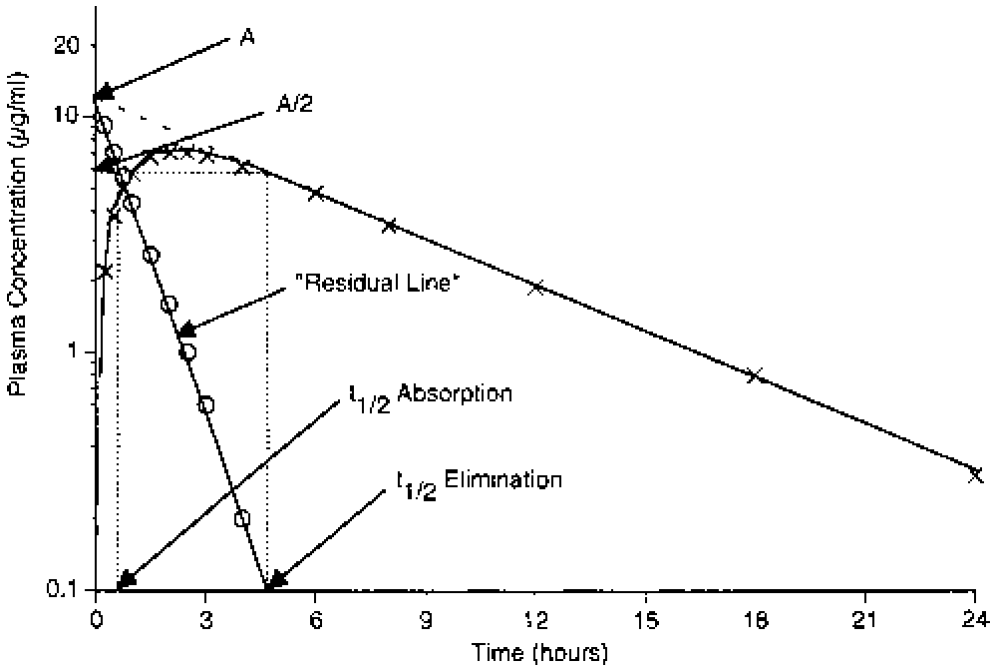

Figure 7a. Typical Plasma Concentration – Time curve of non – intravenous drug administration (Oral, Intra-muscular, Bucal, Rectal, Suppositories), showing the absorption phase, distribution and Elimination phase.

These parameters include; Absorption and Elimination kinetics, Absorption and Elimination half lives, Peak Concentration Cmax, time of maximum concentration, Tmax, Area Under the concentration- time curve, Clearance, Volume of distribution, Residency time.

Area Under the Curve (AUC)

One indicator of the overall exposure of a person to a drug is through the calculation of the area under the curve (AUC). As the name implies, AUC is the mathematically integrated area under the concentration-time curve and is most commonly calculated using the trapezoidal rule of mathematics. Though the shape of the concentration-time profile may affect the AUC for a drug, two drugs with entirely different concentration-time profile shapes may have the same AUC even though the individual may have reached different T_{max} and C_{max} values from those of other individuals.

An additional parameter that can be determined from a concentration-time profile is the half-life of the drug, that is, the time taken for 50% of the drug in the body at a given time to be eliminated over the calculated period. Half-life determination is very useful, since it can readily be used to evaluate how long a drug is expected to remain in the body after termination of dosing, the time required for a drug to reach steady state (when the rate of drug entering the body is equal to the rate of drug leaving the

body), and often the frequency of dosing. The following equation is used to calculate the half-life of a drug following first order kinetics absorption or elimination:

$$T_{1/2} = \frac{0.693}{K_e}$$

Where $t_{1/2}$ is the half-life and K_e is the elimination rate constant calculated from the slope of the declining portion of the concentration-time profile.

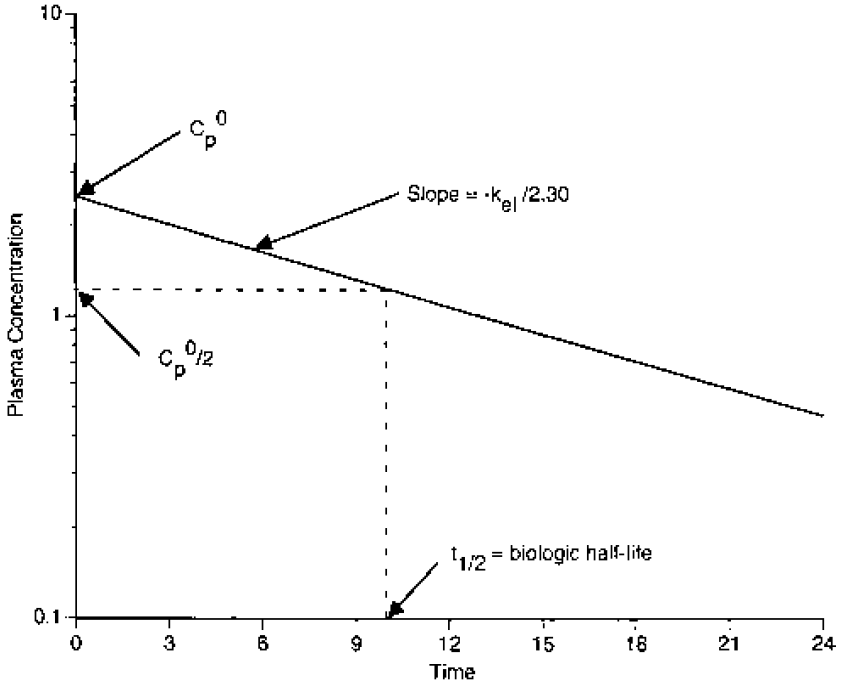

Figure 7b Typical Plasma concentration-Time curve for intravenous administration, showing the initial concentration and elimination phase.

Clearance is defined as the volume of blood from which drug can be completely removed per unit of time (e.g. 100 mL/minute). Clearance can involve both metabolism of drug to a metabolite and excretion of drug from the body. For example, a molecule that has undergone glucuronidation is described as having been cleared, even though the molecule itself may not have left the body. Clearance of drug can be accomplished by excretion of drug into the urine, gut contents, expired air, sweat as well as metabolic conversion to another form. However, uptake of drug into tissues does not constitute clearance. Total (systemic) clearance is the clearance of drug by all routes. Total (systemic) clearance (CI) can be calculated by either of the equations given below:

$$Cl = Vd \cdot Ke$$

Or

$$Cl = \frac{Dose}{}$$

AUC

Volume of distribution (Vd**),** relates a concentration of drug measured in the blood to the total amount of drug in the body. This mathematically determined value gives a rough indication of the overall distribution of a drug in the body. For example, a drug with a Vd of approximately 12 L (i.e., interstitial fluid plus plasma water) is probably able to penetrate cells. In general, the greater the Vd, the greater the diffusibility of the drug. The volume of distribution is not an actual volume, since its estimation may result in a volume greater than the volume available in the body (ca -40 L in a 70-kg man).

Chronobiology and Chronopharmacokinetics

The variations of the timing and duration of biological activity in living organisms occur for many essential biological processes. These occur (a) in animals (eating, sleeping, mating, hibernating, migration, cellular regeneration, etc.), (b) in plants (leaf movements, photosynthetic reactions, etc.), and in microbial organisms such as fungi and protozoa. They have even been found in bacteria, especially among the cyanobacteria (aka blue-green algae). The most important rhythm in chronobiology is the **circadian rhythm,** a roughly 24-hour cycle shown by physiological processes in all these organisms. Different disease conditions also have phase changes over 24 hours period.

The symptoms of rheumatoid arthritis are always worse in the morning. Taking long-acting NSAIDs like flubiprofen, ketoprofen and indomethacin at bedtime optimizes their therapeutic effect and minimizes or averts their side effects. 12-hour sustained-release NSAIDs that are taken twice a day must include a night or bedtime ingestion time to ensure adequate control of the prominent morning symptoms of rheumatoid arthritis. The temporal pattern of pain and stiffness in osteoarthritis sufferers differs between persons. Thus, an individualized chronotherapy of NSAIDs is necessary. The chronotherapy of osteoarthritis involves the administration of once-a-day forms of ketoprofen, indomethacin and other such medicines in relation to the time of day pain is worse. If pain is worse at night or early in afternoon, an evening once-a day NSAID schedule is recommended. If pain is worse in the afternoon or night, a once-a-day morning or noontime treatment schedule is best, provided the amount of side effects produced by the morning one, in particular, is minimal.

If the arthritic condition is severe, synthetic corticosteroids are often of benefit. Morning once-a-day dosing of these medicines is least likely to cause side effects especially if they are taken for a long period of time. Splitting the daily dose of medicine into several small ones for ingestion with meals and at bedtime or taking the entire daily dose at night is not recommended unless absolutely necessary. The risk of severe side effects from these medications increases when they are taken more than 8 to 9 hours after the customary time of awakening, after 15:00 for most people. The later in the day these medications are taken, the greater the risk of side effects. If the relief from the morning symptoms of rheumatoid arthritis sufferers is not attained by a once-a-day morning schedule, an increase in the morning dose is recommended. The result of one study suggest an early afternoon once-a-day treatment schedule might be beneficial for those people who fail to get significant relief from the morning pain and stiffness of rheumatoid arthritis when taking medicine in the morning.

Chronopharmacokinetics deals with the study of the temporal changes in absorption, distribution, metabolism and elimination and thus takes into account the influence of time of administration on these different steps. Temporal changes can be involved at each step of the sequence of pharmacokinetic processes: temporal variations in drug absorption from the gastro-intestinal tract (due to circadian variations in gastric acid secretion and pH, motility, gastric emptying time, gastrointestinal blood flow), plasma protein binding and drug distribution, drug metabolism temporal variations in enzyme activity, hepatic blood flow) and in renal drug excretion (due to variation in glomerular filtration, renal blood flow, urinary pH and tubular resorption). Thus, the time of administration of a drug is an important source of variation which must be taken into account in kinetic studies and particular methodological aspects of chronokinetics are needed.

Drug Absorption, distribution, metabolism and elimination are influenced by many different physiological functions of the body which may vary with time of day. Thus, the pharmacokinetic parameters characterizing these different steps, conventionally considered to be constant in time, depend on the moment of drug administration. However, the time of day has to be regarded as an additional variable influencing the kinetics of a drug since many drugs are affected by time of administration and the

activity or rest period of the human or animal. Chronokinetic studies have been reported for many drugs in an attempt to explain chronopharmacodynamic phenomena and demonstrate that the time of administration is a possible factor of variation in the kinetics of drugs.

When do we need chronokinetic studies? there are some instances in which a chronokinetic study is needed: 1) when possible daily variations in pharmacokinetic may be responsible for time dependent variations in drug effects (e.g. some antimicrobial agents are more effective at a specific time of day), (2) when drugs have a narrow therapeutic range, (3) when symptoms of a disease are clearly circadian phase-dependent (e.g. nocturnal asthma, angina pectoris, myocardial infarction, hypertension crisis, stroke, arthritis, ulcer disease) (4) when drug plasma concentrations are well correlated to the therapeutic effect in case the latter is circadian phase-dependent. (5) when the drug has some serious adverse effects that can be avoided or minimized because they are related to time of administration (e.g. aminoglycosides nephrotoxicity).

Drugs that undergo chronokinetics.

Antibiotics: Many studies have reported temporal variations in the pharmacokinetics of antimicrobial drugs. Experimental animal models have shown that for Antibiotics such as beta-lactams that have concentration-independent killing effects in vitro, the time that the antibiotic concentration remains greater than the MIC (T> MIC) is the most important factor for determining the in vivo efficacy. Therefore, daily Variations in pharmacokinetics may account for impairment in the chemotherapeutic effects. This is of great importance when bacteria with low susceptibility are involved in the infectious processes. Other important aspect of chronokinetics in antibiotics is that not only the efficacy of the drug may increase but also the toxicity of certain drugs may decrease at different time of day as we will see in aminoglycosides. The most important results in chronokinetics studies of antibiotics include: Aminoglycosides: Peak renal toxicity was observed when aminoglycosides were injected in the middle of the rest period of the experimental animals, while lower toxicity was found when they were treated in the middle of the activity period. Based on many studies and on the evidence available in the current literature, it is quite clear that the renal toxicity of aminoglycosides can be reduced by giving the drug as a single daily injection when

patients are active (at day time or in other words in the activity period). The mechanisms responsible for the temporal variation in renal toxicity of aminoglycosides are still unknown. **Gentamicin:** both the effectiveness and the toxicity of gentamicin varied over the 24 h period and the efficacy was best at the time when the toxicity of the drug was the lowest. So the administration of gentamicin in the beginning or the middle of the day in humans may reduce renal toxicity and increase the efficacy of these antibiotics. **Tobramycin:** Tobramycin was administered at 0200 h (dark period), the CL_T was significantly higher and AUC was lower than the values when tobramycin was given at 1400 h (light period). **Amikacin** : amikacin in humans showed higher values for kel in the morning than in the evening. **Ceftriaxone:** total clearance of ceftriaxone varies rhythmically during the day, with its maximum during the dark (activity) period and its minimum during the light (rest) period in rats. **Ciprofloxacin:** In humans, the amount of ciprofloxacin eliminated in urine was greater when the drug was administered at 1000 h than when it was given at 2200 h. **Ampicillin:** Ampicillin biliary and renal clearances were significantly higher during the active cycle of rats than during the sleep cycle.

Antihypertensive drugs: Nearly all physiological functions as well as pathophysiological events display reproducible rhythmic changes within 24 hours of a day, including the cardiovascular system. Clinical chronopharmacological studies with antihypertensive drugs gave evidence that effects on the rhythms in blood pressure and heart rate are also dependent on the time of day. Cardiovascular incidents occur more in the early hours of the day. Chronopharmacokinetic studies with propranolol, oxprenolol, nifedipine, verapamil, etc. also revealed daily variations in the drugs' kinetics.

Drug Interactions

Whenever two or more drugs (xenobiotics) are being taken together there is a chance that there will be an interaction among the drugs. Drug interaction may be between two orthodox drugs, or herbal preparations, food or drink. The interaction may be pharmacodynamic (events consequent on interaction of the drug with its receptor or other primary site of action) or pharmacokinetic. The interaction may increase or decrease the effectiveness of the drugs and/or the side effects of the drugs. The likelihood of drug interactions increases as the number of drugs being taken increases.

Therefore, people who take several drugs are at the greatest risk for interactions. Drug interactions contribute to the cost of healthcare because of the costs of medical care that are required to treat problems caused by changes in effectiveness or side effects.

How do drug interactions occur?

There are several mechanisms by which drugs interact with other drugs, food, and other substances. An interaction can result when pharmacokinetic parameters (ADMET) are altered;

i. absorption of a drug into the body;
ii. distribution of the drug within the body;
iii. alterations made to the drug by the body (metabolism); and
iv. elimination of the drug from the body
v. transport system

Clinically significant drug interactions result from a change in the absorption, metabolism, or elimination of a drug. Drug interactions also may occur when two drugs that have similar (additive) effects or opposite (canceling) effects on the body are administered together. For example, there may be major sedation when two drugs that have sedation as side effects are given, for example, narcotics and antihistamines. Another source of drug interactions occurs when one drug alters the concentration of a substance that is normally present in the body. The alteration or displacement at binding sites of these substances reduces or enhances the effect of another drug that is being taken. The drug interaction between warfarin (Coumarin) and vitamin K-containing products is a good example of this type of interaction. Warfarin acts by reducing the concentration of the active form of vitamin K in the body. Therefore, when vitamin K is taken, it reduces the effect of warfarin.

Change in absorption

Most drugs are absorbed into the blood and then travel to their site of action. Most drug interactions that are due to altered absorption occur in the gastro-intestinal track. There are various potential mechanisms through which the absorption of drugs can be increased or reduced. These mechanisms include:

i. an alteration in hepatic blood flow to the intestine (GIT)
ii. change in drug intestinal wall metabolism

iii. increased or decreased gastro- intestinal motility
iv. alterations in gastro- intestinal pH
v. change in the intestinal bacteria flora
vi. change in gastric emptying rate
vii. presence of complexing agents, (metals, xanthins etc.)

Therefore any drug, herb or food, drink or food supplement that will affect one or a combination of the above (i – vii) may alter the absorption status of an administered drug. Drug absorption also can be affected if the drug's ability to dissolve (solubility) is changed by another drug or if a substance (for example, food) binds to the drug and prevents its absorption.

Change in drug metabolism and elimination

Drugs are predominantly biotransformed in the liver and eliminated by the kidney. Metabolism of drugs is the process through which the body converts (alters or modifies) drugs into forms that are more or less active and are easily eliminated from the body. The cytochrome P450 enzymes are a group of enzymes in the liver that are responsible for the metabolism of most drugs. They are, therefore, often involved in drug interactions. Drugs and certain types of food may increase or decrease the activity of these enzymes and therefore affect the concentration of drugs that are metabolized by these enzymes. An increase in the activity of these enzymes leads to a decrease in the concentration and effect of an administered drug. Conversely, a decrease in enzyme activity leads to an increase in drug concentration and effect.

What are the consequences of drug interactions?

Drug interactions may lead to an increase or decrease in the beneficial or the adverse effects of the given drugs. When a drug interaction increases the benefit of the administered drugs without increasing side effects, both drugs may be combined to increase the control of the condition that is being treated. For example, drugs that reduce blood pressure by different mechanisms may be combined because the blood pressure lowering effect achieved by both drugs may be better than with either drug alone.

The absorption of some drugs is increased by food. Therefore, these drugs are taken with food in order to increase their concentration in the body and, ultimately, their effect. Conversely, when a drug's absorption is reduced by food, the drug is taken on an empty stomach.

Drug interactions that are of greatest concern are those that reduce the desired effects or increase the adverse effects of the drugs. Drugs that reduce the absorption or increase the metabolism or elimination of other drugs tend to reduce the effects of the other drugs. This may lead to failure of therapy or warrant an increase in the dose of the affected drug. Conversely, drugs that increase absorption or reduce the elimination or metabolism of other drugs - increase the concentration of the other drugs in the body - and lead to increased amounts of drug in the body and possibly more side effects. Sometimes, drugs interact because they produce similar side effects. Thus, when two drugs that produce similar side effects are combined, the frequency and severity of the side effect are increased.

Herbs, Herbal preparations and Drug interactions

A strong impetus to look at the problem of herbal preparations-drug interaction more closely came from the finding that grapefruit juice could impair drug metabolism and result in significant changes in the expected drug activity. This observation, first published in 1989, did not come to public attention until several years later, when the use of herbs had become even more widespread. The question then immediately arises: if an ordinary food like grapefruit could cause this response, why not herbs?

Among the other issues of herb-food interactions were these:

- it was recognized that certain foods interacted with a broad class of antidepressant drugs, making people wonder if it was safe to eat pizza while using the drugs (so, how safe can combining with herbs be?)
- it was noted that green vegetables could antagonize the effect of warfarin, the most commonly used blood thinner (many herbs appear no different than green vegetables)

- tetracycline absorption was markedly impaired by ingestion of milk or milk-based food products (perhaps herbs could impair the absorption of drugs as well).

Typical examples of herb-drug interactions and observed effects include: bleeding when warfarin is combined with ginkgo (*Ginkgo biloba*), garlic (*Allium sativum*), dong qual (*Angelica sinensis*), or danshen (*Salvia miltiorrhiza*); mild serotonin syndrome in patients who mix St John's wort (*Hypericum perforatum*) with serotonin-reuptake inhibitors; decreased bioavailability of digoxin, theophylline, cyclosporin, and phenprocoumon when these drugs are combined with St John's wort; induction of mania in depressed patients who mix antidepressants and Panax ginseng; decreased blood concentrations of prednisolone when taken with the Chinese herbal product xaio chai hu tang (sho-saiko-to); and decreased concentrations of phenytoin when combined with the Ayurvedic syrup shankhapushpi. Anthranoid-containing plants (including senna [*Cassia senna*] and cascara (*Rhamnus purshiana*) and soluble fibres (including guar gum and psyllium) can decrease the absorption of drugs (Roby *et al*, 2000, Fugh-Berman and Ernst, 2001, Brazier and Levine 2003). Pharmacokinetic results show that chronic grapefruit juice (GFJ) ingestion has a great influence on paracetamol metabolism, slowing it down (Samojlik, *et al*, 2002). Paracetamol has severally been reported to interact with drug, food, beverages/drinks and oral contraceptives (Villeneuve *et al*, 1983, Adzu *et al*, 2001, Kolawole and Maduenyi, 2004, Tripathi, 2009).

Interaction mode

Plant constituents, mainly secondary metabolites are responsible. Using the common table top grapefruit juice as a typical example. The adverse effects observed in combining grapefruit juice with calcium antagonists (used for lowering blood pressure), the benzodiazepines midazolam and triazolam (for depression), and terfenadine (antihistamine for allergies) are due to the greatly increased amount of drug in the bloodstream due to inhibited drug metabolism. One of the main causative factors in the grapefruit juice effect on drug availability was identified as a group of furanocoumarins that inhibited a major drug metabolizing enzyme system, cytochrome P450 (CYP 450). Furanocoumarins (also called furocoumarins and sometimes designated psoralens, after one of the best known furanocoumarins) and compounds

of similar structure are found in several Chinese herbs. **Therefore, the use of a herbal preparations, which dosage yields a similar amount of the enzyme-inhibiting compounds as grapefruit juice with the same drugs that interact with grapefruit juice could produce the same results.**

Other Herbs and Drug interaction examples

Name of Herb	Some Common Uses	Possible Side Effects or Drug Interactions
 **Echinacea**	Echinacea boosts the immune system and helps fight colds and flu. Aids wound healing.	Echinacea may cause inflammation of the liver if used with certain other medications, such as anabolic steroids, methotrexate or others.
 **Ephedra**	Ephedra is also called Ma-Huang. It is used in many over-the-counter diet aids as an appetite suppressant. It is also used for asthma or bronchitis.	Ephedra may interact with certain antidepressant medications or certain high blood pressure medications to cause dangerous elevation in blood pressure or heart rate. It could cause death in certain individuals.
 **Feverfew**	Feverfew is used to ward off migraine headaches and for arthritis, rheumatic disease and allergies.	Feverfew may increase bleeding, especially in patients already taking certain anti-clotting medications.
 **Garlic**	Garlic is used for lowering blood cholesterol, triglyceride levels and blood pressure.	Garlic may increase bleeding, especially in patients already taking certain anti-clotting medications.
 **Ginger**	Ginger is used for reducing nausea, vomiting and vertigo	Ginger may increase bleeding, especially in patients already taking certain anti-clotting medications.

 **Ginkgo**	Ginkgo, also called ginkgo biloba, is used for increasing blood circulation and oxygenation and for improving memory and mental alertness.	Ginkgo may increase bleeding, especially in patients already taking certain anti-clotting medications.
 **Ginseng**	Ginseng increases physical stamina and mental concentration.	Ginseng may cause decreased effectiveness of certain anti-clotting medications. Persons using ginseng see increased heart rate or high blood pressure. It may cause bleeding in women after menopause.
 **Goldenseal**	Goldenseal is used as a mild laxative and also reduces inflammation.	Goldenseal may worsen swelling and/or high blood pressure.
 **Kava-kava**	Kava-kava is used for nervousness, anxiety or restlessness; it is also a muscle relaxant.	Kava-kava may increase the effects of certain anti-seizure medications and/or prolong the effects of certain anesthetics. it can enhance the effects of alcohol. It may increase the risk of suicide for people with certain types of depression.
 **Licorice**	Licorice is used for treating stomach ulcers.	Certain licorice compounds may cause high blood pressure, swelling or electrolyte imbalances.
 **Saw Palmetto**	Saw Palmetto is used for enlarged prostate and urinary inflammations.	People using saw palmetto may see effects with other hormone therapies.
 **St. John's**	St. John's Wort is used for mild to moderate depression or anxiety and sleep disorders.	St. John's Wort may prolong the effect of certain anesthetic agents.

Wort		
 **Valerian**	Valerian is used as a mild sedative or sleep-aid. It is also a muscle relaxant.	Valerian may increase the effects of certain anti-seizure medications or prolong the effects of certain anesthetic agents.

Table 1. Herbs and reported drug interactions.
(http://www.itmonline.org/arts/herbdrug.htm)

Our Contribution

Drug therapy is a dynamic process involving the right molecule (safe and efficacious drug molecule) in the right environment (devoid of possible adverse interactions) at the right time (chronobiology). Therefore to achieve therapeutic benefit with minimal adverse/side effects, it is essential to optimize drug therapy, by administering medicines that are not fake/counterfeit, safe and bio-available.

At a time that drug faking and counterfeiting was a lucrative business in Nigeria with no scientific baseline study report, we reported to the best of our research limit the problem of fake drugs in Nigeria. Kolawole et al 1994, 2000, 2001, 2002 reported the circulation of unwholesome products of metronidazole, chloroquine, liquid dosage forms (chloroquine, promethazine and paracetamol) for children, and ampicillin respectively. In view of the skeletal reports on the magnitude of the problem of fake and adulterated drugs in Nigeria, Taylor et al 2001, reported in the Lancet, a work we started in 1996. The report became the baseline report for other numerous works thereafter and confirming the fake drug problem in Nigeria. We analyzed 581 drug samples. Table 2, shows the number of individual samples that were within and outside limits stated in the BP and the proportion that failed to comply with the specifications set. 279 (48%) samples contained amounts of active ingredients outside the appropriate limits. For all groups of drugs, antimalarials, antibacterials, and antituberculosis, more than 50% failed to comply with BP specifications. However, for some individual drug preparations all samples assayed were within pharmacopoeia limits. These included proguanil tablets, trimethoprim and sulphamathoxazole tablets

and quinine hydrochloride injection and syrups. No pyrazinamide tablets or metronidazole suspension met pharmacopoeial specifications.

This paper attracted the attention of government and her agencies involved with drug regulation to sit up. Most pronouncements and declarations of the level of fake drug in Nigeria were based on this report. In our immediate environment, I was invited by Dr Thatcher of Family Medicine Department, where I met with his team to discuss the paper.

Table 2: Number and type of drugs tested with number of samples failing to comply with British Pharmacopoeia limits (culled from Taylor *et al* 2001)

ARTICLES

Active ingredient	Dosage form	Total number of samples (n=581)	Number of samples within *British Pharmacopoeia* limits (n=438)	Number of samples outside *British Pharmacopoeia* limits (n=288)
Antimalarials				
Chloroquine phosphate	Capsules	29	9	20 (70%)
	Syrup	20	0	20 (100%)
	Tablets	18	1	17 (94%)
	Injection	15	1	14 (93%)
Chloroquine sulphate	Syrup	11	3	8 (73%)
	Tablets	19	4	15 (79%)
	Capsules	1	1	0
Proguanil hydrochloride	Tablets	19	19	0
Quinine hydrochloride	Injection	10	10	0
Quinine sulphate	Syrup	1	1	0
	Tablets	17	13	4 (24%)
Combination antimalarial				
Pyrimethamine and sulphadoxine	Tablets	50	48	2 (4%)
	Syrup	13	10	3 (23%)
	Tablets	50	39	11 (22%)
	Syrup	13	8	5 (38%)
Antibacterials				
Amoxycillin	Dry syrup	5	3	2 (40%)
	Capsules	32	24	8 (25%)
Ampicillin	Dry syrup	7	2	5 (71%)
	Capsules	39	16	23 (59%)
Benzylpenicillin	Injection	20	9	11 (55%)
Cloxacillin	Dry syrup	3	1	2 (67%)
	Capsules	6	5	1 (17%)
Dapsone	Tablets	3	3	0
Doxycycline	Capsules	19	6	13 (68%)
Metronidazole	Suspension	5	0	5 (100%)
	Tablets	36	10	26 (72%)
Combination antibacterials				
Ampicillin and cloxacillin	Dry syrup	8	8	0
	Capsules	30	21	9 (30%)
	Dry syrup	8	5	3 (38%)
	Capsules	30	7	23 (77%)
Trimethoprim and sulphamethoxazole	Tablets	29	29	0
	Suspension	15	12	3 (20%)
	Tablets	29	29	0
	Suspension	15	11	4 (27%)
Antituberculosis				
Isoniazid	Tablets	4	0	4 (100%)
Pyrazinamide	Tablets	3	0	3 (100%)
Rifampicin	Capsules	15	10	5 (33%)
Streptomycin	Injection	19	9	10 (53%)
Anthelmintic				
Mebendazole	Suspension	2	1	1 (50%)
	Tablets	35	31	4 (11%)
Antifungal				
Ketoconazole	Cream	5	1	4 (80%)
	Tablets	18	18	0

Total number of individual active ingredients recorded was 726, since some samples contained two.

Table 1: **Number and type of drugs tested with number of samples failing to comply with *British Pharmacopoeia* limits**

As a follow up to the above, Kolawole *et al*, 2002, sampled and tested ampicillin capsules and suspensions available in the open market. The result showed the percent drug content to range from 72.73% to 154%, for ampicillin capsules. Six, (42.9%) of the capsules passed the drug content while eight, (57.1%) failed. Out of the eight that failed, six, 54.4% are imported and two are indigenous. Also of the seventeen brands of ampicillin suspension thirteen (76.5%) passed and four (23.5%) failed while 62.5% are imported and 37.5% are indigenous. These results did not show any improvement on the level of fake drugs in circulation in Nigeria despite the effort of the regulatory authorities.

Pharmacokinetics and Method development

Method development and validation is a major step (in fact the "rate determining step") in pharmaceutical analysis. Sample collection and preservation was also a problem especially when dealing with blood samples for pharmacokinetics work. Conventionally, refrigeration at 20^{o}C is recommended. The acceptable procedure of transporting frozen blood and plasma samples is expensive and subject to possible drug degradation, loss of drug and matrix change due to thawing of samples during transport. We developed a simple sample preservation method that kept the integrity of the drug and metabolites till analysis. Plasma or blood samples from volunteers were adsorbed on filter paper, air dried and at the point of use extracted into aqueous mediums. This was done for antimalarial drugs; Quinine, Mefloquine, Proguanil and their analogues and metabolites.

Proguanil

Kolawole *et al*, in 1995, reported a method for the determination of proguanil and its two metabolites, cycloguanil and 4-chlorophenylbiguanide in whole blood and plasma samples obtained by thumb-prick and stored dry on filter paper. The proguanil recovery process involved liquid extraction of plasma and whole blood from the filter paper using 0.9M ammonium hydroxide solution, and supernatant was subsequently extracted by solid-phase extraction using C8 Bond Elut cartridges. Separation and quantification was by ion-pairing Liquid Chromatographic system.Typical range of concentration detected by the method in human subjects were, proguanil 12 – 900

ng/ml, cycloguanil, 16 - 44ng/ml, and 4-CPB 1.5 -10 ng/ml in whole blood. The method was standardized and applied for pharmacokinetic work. Also the stability profile of proguanil and metabolites on filter paper was reported by Kolawole *et al* in 1996, making the sampling, storage and transportation of proguanil and its metabolites in biological fluid easy.

Quinine

Quinine in plasma and whole blood samples dried on filter paper recovery simply involves liquid extraction of plasma and whole blood from the filter paper using 2M ammonium hydroxide solution, votex mixing followed by ultrasonication for 30 minutes. The supernatant was subsequently extracted by solid-phase extraction using C8 Bond Elut catridges. A Reverse-Phase Liquid Chromatography system with UV detection and fluorescence detection was used at the same time. The analytical characteristics of the method are reported, with a quantification limit of 0.1 µg mL (100 ng /mL) and within an assay coefficient of variation of 5.6-8.4% in plasma and 6.5 – 12% in whole blood. Representative chromatograms are shown as a function of time for samples from human subjects after ingestion of a single 400-mg dose of quinine sulphate. Quinidine, dihydroquinine and metabolites are well separated from quinine with a resolution of above 1.

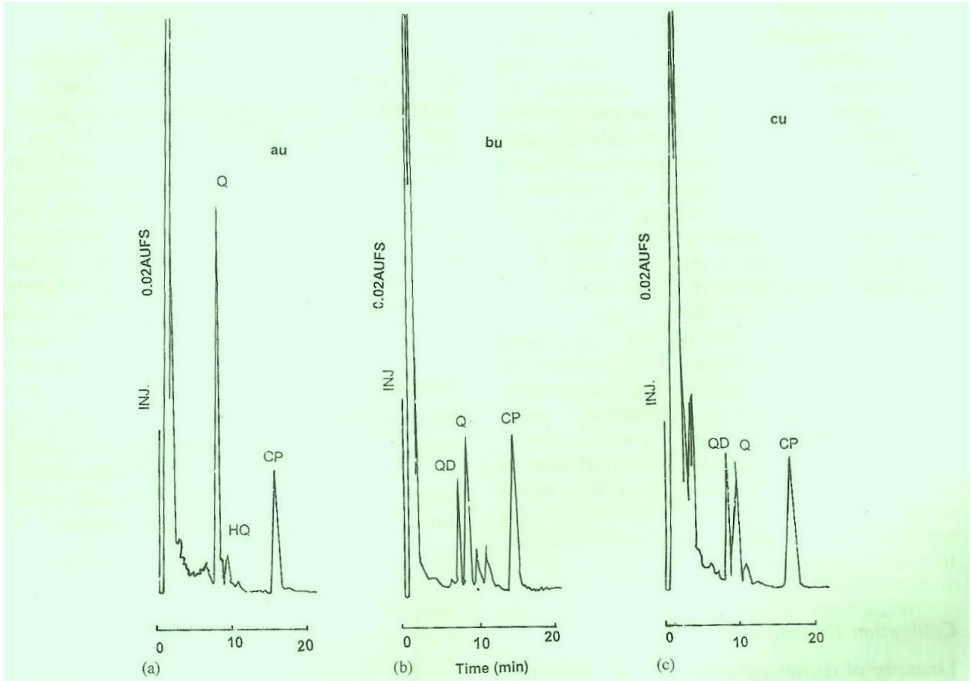

Figure 2. Chromatograms of extracted plasma samples with UV detection of quinine. (a) Plasma spiked with 1.0 μg mL^{-1} quinine (Q) containing internal standard, chlorproguanil (CP) and commercial contaminant hydroquinine (HQ). (b) Concentration obtained 1 h following oral ingestion of 400 mg of quinine. (c) Concentration obtained 24 h following oral ingestion of 400 mg of quinine

Figure 8a: Chromatograms of extracted plasma samples with UV detector of quinine, Chloproguanil, hydroquinine. (Culled from Kolawole and Mustapha 2000)

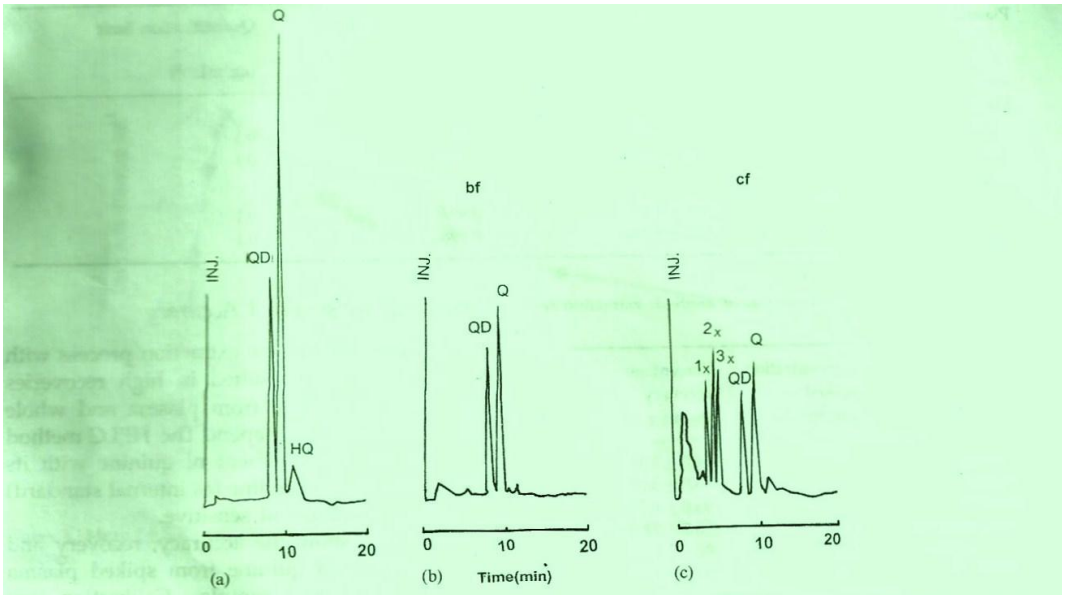

Figure 3. Chromatograms of extracted plasma samples with fluorescence detection. (a) Plasma spiked with 1.0 μg mL^{-1} quinine (Q) containing internal standard, quinidine (QD) and commercial contaminant hydroquinine (HQ). (b) Concentration obtained 1 h following oral ingestion of 400 mg of quinine. (c) Concentration obtained 24 h following oral ingestion of 400 mg of quinine (likely metabolites are shown as 1×, 2× and 3×)

Figure 8b: Chromatograms of extracted plasma samples with Flourescence detector of quinine, quinidine, hydroquinine. (Culled from Kolawole and Mustapha 2000)

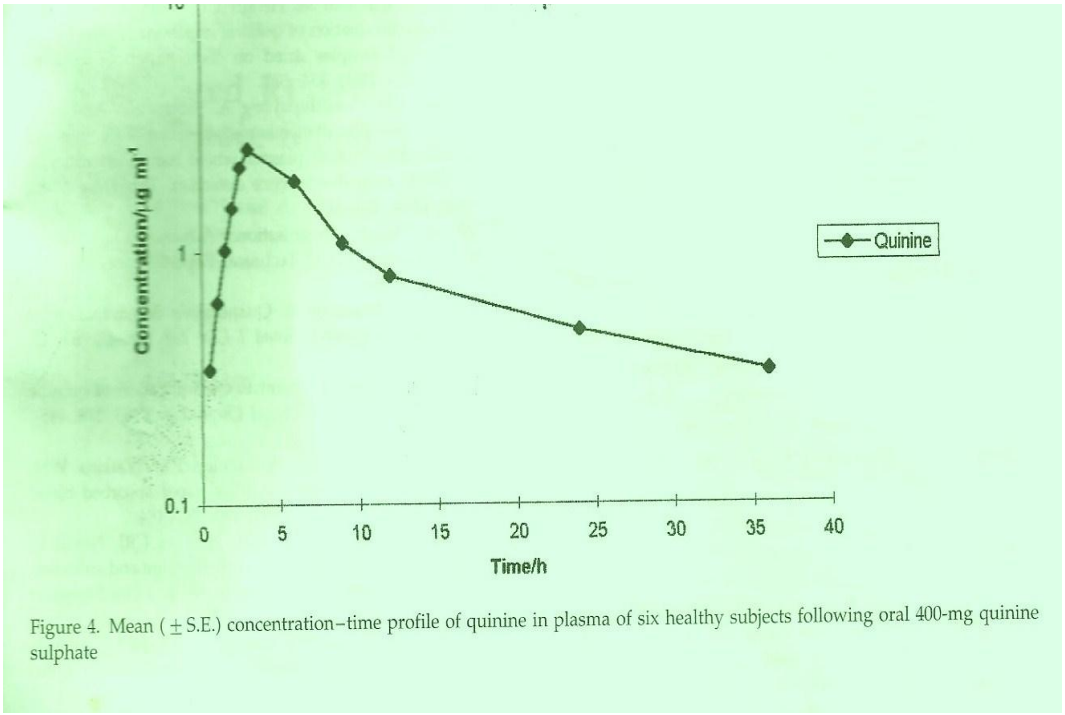

Figure 4. Mean (± S.E.) concentration–time profile of quinine in plasma of six healthy subjects following oral 400-mg quinine sulphate

Figure 8c: Plasma Concentration –Time profile of Qunine in six healthy subjects following oral 400 mg single dose quinine sulphate. (Culled from Kolawole and Mustapha 2000).

Mefloquine

Single dose pharmacokinetics of mefloquuine was determined in six healthy Nigerian male subjects. Mefloquine 500mg single dose was administered and blood samples were collected at intervals. Plasma concentrations were determined by RP-HPLC method after sample pre-treated step by solid phase extraction technique. Analytical characteristics of the HPLC system showed a plasma recovery of between 82.3 and 93% with an intra-day precision of 9.83% and 6.1% inter-day precision. Absorption was rapid with a $T_{1/2a}$ of 4.2 h and a T_{max} of 10 h and a corresponding C_{max} of 1.77 ug/ml and an AUCo-∞ of 19.05 mg/L. day. The volume of distribution Vd/f was small, 9.43L/kg with a corresponding total plasma clearance of 0.453L/day/kg. The elimination was slow with a $t_{1/2B}$ of 18.60 days.

Table 3: Table showing the Pharmacokinetics of Mefloquine in Healthy Human subjects.

Parameter	Mefloquine	± SD
Ka (h-1)	5.27	2.38
B (day-1)	0.0445	0.018
$T_{1/2\alpha}$ (h)	4.20	3.15
$T_{½\,\beta}$(day)	18.56	9.79
T_{max}(h)	8.00	3.10
C_{max} ug/ml	1.77	0.23
AUCo-∞ (mg/L.day)	19.05	7.01
Vd (Lkg-1)	9.43	3.77
CL_T/F (L/day/kg)	0.453	0.151

Drug-Drug interaction

Proguanil and Cimetidine

Proguanil is a prophylactic antimalarial drug widely used in pregnancy irrespective of the presence of peptic ulcers (or it's like such as heart burn). Proguanil is a pro-drug, it is the metabolite – cycloguanil which is the active moiety. The pharmacokinetics of orally administered Proguanil and its metabolites were determined in six healthy volunteers and in six peptic ulcer patients, before and after a 3-day course of Cimetidine (400mg given two times daily for 2 days and 400 mg on the third day 1 h before proguanil). Cimetidine significantly increased C_{max} ($P<0.05$), AUCo-∞ ($P<0.05$) and elimination half-life $t_{1/2b}$ of proquanil in plasma of healthy subjects. In ulcer patients, Cimetidine significantly increased AUCo-∞ ($P<0.05$) elimination half life and C_{max}. Cimetidine significantly reduced ($P<0.05$). Total body clearance in both healthy subjects and in peptic ulcer patients. The C_{max} and AUC o-∞ of the active metabolite cycloguanil was significantly decreased ($P<0.05$) in both the healthy subjects and in the peptic ulcer patients. The C_{max} of the inactive metabolite, 4-CPB was significantly decreased in healthy subjects and AUCo-∞ significantly decreased in peptic ulcer patients.

Table 4a: Pharmacokinetic profile of Proguanil, cycloguanil and 4-CPB, in six healthy subjects following oral 200mg Proguanil before and after Cimetidine

Pharmacokinetic parameter	Proguanil alone (Mean ± S.D)	Proguanil and Cimetidine Mean ± S.D
$T_{1/2\alpha}$	1.00 ± 0.35	0.86 ± 0.42
T_{max} (h)	3.30 ± 1.40	3.00 ± 1.60
C_{max} (ng/ml)	208.30 ± 30.30	393.40 ± 104*
AUC o-∞ (ng.h/ml)	4670 ± 1049	8991 ± 2101**
Vd (L/Kg)	14.00 ± 5.04	10.74 ± 3.37
$T_{1/2\beta}$ (h)	15.27 ± 3.73	22.55 ± 4.19
Cl_T (ml/min)	10.51 ± 2.17	5.47 ± 1.14
Cycloguanil pharmacokinetics after 200 mg oral dose of Proguanil, before and after 400mg cimetidine		
T_{max} (h)	5.00 ± 2.50	8.00 ± 1.70
C_{max} (ng/ml)	43.70 ± 16.00	17.80 ± 13.20*
AUC o-∞ (ng.h/ml)	399.70 ± 186	250.00 ± 104*
Pharmacokinetic profile of 4- Chlorophenylbiguanide after 200 mg oral dose of Proguanil before and after 400mg cimetidine		
T_{max} (h)	7.80 ± 1.60	9.00 ± 2.90
C_{max} (ng/ml)	11.25 ± 7.70	5.73 ± 3.30*
AUC o-∞ (ng.h/ml)	145.20 ± 85.00	114.20 ± 51.00
*P < 0.05	**P <0.005	

Table 4b: Pharmacokinetic profile of Proguanil, cycloguanil and 4-CPB, in four ulcer patients following oral 200mg Proguanil before and after oral 400 mg cimetidine

Pharmacokinetic parameter	Proguanil alone (Mean ± S.D)	Proguanil and Cimetidine Mean ± S.D
$T_{1/2\alpha}$	1.54 ± 0.45	1.46 ± 0.45
T_{max} (h)	4.50 ± 1.70	5.30 ± 1.50
C_{max} (ng/ml)	374.10 ± 54.0	481.45 ± 69.80
AUC o-∞ (ng.h/ml)	8261 ± 1198	12155 ± 2127**
Vd (L/Kg)	7.30 ± 1.09	7.94 ± 2.22
$T_{1/2\beta}$ (h)	14.22 ± 2.75	23.06 ± 8.17
Cl_T (ml/min)	6.00 ± 0.74	4.11 ± 0.68
Cycloguanil pharmacokinetics after 200 mg oral dose of Proguanil before and after 400mg cimetidine		
T_{max} (h)	6.00 ± 2.50	8.00 ± 1.70
C_{max} (ng/ml)	38.80 ± 16.00	26.10 ± 21.20*
AUC o-∞ (ng.h/ml)	459.26 ± 186	462.00 ± 152
Pharmacokinetic profile of 4- Chlorophenylbiguanide after 200 mg oral dose of Proguanil before and after 400mg cimetidine		
T_{max} (h)	7.50 ± 4.00	6.00 ± 2.90
C_{max} (ng/ml)	9.80 ± 6.70	5.60 ± 0.30
AUC o-∞ (ng.h/ml)	160.00 ± 50.00	125.00 ± 44.00*
*P < 0.05	**P <0.005	

Mefloquine and Cimetidine

With the background of indiscriminate use of anti-malarial drugs (especially, newly introduced ones, like Mefloquine in the early 1990s) and Cimetidine as the drug of

first line treatment (in the early 1990s) in peptic ulcer, it was imperative to study possible interactions. The pharmacokinetics of orally administered mefloquine were determined in six healthy male subjects and in six ulcer patients before and after a 3-day course of Cimetidine (400 mg morning and evening).

Peak plasma concentrations C_{max} and AUCo-∞ were similarly and significantly ($P < 0.05$) increased after Cimetidine pretreatment in both healthy subjects and peptic ulcer patients C_{max} was increased by 42.4% and 20.5% while AUC o-∞ was increased by 37.5% in healthy and peptic ulcer subjects respectively. The values of $t_{1/2}\alpha$ absorption and $t_{1/2\beta}$ elimination, total Clearance CL_T/F and volume of distribution were altered to varying levels after Cimetidine treatment but the changes were not statistically significant in both healthy and peptic ulcer subjects.

The established long $t_{1/2\beta}$ and this apparent interaction between mefloquine and cimetidine which resulted in increased mefloquine plasma concentration might be of clinical significant in patients with neurological/psychiatric history or prone to other adverse effects such as abdominal pain, overt psychosis and acute brain syndrome. Today it is no longer in routine use due to these adverse effects.

Table 5a: Pharmacokinetic parameter of Mefloquine (Mean ± SD) in six healthy subjects following oral 500mg Mefloquine before and after oral Cimetidine

Parameter	Mefloquine	Mefloquine and Cimetidine
ka (h^{-1})	5.27 ± 2.38	7.51 ± 3.18
B (day^{-1})	0.0445 ± 0.018	0.0366 ± 0.011
$T_{1/2a}$ (h)	4.20 ± 3.15	2.70 ± 1.59
$T_{1/2\beta}$ (day)	18.56 ± 9.79	20.38 ± 6.34
T_{max} (h)	8.00 ± 3.10	6.50 ± 4.00h
C_{max} (ug/ml)	1.77 ± 0.23	2.52 ± 0.27*
$AUC_{0-\infty}$ (mg/L.day)	19.05 ± 7.01	26.20 ± 18.90*
Vd/F (L/kg)	9.43 ± 3.77	11.60 ± 6.66
CL_T/F (L/day/kg)	0.453 ± 0.151	0.391 ± 0.18
* P <0.05……. significant		

Table 5b: Pharmacokinetic parameter of Mefloquine (Mean ± SD) in six peptic Ulcer patients following oral 500mg Mefloquine before and after oral Cimetidine

Parameter	Mefloquine	Mefloquine and Cimetidine
ka (h^{-1})	11.09 ± 5.82	10.15 ± 2.40
B (day^{-1})	0.0417 ± 0.02	0.0365 ± 0.01
$T_{1/2a}$ (h)	1.90 ± 1.00	1.70 ± 0.30
$T_{1/2\beta}$ (day)	18.70 ± 7.12	19.40 ± 3.30
T_{max} (h)	7.50 ± 3.00	7.00 ± 1.70h
C_{max} (ug/ml)	2.00 ± 0.30	2.41 ± 0.10*
$AUC_{0-\infty}$ (mg/L.day)	19.85 ± 9.48	26.24 ± 9.810*
Vd/F (L/kg)	11.12 ± 4.04	8.50 ± 2.30
CL_T/F (L/day/kg)	0.454 ± 0.19	0.315 ± 0.10
* P <0.05……. significant		

Drug-Herbal Preparation Interaction

Food (drinks inclusive) had been variously reported to affect (decrease or increase) bioavailability of drugs and therefore the success of drug treatment or side effect profiles. Common beverages such as alcoholic drinks, cola, coffee, tea, caffeine grapefruit juice and sour oranges have been reported to affect bioavailability of wide variety of drugs (Williams *et al*, 1996, Malhotra *et al*, 2001, http://www.holistic-online.com, http://www.bmj.com). Grapefruit juice affects the metabolism of nifedipine, cyclosporine, antihistamin atrorvastin through the activity of CYP 3A4, 1A2 isoenzymes in the intestinal wall while caffeine affects antidepressants and acetaminophen. **Paracetamol is primarily metabolised by two isoenzymes of cytochrome P450: CYP2E1 and CYP1A2. The P450 gene is highly polymorphic, however, individual differences in paracetamol toxicity are believed to be due to a third isoenzyme, CYP2D6. Genetic polymorphisms in CYP2D6 may contribute to significantly different rates of production of NAPQI.** Furthermore, individuals can be classified as "extensive", "ultra rapid", and "poor" metabolizers (producers of NAPQI), depending on their levels of CYP2D6 expression

(http://www.wikipedia.org, 2009). **We therefore use acetaminophen as a substrate drug for CYP 1E2 and 1A2, enzymes.** Acetaminophen, a common antipyretic analgesic OTC drug is often administered orally anytime of the day with water or beverages irrespective of possible interactions. Zobo drink, is sweetened water extract of dried calyx of *Hibiscus sabdariffa*. This work was designed to investigate the effect of zobo drink on an oral dose of acetaminophen.

Pharmacokinetic values obtained were found to be in similar ranges as those previously reported. The absorption parameters $t_{1/2}$ Ka, Tmax, Cmax AUC showed no statistically significant changes ($p > 0.05$) after the administration of Zobo. The elimination phase, however was affected. There was statistically significant changes ($p<0.05$) in K_β and $t_{1/2\beta}$ of acetaminophen when administered after Zobo drink. There was also 11.69% increase in Clearance ($C1_T$). Acetaminophen administration should therefore be at least 2-4 hour before or after ingestion of Zobo drink.

Table 6: Pharmacokinetic parameter profile of Acetaminophen in six healthy volunteers before and after administration of water extract of *H. sabdariffa* (Zobo drink)

Pharmacokinetic parameter	Acetaminophen alone(mean ± S.D	Acetaminophen and zobo drink alone mean ± S.D
Lag time (h)	0.23 ± 0.03	-
$T_{1/2}$ (h^{-1})	0.12 ± 0.03	0.12 ± 0.03
T_{max} (h)	0.75 ± 0.14	0.73 ± 0.13
C_{max} (ug/ml)	21.6 ± 3.04	25.08 ± 4.35
AUC (ug.h/ml)	81.35 ± 10.03	74.22 ± 13.04
K (h^{-1})	0.30 ± 0.07	0.56 ± 0.16
$T_{1/2}$ (h)	2.55 ± 0.60	1.35 ± 0.45
Cl_T (ml/h)	207.75 ± 27.84	232.0 ± 46.19
K (h)	5.92 ± 1.21	5.81 ± 1.07

Paracetamol and Yoyo bitters

Paracetamol (acetaminophen) and Yoyo bitters (a liquid oral herbal preparation) are OTC drugs and widely available to "patients" without prescription. They are taken individually or as combination therapy at the slightest signs/symptoms of fever in West Africa. The study was aimed at investigating the effect of Yoyo bitters on the pharmacokinetics of single oral dose paracetamol in human volunteers.

Yoyo bitters did not statistically affect the pharmacokinetics of paracetamol when administered concurrently. However when administered after three days of Yoyo bitters administration, there was statistically significant ($p<0.05$) increase in AUCo - ∞ and $t_{1/2\beta}$ while there was significant ($p<0.05$) decrease in K_β and Cl/F. Elimination parameters (K_β, $t_{½\beta,}$ Cl_T/F) as well as the AUCo-∞ showed significant changes ($p<0.05$) in the delayed protocol indicating that the constituents of Yoyo bitters inhibits paracetamol metabolism.

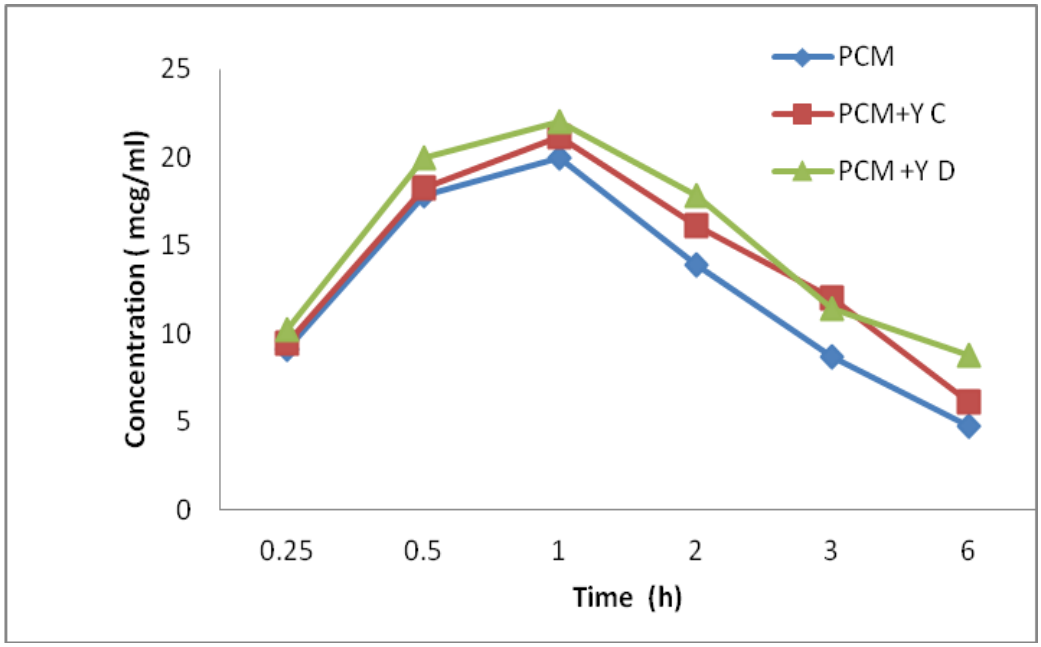

Figure 9. Plot of Plasma Concentration-Time curve of oral single dose (1000 g) paracetamol before, during and after administration of Yoyo bitters.

Key: PCM – Paracetamol alone;

PCM +Y /C- - Paracetamol and Yoyo bitters (concurrent administration)

PCM +Y /D- - Paracetamol and Yoyo bitters (delayed administration)

Chronopharmacokinetics

Bioavailability of orally administered drugs depends on a number of factors such as gastric motility and/or emptying rate, hepatic blood flow, metabolism etc. All of these factors are affected by the body clock. Endogenous circadian rhythms in gastrointestinal pH, intestinal motility, digestive secretion and intestinal blood flow are among other factors responsible for the variation in the pharmacokinetics of many drugs (Markiewicz and Semenowicz 1979, Valli et al., 1980; Kabasakalian *et al.*,1970; Naranjo *et al.*,1980; Kolawole *et al.*,2002; Rebuelto *et al.*,2003).

Metronidazole is a synthetic antimicrobial agent widely used in different clinical conditions. Changes in pharmacokinetic profile of drugs caused by differences in dosing at a time has been known to have led to toxicities, treatment failures and in some cases more beneficial effects, depending on the parameters affected. In 2004, Kolawole and Ameh designed a study to investigate the circadian changes in the pharmacokinetics of metronidazole at three different dosing times; 0700 h, 1300 h,1900 h. The Pharmacokinetic values obtained, though varied, were found to be in similar ranges with previously reported values. There were significant differences ($p < 0.05$), in absorption parameters, Ka and $t_{1/2a}$ values with respect to the time of administration. Absorption was best in the afternoon, 1300 h.

Table 7: Mean Pharmacokinietic values derived from saliva concentration of metronidazole in six healthy volunteers following oral administration of 400mg metrondazole at 0700 h, 1300 h, 1900 h.

	0700 h	1300 h	1900 h
Lag time(h)	0.127 ± 0.091	0.18 ± 0.10	0.24 ± 0.10
Ka(h^{-1})	1.394 ± 0.20	0.72 ± 0.13	1.26 ± 0.56
$T_{1/2a}$(h)	0.51 ± 0.069	0.99 ± 0.22	0.63 ± 0.21
C_{max} (mcg/mL)	8.72 ± 0.90	9.02 ± 0.74	8.32 ± 0.47
T_{max} (h)	2.03 ± 0.052	2.07 ± 0.08	2.77 ± 0.96
AUC(mcg.h/Ml)	75.32 ± 14.71	82.76 ± 10.70	73.69 ± 14.36
Ke(h^{-1})	0.135 ± 0.015	0.130 ± 0.17	0.12 ± 0.02

$T_{1/2e}$(h)	5.19 ± 0.563	5.31 ± 0.89	5.86 ± 1.00
Clt (ml/min)	91.38 ± 17.89	81.88 ± 12.48	92.57 ± 15.19

Acetaminophen is a common antipyretic-analgelsic drug usually administered orally anytime of the day. It was however claimed by Belanger et al, (1985) to have a longer elimination half-life ($t_{1/2\beta}$) in rats when administered by 2100 h, than at 0700 h. this work was designed to investigate this claim in man and to study the circadian changes in acetaminophen pharmarcokinetics at three dosing times; 0730 h, 1300 h and 2100 h. The absorption parameters Ka and T_{max} were significantly altered with dosing at 0730 and 2100 while the mean $t_{1/2\acute{\alpha}}$ and Ka were also significantly changed. There was a significant difference ($P < 0.05$) between the T_{max} values obtained at 2100 h and that of 0730 h, while the 34.9% increase at dosing time 1300 h over 0730 h dosing time is not statistically significant ($P > 0.05$). C_{max} was found to be 22.31 ± 4.24 mcg/ml for 0730h dosing time, while a decrease of 9.64 and 11.7% over this value was recorded for dosing time 1300 h and 2100 h respectively. The changes were however not statistically significant ($P>0.05$). AUC_{o-a} at 0730 h did not show any significant change when compared with values obtained at 1300 h and 2100 h, but there was a significant difference between the values obtained at 1300 h and 2100 h. Elimination half-time, $t_{1/2\beta}$ was found to be 1.68 ± 0.67 h at 0730 h. there was an increase of 13.1% and 20.83% over this value at 1300 h and 2100 h. these increases were not statistically significant ($P > 0.05$), although there are similar increases in $t_{1/2\beta}$ at the 1300 h and 2100 h dosing time as reported by Belanger et al 1985, the increases were not statistically significant in man.

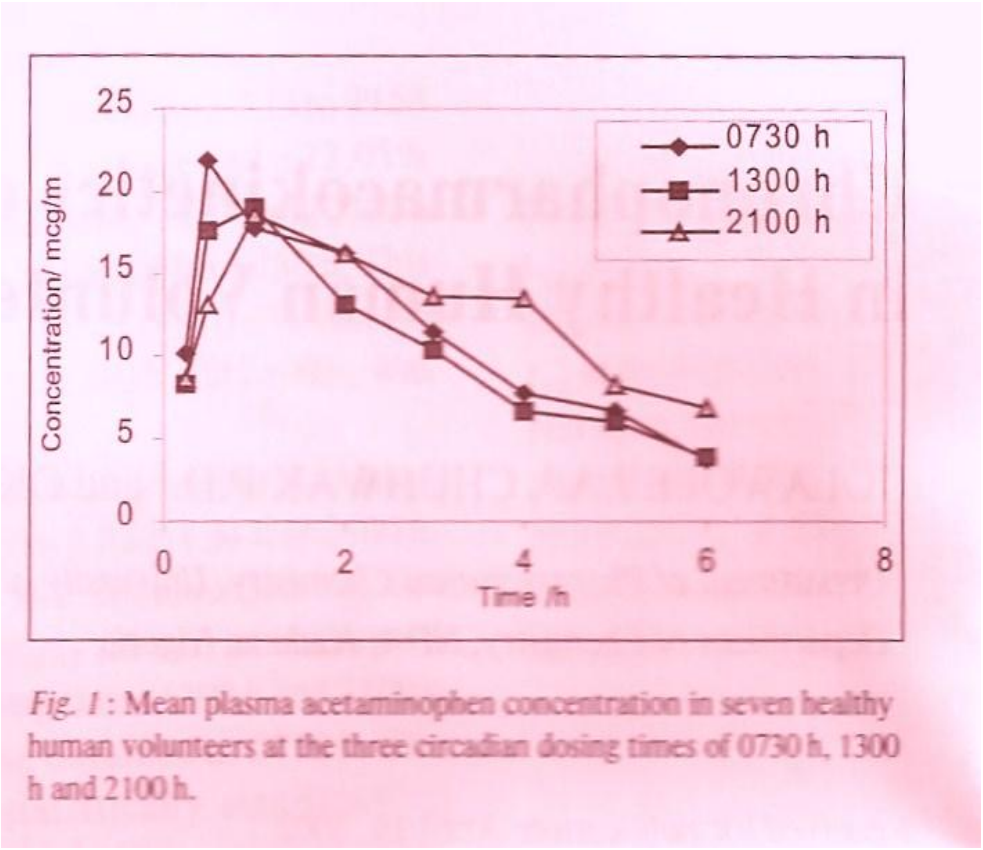

Fig. 1 : Mean plasma acetaminophen concentration in seven healthy human volunteers at the three circadian dosing times of 0730 h, 1300 h and 2100 h.

Figure10. Plot of Plasma Concentration-Time curve of oral single dose (1000 g) paracetamol in Seven healthy volunteers at three circadian times of 0730 h, 1300 h, and 2100 h.

Safety and Toxicity

Metronidazole, was just an antiprotozoan in its early days of medical use. It has over time, gained wide spread use as antibacterial agent in addition to the above. This made it important pre and post surgery anti –infective agent. It was however reported to affect fetal liver hexokinase and glutathione reductase in man and proliferation of the smooth endoplastic recticulum with symptoms of cell damage in rats. It therefore became necessary to study possible biochemical changes in human liver with high doses of metronidazole. In 1997, we reported the effect of oral doses of metronidazole on liver function test parameters in man. A dosage of 200 mg oral metronidazole administered 8 hourly for 15 days did not significantly alter serum albumin level and the levels of alanine aminotransferase (ALT) aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Higher does, (400 mg hourly for 15 days and a single 2 grams dose) significantly depressed serum albumin levels and increase the level of

ALT, AST and ALP. The enzyme activities did not fully return to initial levels five days after administering the last does. Therefore, the need for caution when there is the need for high doses of metronidazole for a long period.

Conclusion

Mr. Vice Chancellor, Ladies and Gentlemen, an attempt was made in this lecture to enlighten the audiences on some of the likely reasons for administered medicines (drugs) not to elicit its desired pharmacological effect. From the point of view of a pharmaceutical chemist, that I am, optimizing drug therapy, require that the drug contains the chemically right active ingredient, in the specified amount (right dose and dosing) and also readily made available to the human system (active site) at the right time and in the right environment. Therefore any condition such as counterfeit drug, drug-drug interaction, drug-food/drink interaction, chronobiological influence, that will cause the plasma concentration to be outside the therapeutic range (curve A, C or D, of Figure 6, page11) will result in therapeutic failure, possible heightened side effects and adverse effects. Our focus was on, quality control/assessment that will result in the availability of genuine drug to Nigerians. Optimum availability is a function of having the correct drug administered at a time that the pharmacokinetics parameters are not subverted or compromised. Professionals in the healthcare system should employ the knowledge of the Pharmacists to optimize drug treatment for our patients. Therapeutic drug monitoring is a must for our Teaching Hospitals, if the patients are to get the best of drug treatment. We all must join the fight against fake/counterfeit medicines.

Thank you for listening.
God bless the University of Jos
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God bless Nigeria.

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