COMPARISON OF THE INFLUENCES OF CHLOROQUINE AND CIMETIDINE ON THE PHARMACOKINETICS OF PARACETAMOL IN HEALTHY HUMAN SUBJECTS

BY

AMINA SANI YAKASAI

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE

DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY
FACULTY OF PHARMACEUTICAL SCIENCES
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA

AUGUST, 1999

DECLARATION

I hereby declare that the work reported in this thesis has been carried out by me under the vigilant supervision of Dr. M. Garba and Dr. M. Mamam.
It has not been presented in any previous publication for higher degree. The work of others are accordingly referred to and acknowledged.

Amina Sani Yakasai
Department of Pharmaceutical and Medicinal Chemistry
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria – Nigeria

CERTIFICATION

This thesis entitled "COMPARISON OF THE INFLUENCES OF CHLOROQUINE AND CIMETIDINE ON THE PHARMACOKINETICS OF PARACETAMOL IN HEALTHY HUMAN SUBJECTS" by Amina Sani Yakasai, meets the regulations governing the award of the degree of MASTERS OF SCIENCE (PHARMACEUTICAL CHEMISTRY) of AHMADU BELLO UNIVERSITY, and is approved for its contribution to science, knowledge and literary presentation.

MGarba — 13/10/99

Dr. M. Garba
B.Sc (Pharm), M.Sc. Ph.D, MPSN,
Head, Department of Pharmaceutical and
Medical Chemistry,
Deputy Dean, Faculty of Pharm. Sciences.

Date

13/10/99

Dr. M. Mamman
DVM Msc. Ph.D
Department of Vet. Physiology,
Faculty of Vet. Medicine,
A.B.U. Zaria.

Date

13/10/99

Dr. J. A. Kolawole
External Examiner
BSc. (Pharm), MSc. Ph.D
H.O.D (Pharm Chem)
University of Jos.

Date

24/03/01

Dean, Postgraduate School,
A.B.U, Zaria – Nigeria

Date

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DEDICATION

To all suffering patients – the world over.

ACKNOWLEDGEMENT

My immense gratitude goes to my major supervisor Dr. M. Garba for his diligent guidance and constructive criticism.

My appreciation also goes to my second supervisor – Dr. M. Mamman.
I am very much indebted to the other side of myself – my husband, who has been my source of courage, security and happiness.

To twins (my bubbles of joy and laughter), Yafendo, Aisha, Umar, Abdullahi and Islam – I thank you very much for your patience and understanding.

ABSTRACT

Malaria is very prevalent in the world especially in Africa and chloroquine is still a leading drug in the treatment of this ailment. Patients go to hospital with multiple illnesses, malaria inclusive. Thus other drugs are frequently prescribed with chloroquine. This work is designed to find out the interaction of chloroquine with other drugs with which it is commonly prescribed. This effect is compared with that of cimetidine on paracetamol absorption.

Paracetamol (1g) was given orally alone as control (first stage). Cimetidine (400mg) was given concurrently with paracetamol (1g) orally (second stage) and then cimetidine was given orally one and a half hours before paracetamol (third stage). Chloroquine sulphate (0.8g) was given orally one and a half hours before paracetamol (fourth stage). All pharmacokinetic parameters of paracetamol were determined statistically for all the different stages of the study. They were then compared with control and those of chloroquine treated compared with those of cimetidine treated (delayed and concurrent administration).

Oral administration of 400mg cimetidine an hour before paracetamol significantly ($P<0.05$) decreased Cmax, Tmax, T½ab, Kab, T½el, Kel, Lagtime, Clearance. With the concurrent administration, cimetidine insignificantly ($P>0.10$) decreased all the pharmacokinetic parameters. Chloroquine significantly ($P<0.05$) decreased Cmax, Tmax, T½ab, Kab, Lagtime.

When the influences of Chloroquine (delayed) and cimetidine (delayed) on paracetamol kinetics were compared, only clearance, Cmax and AUCo-oo in chloroquine treated were significantly ($P<0.05$) decreased, while all other parameters were insignificantly ($P<0.10$) decreased.

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ABBREVIATIONS

A.B.U	-	Ahmadu Bello University
B.P.	-	British Pharmacopoeia
CQ	-	Chloroquine
Cim	-	Cimetidine
°C	-	Degree Centigrade
et al	-	and others
g	-	Gramme
fig	-	Figure
HCl	-	Hydrochloric Acid
Hr	-	Hour
I.M.	-	Intramuscular
I.V.	-	Intravenous
L.	-	Litre
In	-	Natural Logarithm
Log	-	Logarithm
Min	-	Minutes
ml	-	Milli-litre
mwt	-	Molecular Weight
NaoH	-	Sodium hydroxide
nm	-	Nanometer
PCM	-	Paracetamol
pH	-	Hydrogen-ion concentration
r.p.m	-	Revolutions per minute
U.V.	-	Ultra violet
λ	-	Wavelenght
µg	-	Microgramme

CHAPTER ONE

INTRODUCTION

1.1 DRUG INTERACTIONS

Drugs are frequently administered to correct an altered physiology or for treatment in diseased states. Unfortunately, the eagerness to use "shotgun" therapy in the treatment of such diseases has necessitated a greater knowledge of drug interactions.

The proliferation of both multiple and single entity medications and combinations of drug therapy has created a situation where patients are consuming medications at an ever alarming rate. In addition to drugs prescribed by their physician, patients have the ability to purchase over the counter (OTC) medications that can complicate their management. Concerning poly pharmacy, Jick et al (1970) in a drug utilisation study of six hospitals, found out that the average patient admitted received 8.4 drugs. To understand the effects of these interactions, a systemic approach must be developed to identify and evaluate a recognized drug interaction.

The recognition of a drug interaction is usually the result of either an enhanced or diminished therapeutic effect. This may be from concomitant administration of another agent, an alteration in physiology or from the effects of diseases.

However, drug interactions may be useful in advancing the treatment of some diseases. Examples:

1. Co-administration of a beta-blocker (Propranolol) to decrease cardiac output with a vasodilator (Hydralazine) to decrease peripheral vascular resistance.
2. Concurrent administration of probenecid and penicillin to achieve adequate serum level of penicillin for the treatment of venereal diseases.

2.4.1.12 Imipramine and Chloroquine (CQ)

This interaction was investigated in six healthy volunteers who received 300mg of Chloroquine and 50mg imipramine, and combined doses of both drugs in a randomized crossover design. Blood and urine samples were collected at predetermined time intervals and were analyzed for the drugs and their metabolites by High Performance Liquid Chromatography (HPLC) method. The results revealed that the plasma concentrations of chloroquine and its pharmacokinetic parameters were not significantly (P>0.1) altered when chloroquine was co-administered with imipramine. The plasma conc-time profiles and the disposition characteristics of imipramine also were not altered after co-administration with chloroquine. The results suggest that there appears to be no pharamacokinetic interaction between chloroquine and imipramine given as single oral doses (Ogunbona et al, 1993).

2.4.2 PARACETAMOL DRUG INTERACTIONS

2.4.2.1 Paracetamol with Alcohol

Hepatic Enzyme induction which accompanies prolonged alcohol ingestion leads to enhanced formation of hepatotoxic metabolites of paracetamol under conditions of paracetamol overdosage.

<u>Chemical Significance:</u> In chronic alcoholics, hepatic enzyme system is induced. These subjects are prone to paracetamol hepatotoxicity. Whether alcoholism could predispose alcoholics to hepatotoxicity in patients chronically receiving large therapeutic doses of paracetamol is not established.

Drug interactions are most often considered significant only when they result in several problems such as hemorrhage, hypoglycemic coma, seizures or a hypertensive crisis. However, a number of lesser reactions occur that may go unreported. Failure of a patient to respond to an antibiotic may be the result of a drug interaction resulting from either an intravenous incompatibility or an in vivo drug-drug interaction (Weibert, 1977).

Hurwitz (1969), showed that the incidence of adverse drug interactions rises with the number of drugs prescribed.

Therefore, it is reasonable to suspect that a very large number of patients may be at risk of having potentially harmful drug interactions and that an important problem in modern therapeutics might exist (Graham, 1977).

1.2 DRUG-CELL CONSTITUENTS INTERACTIONS

1.2.1 *Drug-receptor Interaction:*

The most important of these interactions is the drug-receptor interaction. Receptors are macromolecular components/molecules on the cells with which a drug combines reversibly to elicit its pharmacological effect.

Receptor + drug ⟷ Drug-Receptor Complex.

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Receptor site interactions are other areas of drug interactions with clinical significance. The amount of drug interacting with receptor sites is dependent on the amount of drug in the body, its accessibility to the receptor site and the drug's affinity constant for the receptor. A drug may act on a receptor site and result in a detectable effect, such as dopamine that acts on dopaminergic receptors to elicit a vascular response. Other drugs may elicit no detectable effect of their own but may either have a large affinity for receptor sites or be present in high concentrations to act on a receptor. Rather than eliciting a direct effect, these compounds may prevent access of pharmacologically active compounds to the

receptor. This effect is often observed with many therapeutic agents such as atropine. These agents can occupy a receptor and block the effects of an active substance such as acetylcholine. Blockade of a receptor by atropine can be reversed with agents that allow acetylcholine to accumulate and compete competitively for receptor sites. These agents include the cholinesterase inhibitors such as neostigmine and physostigmine (Cupit et al, 1980).

Patients taking isoproterenol by inhalation may exhibit a profound receptor-site interaction with prolonged use of the drug. Isoproterenol is metabolized to 3-methylisoproterenol, a weak beta adrenergic blocker, which blocks the effects of isoproterenol and increases airway obstruction. Increasing the use of isoproterenol leads to an increased accumulation of 3-methyl isoproterenol and the progressive deterioration of asthmatic patients (Paterson, et al 1968).

Another method of receptor site interaction is modification of the intensity of a response through a non-competitive process. Administration of diuretics can cause hypokalemia. Patients who are also taking digitalis preparations may exhibit toxicity during hypokalemia. It is proposed that the mechanism of action of digitalis is by inhibition of the enzyme (sodium-potassium adenosine triphosphatase) that regulates the sodium-potassium pump. Extracellular potassium competes with digitalis for binding to the enzyme which controls the sodium-potassium pump. If extracellular potassium is decreased, more digitalis is bound to the enzyme inhibiting the sodium-potassium pump. This causes less potassium to enter the myocardial cell. Myocardial contractility then increases and arrhythmias can occur (Mason , 1974).

In some cases the combination is irreversible e.g. alkylating agents. Here covalent bonds are formed. In the reversible cases, forces such as electrostatic

forces, hydrogen bonds, dispersion and van der Waal forces are involved. (Bryan et al, 1980)

1.2.2 Protein-Drug Interaction

The extensive distribution of different types of protein molecules in the various tissues of the body makes it unsurprising that many drugs interact with and are bound to them for a time. Serum albumin is the protein which is easy to get in relatively pure form and which has been studied with respect to drug binding. Bovine and human albumins were analysed and it was found out that a particular drug binds differently to these types of protein. This varied protein binding percentage among compounds is a factor in determining the duration and intensity of the effect of a drug.

A bound drug is pharmacologically inactive as its active groups are already bound to the side groups of various macro-molecules. The bound drug cannot pass through cell membranes and is therefore protected from both excretion and metabolism. However, since the forces involved in the binding are weak this interaction is reversible and a shift of equilibrium can easily release free drug molecules. This binding, thus serves as a drug reservoir. A drug which binds strongly to proteins of muscle cells when given intramuscularly will provide a reservoir from which the free drug will be released slowly into the blood stream. A decrease in this binding may occur due to the presence of a second drug which binds at the same site. This would depend on the relative concentration and affinity constants of the drugs. Displacement from binding sites might increase, decrease or have no effect on the amount of drug at the receptor site (Bryan et al, 1980). In older paediatric patients, the displacement of drugs from protein binding sites can be a problem. Concomitant administration of methotraxate with either aspirin or sulfisoxazole, which increases the displacement of methotrexate from its binding site could be hazardous (Dixon, 1965). Since there is a narrow

therapeutic index for this drug, there can be enhanced toxicity from prolonged elevated levels of methotrexate. These two drugs should be avoided during methotrexate infusions.

Total concentration of the drug in plasma does not reveal intracellular concentration of the drug. This leads us to loading dose of some drugs e.g. quinacrine which binds extensively to macromolecules. Thus at normal doses, there will not be enough drug levels in the plasma to clear the parasites, small doses then follow to balance excretion of the drug.

1.2.3 Other Cell Constituents with which Drug Interacts.

A number of other cell constituents exist with which drugs interact. Examples of such constituents are:- Nucleic acids, Mucopolysaccharides, lipids and minerals. Mineral structures such as bones and teeth bind with certain drugs. Example is tetracycline. It appears as a colouration of teeth in children. However, this binding does not serve as a reservoir of the drug.

1.2.4 Consequences of Reversible Drug Binding

Different drugs have different affinity for a particular protein. Consequently drugs compete for binding sites on the protein e.g. salicylic acid can displace long-lasting sulphonamide from albumin. The free sulphonamide which is released can diffuse into tissues where it can exert continued antibacterial action with prolonged effect. Certain acidic drugs also release anticoagulants of the coumarin type from albumin binding. This causes a profound bleeding with serious consequences.

Bilirubin is a naturally occurring substance and can be displaced from its binding sites on albumin by the presence of competing drugs such as salicylates (Stern, 1972), sulphonamides (Malaka, 1965), sodium benzoate (Cupit, 1975) found in agents such as caffeine and in the injectable form of diazepam, novobiocin,

oxacillin and cephalothin (Stern, 1972) and methylparaben, which is found in injectables such as gentamicin (Rasmussen , 1976). Many of these studies, however, suffer from variations in techniques that assess bilirubin binding. Many of the studies were performed in vitro and have not been clinically assessed. The result of this in the infants is very fatal since the enzymes responsible for detoxification of bilirubin are not fully developed. This leads to hyperbilirubinemia and kernicterus - a kind of brain damage due to passage of bilirubin into the tissue. If it were not for binding to proteins the intravenous administration of therapeutic doses of certain drugs might be dangerous.

1.3 Drug Interactions in Anaesthesia

The interaction of general anaesthetic agents (halothane, divinylether, etc) varies with the drug they interact with.

1.3.1 **With Alcohol:** If tolerance has set in, then more of the usual dose of the anaesthetic agent will be required for the general anaesthesia even if the patient is free of alcohol at the time. But alcohol and anaesthetics have additive CNS depressant effect (Dunphy, 1969).

1.3.2 **With Antibiotics:**

Antibiotics which cause neuromuscular blockade e.g. gentamicin, bacitracin, streptomycin etc sensitize the motor and endplates to anaesthetics. This will lead to prolonged response (JAMA, 1963).

1.3.3 **With Barbiturates:**

The combination of Barbiturates and some general anaesthetics can be hazardous. Recovery from CNS depressant effects may be prolonged and collapse is possible (Pharm J. 1967).

1.4 PHARMACOKINETIC INTERACTIONS

1.4.1 *Drug Absorption Interaction*

During the absorptive phase, interactions of drugs may result in either or both of the following potentially significant effects.

(I) An increase or decrease in the amount of drug absorbed

(ii) An increase or decrease in the relative rates of drug absorption.

When a decrease in the amount of drug absorbed results, this is equivalent to a decrease in the dose given. When a drug has its rate of absorption decreased, this may result in either ineffective serum concentrations or a rate of onset that may be greatly delayed. Another consequence of a slow rate of absorption is to sustain release and perhaps prolong an undesirable effect. A delayed onset is deleterious when prompt relief is necessary for acute symptoms such as pain.

Generally, a decreased rate of absorption is not desired for drugs given as a single dose in clinical situations requiring a rapid onset of activity e.g. analgesics such as acetaminophen or aspirin and anti-asthmatics such as theophylline (Cupit et al, 1980).

A rapid rate of absorption is usually not important for drugs administered in multiple dose regimens to achieve a constant serum levels such as tranquilizers. Rather, the average steady state serum levels in a multiple dose regimen is affected by the fraction of the drug absorbed but not usually by the relative rate of absorption. An example of this can be found in examining the interaction of food and aspirin. Food affects the rates of absorption of aspirin but not the total amount absorbed. This effect would be clinically significant when treating an acute process such as a headache where a fast rate of drug absorption is necessary but not significant when treating juvenile rheumatoid arthritis where amount absorged is required. (Cupit et al, 1980).

Many mechanisms have been elucidated to explain how drug absorption can be altered. These include:

1. Alterations of ionization, affecting transport across cell membranes.
2. Alterations in pH that change the dissolution rates of drugs.
3. Alterations in gastric emptying times and /or intestinal transit times.
4. Physicochemical interactions during absorption.

Examples of alteration in pH that cause alterations in drug absorption usually involve antacid administration. When an antacid is concomitantly administered with an enteric-coated product, the antacid raises gastric pH so the enteric-coated medication begins to dissolve. This may cause unwanted side effects.

An alteration in body acid-base balance is known to have significant influence on the action of drugs when used as single agents or in combination. Treatment of poisoning or the drug addiction is the most common example of drug abuse in this category. Phenobarbital poisonings have all benefited from appropriate adjustment of urinary pH (Milne, 1955). Drugs causing pH change can alter the absorption of drugs from the between plasma and cells, and the excretion through the kidney.

Many drug compounds are either weak acids or weak bases where the proportion of ionized or un-ionized is pH-dependent. Alterations in these characteristics (such as affinity for plasma and tissue proteins) and their partition co-efficient and physiological pH will alter their availability at receptor sites (Morrelli, 1978).

The weak acid salicylic acid in the stomach in almost entirely un-ionized, diffusing across stomach lining cells into capillary blood. Shore et al (1957) compared this to the diffusion of barbiturate of a higher pKa where the plasma gastric diffusion is less rapid.

Alteration in pH by ingestion of ammonium chloride, sodium bicarbonate, etc influence the absorption, distribution, and excretion of weak acids and bases. Again, it is recognition and monitoring, more often than predictability, that make this a factor when dealing with drug interactions.

A prime interaction involving pH and gastrointestinal absorption involves the concomitant administration of sodium bicarbonate and tetracycline. The lowered pH results in a decreased absorption of tetracycline (Barr et al, 1971).

Several substances can bind or solubilize drugs that may alter absorption. Cholestyramine an ion- exchange resin used to bind intestinal bile salts and reduce serum cholesterol levels, also binds many drugs. If it is necessary to administer this agent with other medications, it is advisable to space drug administration times between cholestyramine administration times (Gaffo et al, 1965).

Chelation of tetracycline with antacids containing polyvalent cations (aluminum, calcium or magnesium) results in decreased absorption of tetracycline. If antacids are indicated, they should be administered at least three hours after tetracycline administration to allow adequate absorption.

Another chelation interaction involves the administration of iron products and tetracycline. When these two are administered simultaneously there is a decreased absorption of both compounds (Nevonen et al 1970). Again, this interaction may be avoided by administering the ferrous sulfate not less than three hours after tetracycline.

Perhaps one of the most potentially severe drug interactions involves antacids and digoxin absorption. It has been shown that both antacids and kaolin pectin can markedly reduce serum concentration of digoxin (Brown et al, 1976).
Drugs which are analogues of naturally occurring purines, pyrimidine, sugars, and amino acids may be absorbed by small intestinal active transport (Prescott, 1974), and it had been suggested that absorption may be reduced by competition between substrates such as L-dopa and phenylalanine derived from dietary sources (Bianchine et al, 1971). It is also possible for a drug to interact with the enzyme responsible for the transport of a second drug. This leads to decreased absorption of the second drug. Example of such case is between chlorpromazine and L-dopa.

Some drugs have toxic effects on the gastrointestinal mucosa and may cause a malabsorption syndrome with impaired absorption of other drugs. Example is the megaloblastic anemia caused by colchicine through interference with vitamin B_{12} absorption (Webb et al, 1968).
Gastric emptying and gastrointestinal motility play an important role in the absorption of drugs. This is because extensive drug absorption takes place in the small intestine (Kekki et al, 1971) due to the greater surface area of the small intestine. The rate of gastric emptying may limit the rate of drug absorption and is therefore crucial in the context of interactions since it can be influenced by many drugs e.g. Propantheline. Paracetamol and ethanol absorption is related to the rate of gastric emptying and absorption rate is decreased when emptying is slowed down with propantheline (Nimmo et al, 1973).

Interactions involving antacids are probably significant, since they are very widely used and are obtainable without prescription.

Indirectly drug interactions are those where effects on the kidney by an agent will ultimately change a therapeutic potential for another drug in the body. The most common example here is the excretion of potassium (Hypokalaemia) associated with the thiazide and its resultant implication. Skeletal muscle relaxant potency which increases with hypokalaemia is also another example of the indirect drug interaction of the thiazide diuretic. Any drug which damages the kidney will have an effect on subsequently drug secretion (Harshorn, 1973).

1.5 BIOTRANSFORMATION OF DRUGS

Once a drug gains access to the body, its continued presence in the body depends upon how strongly it interacts with various cell constituents throughout the body and upon its ability to pass through cell membranes. Drug molecules that are poorly soluble in lipids do not readily pass through cell membranes and do not interact extensively with non specific cell constituents and therefore they are generally excreted rapidly.

The less polar, or more lipid soluble, a drug molecule is, the more readily it will be re-absorbed by cell from the extracellular fluid. However, this drug (non polar) has its action terminated by the biotransforming enzymes. It undergoes a series of reactions in which it is made progressively more polar. This facilitates its excretion and thus protect the body (Gourley, 1971).

If the parent drug is inactive pharmacologically (prodrug), then it has to be converted to an active metabolite to exert its pharmacological action. This means that drug metabolism is not always detoxification.

The general pathways of the progressive polarization of foreign compounds in biotransformation are summarized below.

Table 1.4.1 **Drugs that might influence gastric emptying**

* Atropine and anticholinergics.
* Antihistamines
* Phenothiazines
* Narcotic analgesics
* Nitrites
* Chloroquine
* Propantheline
* Metoclopramide
* Antacids
* Caffeine
* Antihypertensives.

1.4.2 INTERACTION IN RENAL CLEARANCE

Drug interactions may occur as a result of change in the urinary excretion of one or more pharmacological agents. Alterations in urinary excretion occur by changes in three mechanisms, glomerular filtration, tubular reabsorption, and active tubular secretion. Changes may be the result of an alteration in a normal physiologic process in the kidney occur from competion in urinary pH. One of the most known examples of this is where probenecid and penicillin compete for tubular transport; thus increasing the level of penicillin. Drugs which are actively secreted include salicylic acid, hippuric acid, sulphonic acids, sulphonamides, acetazolamide, probenecid, phenylbutazone, oxyphenbutazone, indomethacin, penicillins, thiazide diuretics, and chlorpropamide, and often their metabolites (Warren et al 1977).

Administration of probenecid has been shown to double the concentration of indomethacin when these two agents are administered simultaneously.

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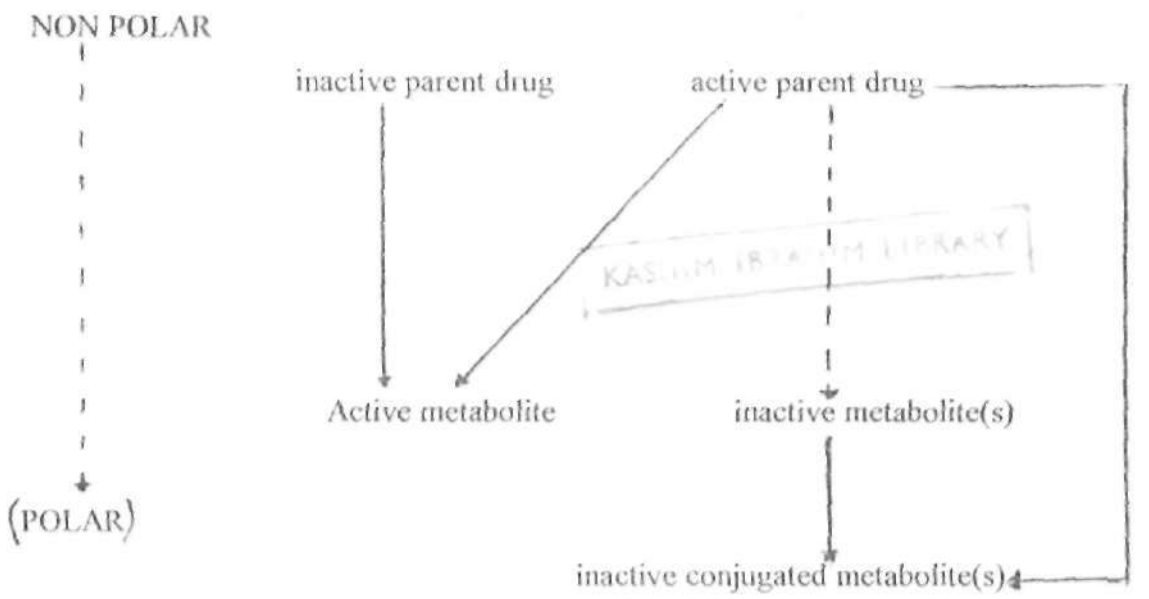


Fig.1.5 The general pathways of biotransformation of a drug molecule which becomes progressively more polar at each step. The broken arrows indicates the possibility of a series of reactions.

1.5.1 Site of Biotransformation

The metabolic transformation of drugs are catalyzed by enzymes that occur in the cells of a wide variety of body tissues-liver, placenta, small intestine, thyroid gland, plasma, skin, lungs, kidney. Of these, liver is the most important organ for drug biotransformation.

1.5.2 FACTORS AFFECTING BIOTRANSFORMATION

Marked variations in responsiveness to drugs occur in man which can have significant clinical consequences. For the most part, this is due to large interindividual variations in drug disposition. Administration of a drug in a fixed dose, or a weight adjusted dose, fails to compensate for these large interindividual

variations. This can result in drug toxicity on the one hand, or a failure to attain the full therapeutic benefit on the other.

Each subject represents a special mosaic of multiple interacting genetic and environmental factors which can cause differences in both drug disposition and response. The significant role played by genetic factors in the inter-individual variations in disposition of some commonly used drugs, has been shown in studies of identical and fraternal twins (Vestal et al, 1980). Marked variation was observed in the half - lives of a number of drugs between fraternal twins, which were 6 - 22 times greater than those observed between identical twins. These variable factors affecting biotransformation are as follows.

1.5.2.1 Genetic Factors

Hereditary difference in the amount or structure of a key enzyme may result in significant variations in the rate of drug metabolism in man. This can be of two distinct types, continuous or discontinuous. The continous type is more common, and arises when the inherited characteristics are controlled by several genes of small effect (multifactorial). Monofactorial inheritance (discontinuous) is much less common. Example of such is the hepatic enzyme N-acetyltransferase which catalyses the transfer of an acetyl group to a number of drugs e.g. isoniazide, sulphadimidine, dapsone. In case of isoniazid, two sub-population were identified as "rapid" and "slow" acetylators. Other heritable factor recognised by the use of drugs include defective plasma cholinesterase, resulting in prolonged action of suxamethoniumm, and the haemolytic activity on red cells deficient of glucose-6-phosphate dehydrogenase of some drugs with oxidizing capacity, e.g. the antimalarial drug primaquine.

Inter-specie difference in metabolism also occurs. This can be either qualitative or quantitative. Certain biotransforming reactions that are common to most

species may be completely absent in others. With drugs that enter two or more routes of administration, the relative importance of the different results can vary from one specie to another. Such differences are particularly important if one of the metabolites is pharmacologically active.

Most species differences, however, are accounted for by variations in the rates of drug biotransformation (quantitative). For example, the mouse metabolizes the centrral nervous system depressant drug, hexabarbital, twenty times faster than man and therefore the duration of the anaesthetic action of given dose per kilogramme of body weight is longer in man by about the same proportion.

Rates of drug biotransformation vary also from one strain to another within a specie. There are marked differences in the duration of action of hexabarbital in various mouse strains

(Kwanashie et al, 1997).

1.5.2.2 **Environmental Factors.**

Numerous environmental factors, each with differing capabilities of altering the "basal" genetically controlled level of drug metabolism, impinge on almost every person. This makes it exceedingly difficult to attribute quantitatively different portions of the total individual variation in rate of drug metabolism to specific environmental factors. The antipyrine test is used to assess the influence of environmental factors on biotransformation. This test consists of measuring antipyrine clearance in a subject under environmentally stable controlled conditions and then again after imposition of a single environmental change (Alvates, 1979). Since antipyrine has a low hepatic extraction ration, is negligibly bound by plasma proteins, and is eliminated entirely by hepatic metabolism, its clearance will depend solely on the activity of the various metabolising enzymes.

Using the antipyrine test, environmental factors such as the diet content of certain macromolecules (protein or carbo-hydrates) and even the mode of preparationm such as charcoal-broiling of meat have been shown to markedly influence drug biotransformation in man (Alvates, 1979). Certain vegetables such as cabbages and brussels have also been shown to stimulate the metabolism of certain drugs (Alvates, 1979). Other well known inducers of the hepatic microsomal drug-metabolising system include many drugs such as phenobarbitone and phenytoin, alcohol, adrenocortical steroids, chlorinated hydrocarbon insecticides such as DDT, and polycyclic aromatic hydrocarbons such as 3-4 benzpyrene. These latter compounds are products of incomplete combustion, and are widespread in man's environmental, occuring in particularly high concentration in tobacco smoke and polluted city air.

Inhibition of the hepatic microsomal enzyme system by environmental compounds is less well known.

1.5.2.3 **Age**

The pharmacokinetics of a drug in a particular individual is not a constant feature but changes with age and development. There are significant differences in drug tolerance between children especially infants and adults.

A reduced capacity (20-30% adult values) to dispose drugs is constantly observed during the first 15 days of life in premature and full term neonates, unless there is exposure to inducing agents before or immediately after birth. This is followed by a dramatic increase in the metabolic rate of mainly phase I reactions (oxidation, hydroxylation, reduction) to a rate 2 - 6 times that of adults. This period of increased metabolic disposition is usually evident from 2 - 3 months up to 2 - 3 years, after which values tend to decline gradually to reach those of adults after puberty.

In premature infants, the elimination rates of certain drugs appear to be even more retarded than in full-term heathly new borns. For example, in two premature infants plasma indomethacin half-lives were 21 and 24 hours (Friedman, et al, 1978), whereas in full-term healthy newborns the mean value was 14.7 hours (Traeger, 1973) and in healthy adults 2-3 hours (Duggan, 1972) or 7.2 hours (Palmer, 1974). Obviously, these marked differences in elimination rates of drugs among premature infants, full-term newborns, children, and adult have practical significance in the calculation of appropriate dosages of drugs. They also indicate the need to investigate pharmacokinetic and pharmacodynamic processes for many more drugs in these age groups.

Glomerular filration and tubular secretion are also impaired in the neonate, with childhood values not being reached until 30 - 40 weeks. Thus, during this initial period there is an increased from drugs such as gentamicin, kanamycin, indomethacin and digoxin, which are dependent on glomerular filtration for their excretion, and drugs like penicillin and sulponamides whose excretion depends on tubular secretion.

Apart from maturation of the two main eliminating organs, many physiological changes are taking place in the developing neonate the extracellular fluid amounts to 40 per cent of body weight, compared to approximately 20 per cent in adults, while fat tissue and skeletal muscle are also much reduced in the young. Other factors which vary during the first days of life include blood pH, acid-base balance cardiac output and regional blood flow, all of which may influence drug disposition.

Since it is difficult to predict plasma drug concentrations in neonates and young infants then for any drug with a low therapeutic index e.g. digoxin, phenytoin and theophyllin, monitoring of its effects and its concentration is necessary.

During the aging process, patient variables may be altered beyond the range of values experienced in the young adult. Changes that accompany old age include decrease in cardiac output, hepatic blood flow, glomerular filtration rate, plasma albumin concentration, lean body mass, etc. These have been shown to affect drug disposition. The primary factor that decreases in the elderly is clearance. This may be due to the diminished ability of the kidney to excrete, or of the liver to metabolise drugs, or both. Renal function over 20 - 80 years gradually declines to approximately 50 per cent of its initial value. Drugs which are primarily eliminated by the kidney will also have their elimination affected to similar extent since there is a similar reduction in renal blood flow over the same period.

The impairment of hepatic elimination of a number of drugs is not due to a reduction in drug metabolizing enzyme activity per se, but rather to a reduction in blood flow, and to the fact that the elderly appear to become relatively resistant to the effects of enzyme inducers present in the environment (Vestal, 1980). The reduction in blood flow is very significant especially in anaesthesia where the onset of anaesthesia is delayed. The delay does not call for addition of more anaesthetic agent.

1.5.2.4 Pregnancy:

With advancing pregnancy, important physiological changes occur in the haemodynmics, the relative volumes of body water compartments, the hormonal status, the concentration of plasma proteins and endogenous compounds such as non-esterified fatty acids, all of which are known to infuence drug disposition and kinetics (Krauer, 1977)

non-esterified fatty acids, all of which are known to influence drug disposition and kinetics (Krauer, 1977)

There are differences in gastrointestinal function such as intestinal motility, and increase of about 30-50 per cent in the gastric and intestinal emptying time. These might affect absorption of drugs during this period. Since plasma volume increases by about 50 per cent, cardiac output increases by about 30 per cent, and by the end of the first trimester renal blood flow increases by about 50 per cent, then distribution and elimination will also be affected. Drugs which are eliminated by the kidney, could have their elimination increased due to the observed increase in creatinine clearance. Increase in hepatic blood flow has not been established.

The free fraction of many drugs rise during pregnancy (Dean, 1980) which will affect their distribution and also their elimination rate depending on the characteristics of the drug. For example "Capacity limited" drug will have its systemic clearance increased if its free fraction is increased. Steroidal production is significantly increased in pregnancy.

A greater rate of hepatic metabolism for some drugs might occur since progesterone is known to stimulate hepatic microsomal enzyme activity. However, oestradiol steroids are competitive inhibitors of microsomal oxidation of some drugs, such as ethylmorphine and hexobarbitone.

1.5.2.5 **LIVER DISEASE:**

It is firmly established that hepatocellular disease is associated with decreased capacity to metabolize some drugs. It has not yet been determined, however, whether the extent of depression of hepatic drug-metabolizing function in an

individual with liver disease is similar for all drugs biotransformed by the organ (Vestal et al, 1980).

The metabolic pathway for hepatic biotransformation of prednisolone was shown to be affected by liver disease, the extent of this effect depending on the severity and nature of the disease (Cooksley et al, 1971). In 1972, patients with severe bilharziosis were shown to have impaired metabolism of the schistosomacide, niridazole, as revealed by higher concentrations of the drug and a higher incidence of adverse effects than in normal subjects (Faigle, 1972). Also in 1974, aminopyrine metabolism was shown to be deranged in a large percentage of patients with liver disease (Hapner et al, 1974)

Serum antipyrine half-life was shown to be prolonged in patients with liver disease (Branch 1973), an observation confirmed in 1974 (Andreasen et al, 1974)

Observation that rates of aminopyrine elimination are reduced in almost all patients with parenchymal liver disease and that liver disease is also accompanied by reduction in rates of hepatic antipyrine metabolism can be harmonized with observations that warfarin disposition during acute viral hepatitis is unchanged (Williams, et al 1976) and that oxazepam disposition is normal during acute viral hepatitis and cirrhosis (Shull, 1976). Between these extremes, a group of drugs of which clindamycin is an example, apparently exhibits intermediate or moderate changes in disposition in liver disease (Avant, et al, 1975). A wide range, from no change whatever in disposition to significant retardation, has been reported in liver disease depending on the drug studies. For drugs with high hepatic extraction ratios (>0.8), such as propranolol and lidocaine, alterations in blood flow accompanying liver disease can produce large changes in hepatic clearance of the compound. For those drugs having low hepatic extraction ratios (<0.2),

such as antipyrine and aminopyrine, large variations in the extent to which hepatocellular disease alters their rates of metabolism cannot be due to abnormal liver blood flow. They may be attributed in part to multiple molecular forms of hepatic cytochrome p-450 and to the differential effects that a particular hepatic disorder might exert on these forms (Vestal, 1980).

1.5.2.6 **Other Factors**

I) **Hormonal Status:**

The hormonal status or balance is varied depending on the stress. For example less than four days of sleep deprivation failed to alter plasma 11-hydroxy corticosteroid (Vesell ES, 1977) or 17-hydroxy corticosteroid (Kollar, 1966) concentrations but longer periods of sleeplessness (205hours) disrupted diurnal rhythms of plasma 11-hydroxycorticosteroid concentration and glucose metabolism (Rubin, 1969). Other forms of stress affect adrenocortical function by markedly increasing hydrocortisone concentrations, i.e. alcoholism (Kissin, 1959), general surgery (Persky et al 1956), intense physical activity (Crabbe, et al 1956), electroconvulsive therapy (Bliss et al, 1954), and myocardial infarction (Oka, 1956).

Swartz et al (1974) reported that acute physical stress alters the disposition of drugs eliminated by the liver in man. Heat, fluid deprivation and exercise decreased indocyanine green clearance primarily by reducing liver blood flow, but these factors failed to alter greatly antipyrine clearance, probably because antipyrine is a drug with a low hepatic ratio.

ii) **Diet:**

The effect diet has on hepatic drug elimination is very complex. Starvation, was reported to affect hepatic metabolism of drugs in different ways, some drugs being accelerated, others being retarded in their elimination rate. Furthermore, a sex dependency exists for this starvation effect in rats (Zannoni'et al 1972). Starvation of rats for 72 hours diminished or abolished the sex differences in rats of metabolism that occur in normally fed rats for many drugs (Kato, 1965). Starvation effects on drug metabolism in rats has been shown to be mediated by interference with the stimulatory action of the androgenic steroids (Kwanashie, 1998).

Effects on drug elimination of the relative proportion of carbohydrate to protein in anisocaloric diet were investigated (Kappas, 1976). Careful diet control was observed for two weeks in each of two different studies. Non medicated, normal male volunteers aged 22-27 years were involved. In the first study, protein constituted 44 per cent of total caloric intake while carbohydrate was 35 per cent of total caloric intake. Theophylline and antipyrine elimination rates were accelerated by about 40 per cent. In the second study, the protein content was changed to 10 per cent of total caloric intake and carbohydrate was elevated to 70 per cent. The half lives of the two drugs were prolonged by about 40 per cent of their values in the first study.

1.5.3 Drug Metabolism Interaction

Drug interactions involving metabolic process are perhaps the most complex area of drug interactions.

Drug effect is terminated either through excretion (via the kidney) or through metabolism (e.g. via the liver). There are several enzymes in the liver which metabolise drugs and then eliminate them from the circulation. These enzymes

can be induced or inhibited by several drugs and this leads to altered effects of other drugs concomitantly administered. However, it is important to consider not only the effect of two drugs and the changes resulting from their concomitant administration, but also the state of the metabolic process before and after the drugs impart their activity. An example is a patient with diminished liver function due to age or pathophysiological processes who receives a microsomal enzyme inducing agent. In the normal liver, one would expect the inducing agent to yield a lower level of a second drug utilizing the same pathway. In this example, the second drug may only approach normal metabolic degradation (Cupit et al, 1980).

1.5.3.1 **Enzyme Induction:**

Some drugs have the ability to induce the activity of the microsomal (hepatic) enzyme. If another drug which is metabolised by this hepatic enzyme is concomitantly administered with an enzyme inducer, the second drug will be quickly metabolized leading to diminished effect of the second drug. This type of metabolic change takes several days sometimes even weeks to develop fully. Warren and Bender (1977) cite many recognised drug interactions with antineoplastic agents. Interactions occur via alterations in metabolic process, either by induction or inhibition of enzyme metabolism. Phenobarbital, for example, which is known to interact with cylophosphamide, has been shown to both increase and decrease the activity of this agent (Oira, 1974). Morphine, when tested in the animal model, has been shown, to decrease cyclophosphamide metabolism as has the known enzyme inhibitor chloramphenicol (Hansten et al, 1975). In these studies, except for very extreme levels of cyclophospamide that could be toxic to the patient, the therapeutic range is so wide and the response so variable in an individual patient that these drug interactions can only be monitored and cannot be quantified to allow dosage or serum-level adjustments (Alberts,et al 1976).

Table 1.5.3.1 **INTERACTIONS DUE TO ENZYME INDUCTION (Summer 1980)**

Drug(s) Affected	**Inducing Agent(s)**	**Effect of Interaction**
Anticoagulants	Aminoglutethimide Barbiturates Carbamazepine Dichlorophenazone Rifampicin	Anticoagulant effects reduced.
Contraceptives (Oral)	Barbiturates Phenytoin Rifampicin	Contraceptive effects reduced, break through bleeding, contraceptive failure
Corticosteroids	Barbiturates Carbamazepine Phenytoin Rifampicin	Corticosteroid effect reduced
Holoperidol Pentazocine Phenytoin	Tobacco smoke Tobacco smoke Rifampicin	Haloperidol effect reduced Pentazocine effect reduced Phenytoin effect reduced. Seizure risk increased.
Theophylline	Barbiturates Rifampicin Tobacco smoke	Theophylline effect reduced

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1.5.3.2 **ENZYME INHIBITION**

Some drugs have the ability to inhibit the activity of the microsomal enzyme. These drugs are termed enzyme inhibitors. The normal pace of drug metabolism is slowed down so that the metabolism of other drugs given concurrently is also reduced-leading to accumulation of the second drug. The manifestation is like that of overdosage. Enzyme inhibition can occur within two or three days resulting in rapid toxicity.

Inhibition is much more dangerous than induction because by the time it is noticed, hazard may have occurred. Example is concomitant administration of an enzyme inhibitor such as cimetidine and an anticoagulant such as warfarin in the usual doses. This leads to profuse bleeding due to accumulation of the anticoagulant in the body resulting in exaggerated effect. So if they have to be given together, the dose of the anticoagulant must be reduced throughout the course of the inhibitor (Kwanashie, 1997)

Dixon and Fouts (1962) have shown that chloramphenicol decreased the rate of metabolic transformation of hexobarbitone, acetanilide, codeine and aminopyrine in mice. In vitro experiments showed an inhibitory effect of a non-competitive nature on the microsomal drug - metabolizing enzymes.

Table 1.5.3.2 (**INTERACTIONS DUE TO ENZYME INHIBITION (Summer1980)**

DRUG(s) AFFECTED	INHIBITING AGENT(S)	CLINICAL OUTCOME
Alcohol	Chlorpropamide Disulfiram Metronidazole	Disulfiram - effect due to a rise in blood acetaldehyde level
Anticoagulants	Metronidazole Phenylbutazone	Anticoagulant effect increased. Possible bleeding
Azathioprine Mercaptopurine	Allopurinol	Toxicity due to increased effects of the drugs affected
Phenytoin	Chloramphenicol Isoniazid	Phenytoin effects increased Intoxication possible

1.6 PHARMACOKINETIC STUDIES

Pharmacokinetics is the summation of all the physiological activities which the drug

undergoes in the body. These are changes in concentration of the drug and/or its metabolite(s) in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage, binding, metabolism and excretion.

The objective of prescribing any medication to a patient is to achieve a specific therapeutic response while minimizing adverse effects. Since patients' response to drugs are known to show wide variation, most patients are initially treated with a standard dosage regimen and then clinically evaluated periodically to check if modification of the regimen is necessary.

Measuring plasma drug concentrations and utilizing pharmacokinetic tools to predict
future plasma levels can be valuable aids in the proper adjustment of dosage.

Precise determination of the biologic rate processes involved in drug distribution and elimination require knowledge of complex compartment models. The validity of this compartment model depends on some assumptions:

1. Drugs enter the system only via the central compartment and are eliminated only from that compartment.
2. Reversible transfer occurs between central and peripheral compartments.
3. Exit of drugs from all compartments in the system is described by first order kinetics.

All the three assumptions are true for majority of drugs. These enter the system via the blood and are removed from the blood via the kidney or liver. Majority of drugs are eliminated by first order kinetics. However, there are exceptions where drugs when given in high enough doses cause saturation of the liver enzymes thereby reverting their biotransformation from first to zero order kinetics.

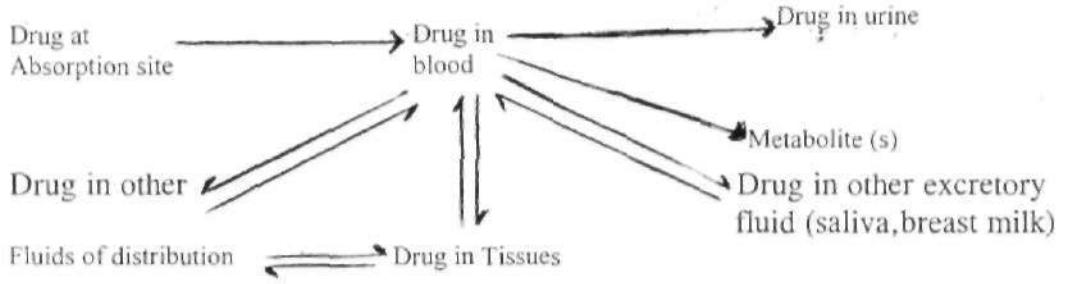


Figure 1: **Distribution and Elimination of Drugs in the Body**

1.6.1 FIRST ORDER KINETICS

Following drug administration, a drug must cross several membranes to reach its site of action. This transfer is usually accomplished by passive diffusion, the driving force of which is the concentration gradient between both sides of the membrane. Drugs in which the rate of transfer is proportional to the amount of drug remaining to be transferred are described by a first - order process. Drug

transfers (between body 'compartments') and drug elimination involve rate constants (k) specific to each transfer.

When a semilogarithmic plot of drug concentration versus time results in a straight line, then drug transfer can be considered to proceed by first -order Kinetics.

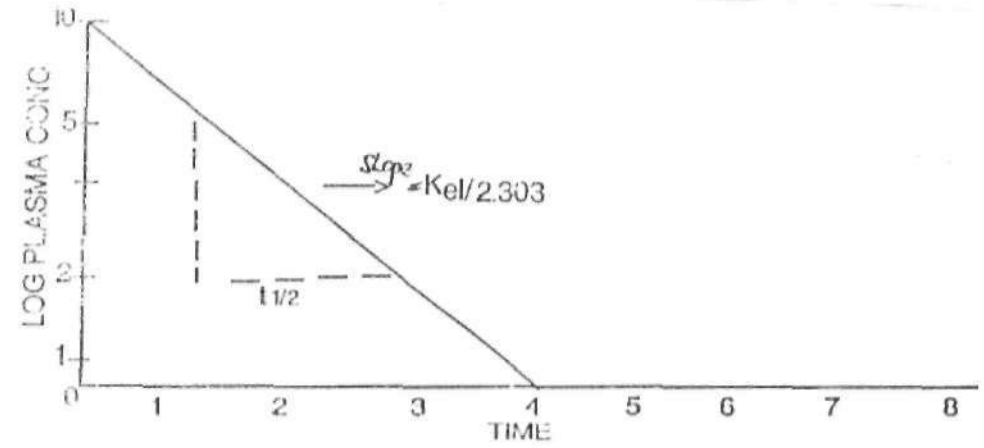


Fig.1.6.1. **A typical first-order decline of plasma drug concentration versus time**

The decline of drug concentration in the plasma is usually accompanied by a parallel
decline of drug concentration in the major drug storage tissues.

$$\log C_t = \log C_o - K_{el} \times t/2.303$$

This equation describes the plasma decay curve where

C_t = drug concentration at time t

Co = drug concentration at time zero

the slope of the linear portion is the elimination rate constant K_{el} divided by 2.303

$$K_{el} = \frac{0.693}{t½}$$

$t½$ = half - life

For a drug following first order kinetics, the half - life is independent of dose, plasma concentration or route of administration, and is often directly related to the duration of pharmacologic effect.

For most drugs, absorption, distribution, metabolism and renal excretion follow first order kinetics.

1.6.1.1 Single Compartment Model

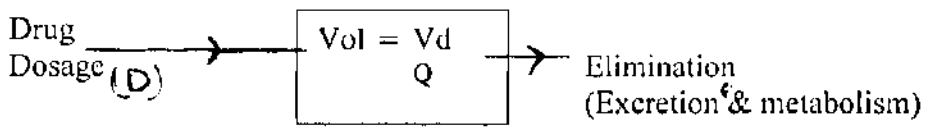
This is the simplest model. When the exchange of drug between the plasma and tissues proceeds rapidly compared with the rate of elimination, the whole body may be considered mathematically as a single compartment. Assuming instantaneous distribution after an intervenous (iv) injection of a drug into this model, the concentration Co in the plasma immediately after injection is equal to the dose (D) divided by the volume of this compartment (Vd)

$$Co = \frac{D}{Vd}$$

Co → is obtained by extrapolation of the graph

The apparent volume of distribution (Vd) of the drug is not a true volume but that volume into which all the drug in the body would appear to be distributed to achieve a concentration the same as that in the plasma.

CENTRAL COMPARTMENT

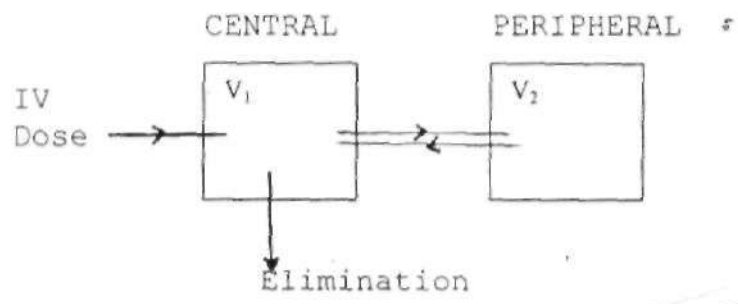


After instantaneous distribution, the concentration falls according to first-order kinetics i.e. a constant fraction of drug present is eliminated per unit time.

In this assumption it does not mean the drug concentration in body tissues at any given
time are the same. However, it assumes that any change that occurs in the plasma quantitatively reflects changes occurring in tissue drug - levels.

1.6.1.2 Two Compartment Model

Two compartment model contains a central compartment and at least one other peripheral compartment and this is usually considered when the distribution of the drug is so slow that it can not be disregarded. Although, these compartments lack physical or anatomical reality, for many drugs the central compartment may correspond to the plasma or blood volume together with the extracellular fluid of highly perfused tissues such as muscle, skin or adipose tissue into which drugs may enter more slowly. The combined effect of two compartments results in a biphasic curve on IV injection with two distinct linear portions when drawn on a semi ordinate scale.



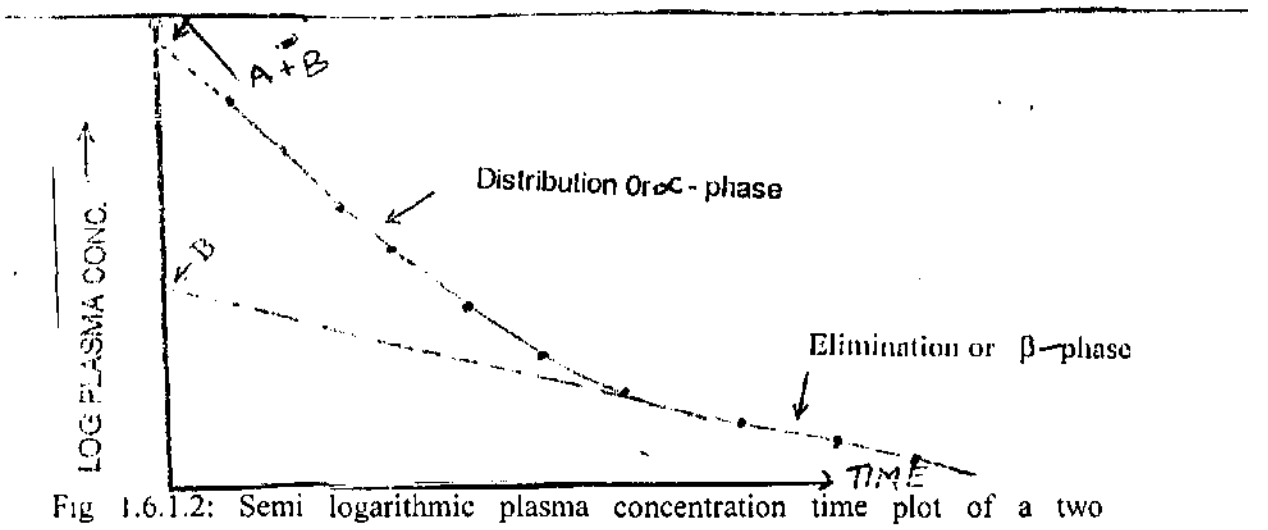
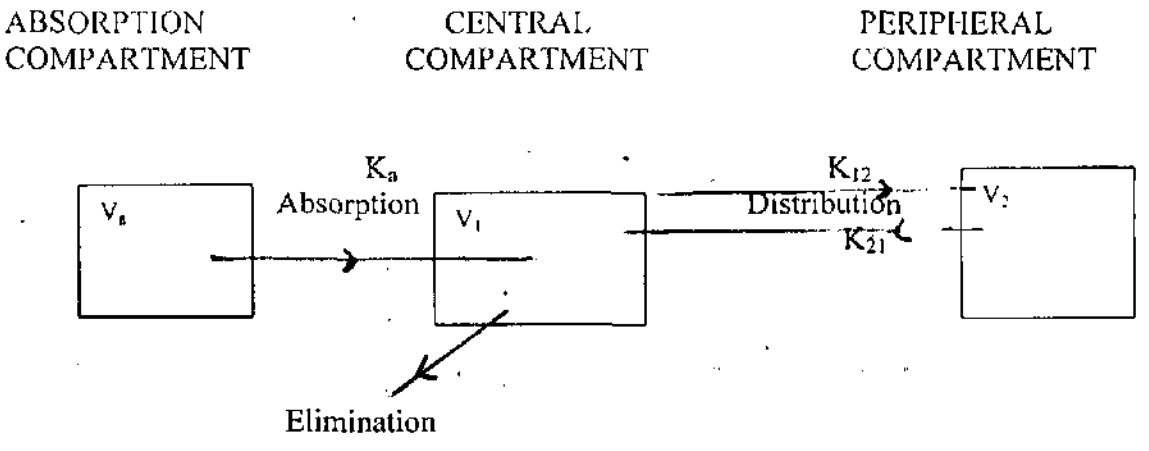


Fig 1.6.1.2: Semi logarithmic plasma concentration time plot of a two compartment model.

1.6.1.3 Multicompartment model

The two compartment model is the most popular in assay techniques. However, it may be expanded to contain additional compartments which can be described mathematically as the sum of as many individual exponential functions as there are relevant compartments. In reality, a maximum of three compartments is allowed in assay techniques (Paxton, 1981).

With an oral dose an additional absorption compartment is incorporated into the model to represent the volume (Va), from which absorption occurs at a first-order rate.



A three Compartment model

In this model it is assumed that the entire dose is rapidly introduced into the site of absorption, from where it is absorbed into the central compartment.

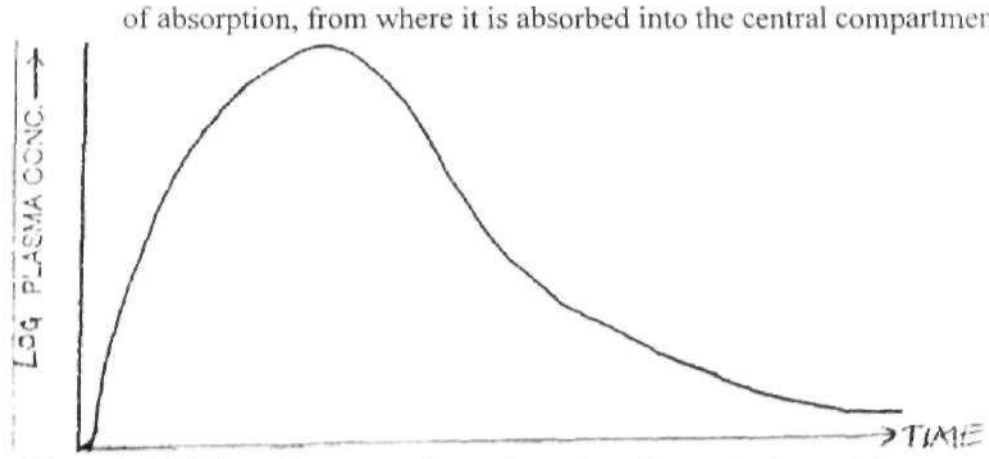


Figure 1.6.1.3 **Log Concentration - time plot after a single oral dose.**

Similar plots as above are obtained for other administration routes, such as intramuscular or subcutaneous, which involve a preliminary absorptive phase.

1.6.1 PHARMCOKINETIC PARAMETERS

Area under the curve (AUC) of blood level versus time, systemic and total clearance, half-life, apparent volume of distribution and rate constants are some of the most useful pharmacokinetic parameters obtained after drug administration.

Area under the curve (AUC) of blood level versus time is the integral of drug serum levels over time from drug administration (time zero) to infinity. Since oral preparations account for the majority of all drugs prescribed, the efficiency of this route for a medicament, must be determined by measurement of the rate and extent of absorption of a drug from a pharmaceutical dosage form i.e. bioavailability. Hence AUC becomes a useful parameter because it is directly proportional to the amount of drug that is absorbed.

Multiple drug serum levels following a single dose are required for the calculation of AUC.

Methods to calculate AUC are planimetry, cut and weigh method, computer application and trapezoidal rule (Wagner, 1971).

The trapezoidal rule is frequently used. This rule gives the value of the sum of the areas of serial trapezoids whose bases are the time ordinates from time zero to time infinity.

For quantitation of drug bioavailability from a product it is necessary to compare the calculated AUC to a standard, preferably an I.V. preparation (AUCoral/AUC i.v.). An oral reference standard may also be used to determine relative bioavailbility. Significant differences in plasma drug levels following administration of different brands of phenytoin have prompted the Food and Drug Agency (FDA), New York, to revise prescribing directions for all oral phenytoin products i.e. to maintain patients on one product and to monitor blood levels if switching dosage forms is necessary (Ebert et al, 1976).

This points it out that formulation differences causing bioavailability discrepancies may be less important at the start of therapy than if substitution occurs during an established course of therapy (Michele, 1980).

Half-life is the time required to reduce the drug concentration in plasma by one-half of the original concentration (Michele, 1980). It can be determined for both one and two compartment models from the terminal data of the log concentration time plot. This is calculated by dividing 0.693 (natural log of 2) by the calculated slope of each exponential phase of multiphasic blood level versus time curve.

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

Even though this parameter is valuable for a clinical judgement of the duration of the

effect (which is not necessarily the same as the duration of measurable drug concentrations in plasma) it gives no information about the efficiency of drug elimination. It is used in the determination of suitable dosage intervals and the time required to attain the steady-state during constant intravenous or chronic oral therapy.

Systemic or plasma clearance is the volume of blood or plasma that is irreversibly cleared from the drug per unit time. This parameter is a direct index of elimination. The sum of processes of elimination (hepatic metabolism, renal and faecal excretion, exhalation by lungs) represents total plasma clearance. The easiest way of measuring drug clearance is to measure the drug concentration in plasma (or blood) repeatedly after a single I.V. dose and calculate the area under the plasma concentration versus time curve (AUC). The clearance (Cl) is related to the AUC according to

$$Cl_{i.v.} = \frac{D}{AUC_{i.v.}}$$

where D is the dose given. If the drug has to be given orally, a correction factor (F) has to be introduced.

$$Cl_{oral} = \frac{FD}{AUC_{oral}} \quad ; \quad F = \frac{AUC_{oral}}{AUC_{i.v}}$$

F represents the fraction of the oral dose that is available to the systemic circulation. The remainder of the dose (I-F) is the fraction that is bound/metabolized in the liver or gut wall during the passage from the gut lumen to the systemic circulation. This phenomenon is called "first pass elimination" or first pass effect," (Anders, 1980).

Under certain circumstances the plasma half-life may serve as an estimate of the hepatic drug metabolizing activity. If the systemic drug clearance is equal to the hepatic ($Cl_s = Cl_H$) i.e. the drug is only eliminated via hepatic metabolism, then

$$Cl_H = \frac{0.693 \times Vd}{t_{1/2}} \quad \text{or } t_{1/2} = \frac{0.693 \times Vd}{Cl_H}$$

Hence, the t½ depends on Cl_H and the apparent volume of distribution (Vd). Under the assumption that Vd is constant, then the t½ is virtually proportional to the hepatic enzyme activities (for "low extraction" drugs), the hepatic blood flow (for "high extraction drugs) or both (for drugs with intermediate values of intrinsic clearance). Since the Vd is subject to large developmental variations, caution must be exercised in interpreting t½ data in infants and children, and comparing these to adult values.

Systemic Clearance $Cl_s = \beta \times Vd$ ie the apparent volume of distribution multiplied by the elimination constant (β).

Clearance has dimension of volume per unit time.

The apparent volume of distribution (Vd) is defined as that volume in which the amount of drug in the body would need to be uniformly distributed to produce the observed plasma concentration. This volume of distribution is inversely proportional to blood drug level, i.e. the larger the volume of distribution, the more extensively the drug distributes from the blood stream into the various tissues, organs and binding sites of the body. Calculation of the volume of distribution may exceed total body water. This is the case with digoxin. Exchange transfusion in neonates has been reported to remove less than 6 per cent of a given dose of digoxin. This indicates that the drug is extensively stored in extravascular sites. Protein binding also affects the distribution of many drugs. Drugs highly bound to serum proteins have a small volume of distribution.

The volume of distribution, however, is usually more dependent on the physicochemical properties of the drug than on the patient's biologic processes.

There are many different methods employed in calculation of Vd. The following equation, proposed by Gibaldi et al, 1980 is valuable for estimating Vd in a single dose study.

$$Vd = \frac{Dose}{AUC_{0-\infty}}$$

The estimation of Vd will be affected by changes in the elimination rate and AUC.

1.6.2 BIOAVAILABILITY

This is defined as the rate and extent to which active drug ingredient or therapeutic moiety from a drug product becomes available at the site of action which is normally estimated by its concentration in fluids, rate of excretion or acute pharmacological effect (Skelly, 1976). Accordingly, the absorption of drug administered intravenously is rapid and complete. However, for convenience and stability reasons, majority of drugs are administered orally. For such drugs the appearance of the plasma (or serum) concentration curve depends upon a very large number of factors. These include physicochemical properties of the drug, type of manufacturing technique, nature of excipient used, type of dosage form, physiological conditions such as stomach emptying rate, gastrointestinal pH, enzyme concentrations, etc.

Bioavailability studies are usually based on measurement of the active drug moiety and/or its metabolite(s) in biological fluids as a function of time. In vivo bioavailability testing in humans usually states that the biological fluid sampled, be limited to blood, urine or saliva. The dosage form is administered to healthy volunteers in the fasting state. They are not allowed to take food for at least four hours following administration. This is essential as food is known to reduce gastric motility. Blood, saliva or urine samples are collected before and after drug administration at a frequency sufficient to permit an estimate of peak plasma or serum concentration of the active ingredient and the total area under the plasma level versus time curve. In most studies where the oral dosage forms are compared, the sampling times after each drug administration will be identical. The following figure illustrates a typical plasma concentration versus time curve that may be obtained after oral administration of a dosage form.

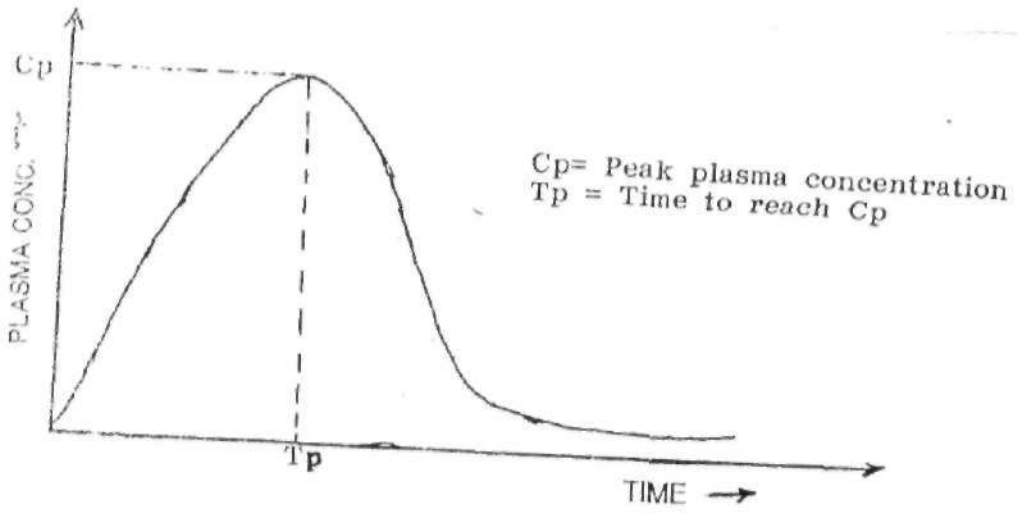


Figure 1.6.1.4 **Plasma - Concentration Time Curve**

Many factors combine to modify the shape of the curve. Absorption, distribution and elimination are some of such factors.

Cmax (Cp), the maximum concentration in the plasma and Tmax (Tp) the time to reach Cmax are related to and used as a measure of the rate of bioavailability from the dosage form. The area under the curve (AUC) signifies the extent of absorption from the dosage form.

For "absolute" bioavailability determination, AUCs obtained in oral and intravenous administration of the drug must be compared. I.V. administration is not always possible, therefore "relative" bioavailability is determined by comparing the AUCs of the test drug and a secondary reference standard such as an orally administered solution of the drug or a dosage form which has been accepted as a standard (Gibaldi and Perrier, 1975a).

In some instances, this bioavailability determination is difficult following a single dose. Such instances include:

*When the plasma concentration of the drug is very minute such that the usually available assay method fails to detect it.

*When the plasma half-life of the drug is very long, the AUC will require plasma samples to be obtained for excessively long periods of time.

Thus, it is sometimes desirable to determine availability based on steady state plasma drug levels following multiple dosing (Gibaldi and Perrier, 1975a)

The area under the plasma concentration time curve (AUC) determination is necessary for availability estimation. The most commonly employed methods for calculating AUCs are the 'cut and weigh', planimetry, trapezoidal rule and computer application.

1.7 FACTORS AFFECTING BIOAVAILABILITY OF DRUGS

Compressed tablets today make up the bulk of oral dry product medication. This is so

because they provide accurate dosage from a chemical analysis point of view, they are convenient to carry, provide good stability for most active ingredient and are economical. The tablet is commonly involved in drug bioavailability and bioequivalence problems due to the reduction in drug surface area in the compressed formulation and the wide variety of drug forms, tabletting methodologies, excipient and coating that are used. Formulation differences causing bioavailability discrepancies may be less important at the start of therapy than if substitution occurs during an established course of therapy. Significant differences in plasma drug levels of phenytoin following administration of different dosage forms and different brands of phenytoin, have prompted the Food and Drug Agency (FDA) to revise prescribing directions for all oral phenytoin products i.e. to stick to one product and to monitor blood levels of switching dosage forms is necessary. Other drugs known to have bioavaliability problems include thyroid, prednisone and Digoxin (Colaizzi, 1975).

Dosage form related factors which can produce profound differences in bioavailability

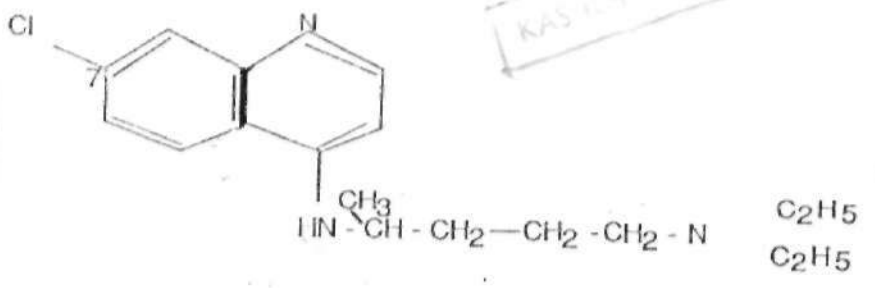
include formulation and manufacturing variables such as particle size, chemical form and solubility of the drug, type and quantity of excipient used and the compaction pressure (Blanchard et al, 1979). Crystal form, salt and pH of the active ingredient also bring about variability in bioavailability.

CHAPTER TWO

LITERATURE REVIEW

2.1 CHLOROQUINE

Structure



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2.1.1 Chemistry

7 Chloro -4 -(4′ - diethylamine - 1′ - methylbutylamino) quinoline.

It is a 4 aminoquinoline derivative. The d and L forms of chloroquine are indistinguishable in potency tests in duck malaria, but the d form is less toxic than the L-form in mammals. Melting point of the d-form is 213°C, while that of the L-form is 194°C.

Description Chloroquine is a whitish almost white powder, odourless and it is soluble in four parts of water.

Chloroquine is an antimalarial and it is also used in rheumatism and liver necrosis. It is also used in photoallergic reactions where high doses are employed. This calls for a close monitoring for toxic effects.

It is used in the treatment of cardiac arrythmias instead of quinidine. This is due to its ability to increase the threshold of excitability of the papillary muscle.

It inhibits competitively, the enzyme alcohol dehydrogenase which is important in the visual cycle. This leads to the retinopathy frequently encountered when the drug is used in large doses for long periods (Fiddick 1967).

Apart from its effects on the plasmodium (Malarial causative agent). chloroquine also possesses a direct relaxant action on isolated guinea pig smooth muscle, to prevent histamine- induced contractions (as in ulcers) and to block anaphylactic contractions of tracheal muscle (Agarwal et al, 1963).

2.1.2 **Absorption:**

Absorption is rapid and complete from the gastrointestinal tract. It has a high volume ofdistribution. Plasma half-life is 3-7 hours.

2.1.3 Distribution:

Tissues bind less of the drug. Plasma concentration is high. About ten to 25 times plasma concentration is in red blood cells while the liver concentrates about three hundred times the plasma concentration. Hence its use in amaebiasis.

Only 10 -25% of the dose is excreted renally and the rate of this excretion is increased with acidosis.

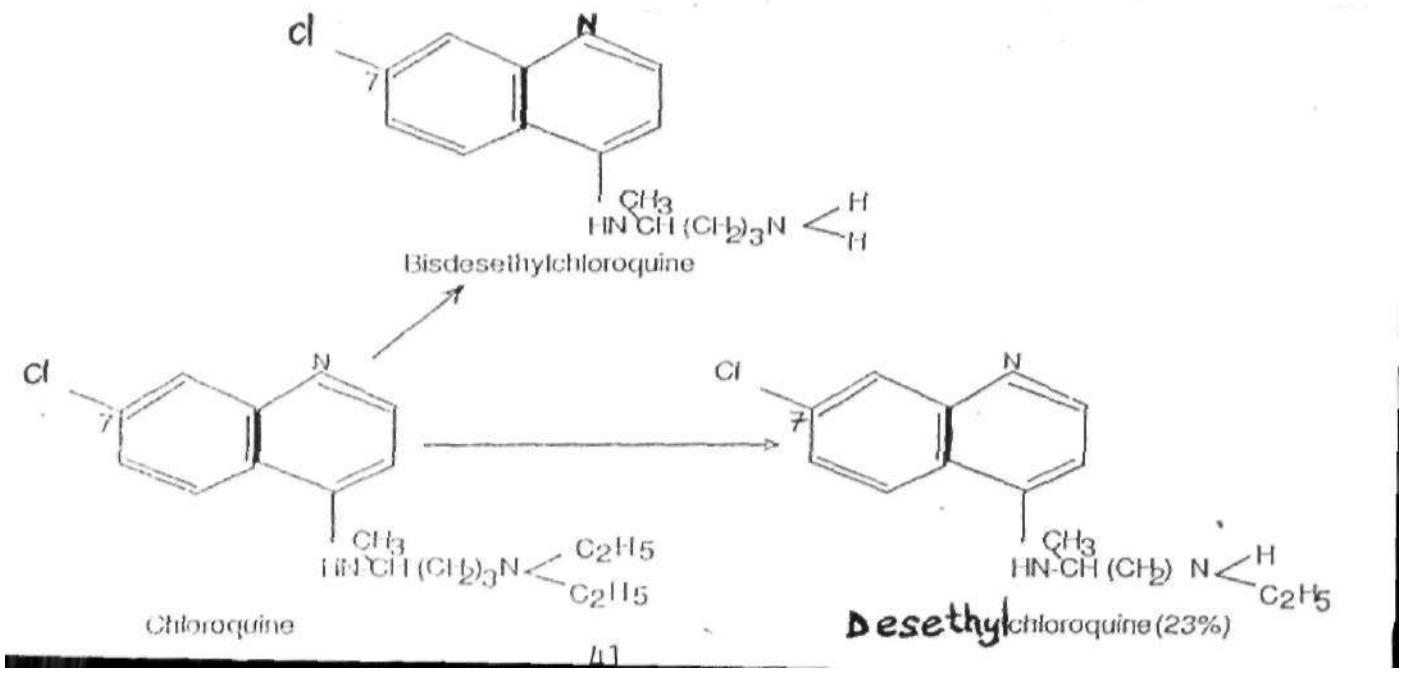
2.1.4 Metabolism:

This is the major pathway of chloroquine excretion. The following diagram shows the types of metabolites and their proportions.

2.1.5 STRUCTURAL ACTIVITY RELATIONSHIP

Marked clinical activity is achieved with chlorine atom in the 7 position of the quinoline nucleus.

Methyl (CH_3) substitution at 3 - position of the nucleus reduces activity. Additional methyl group at position 8 leads to complete loss of activity.



2.1.6 Pharmacological Actions And Uses:

Chloroquine is not a causal prophylactic agent. It is highly effective (Schizonticidal) against the asexual erythrocytic forms of P. vivax and P. falciparum. It is therefore extensively used as an antimalarial in the tropics, although some cases of resistance to it have been reported in some parts of the tropics. In malaria and extra intestinal amebiasis the dose of chloroquine is 600mg base once daily for two days then 300mg base once daily for 2-3 days. In children not more than 5mg base per kilogramme body weight (single dose) should be given. It can be repeated after six hours. Total 24 hours dose should not be more than 10mg base per kilogramme body weight.

2.1.7 Overdose and Management:

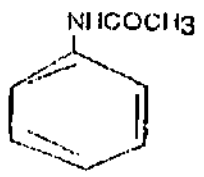
Since chloroquine absorption is rapid, toxic symptoms may also occur rapidly. These symptoms include headache, drowsiness, visual disturbances, cardiovascular collapse, convulsions followed by sudden and early respiratory and cardiac arrest.

Treatment includes prompt symptomatic measures with immediate evacuation by emesis (at home) or gastric lavage. Finely powdered charcoal can be introduced by stomach tube after lavage and within thirty minutes after ingestion of the chloroquine. This may inhibit further intestinal absorption of the Dose of the charcoal powder should be five times the estimated dose of chloroquine ingested. Convulsions should be treated before lavage.

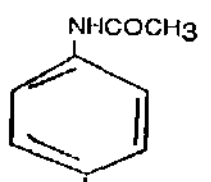
2.2 PARACETAMOL (ACETAMINOPHEN)

2.2.1 General

Due to the excessive toxicity of acetanilid its use as an antipyretic was stopped and derivatives with analgesic promises were researched for.



Acetanilide



Paracetamol

Because the antipyretic effect resides in the amino benzene structure other manipulations on this basic structure were made. Paracetamol is one of such research output.

2.2.2 Chemistry/ Properties

It is a derivative of acetanilide. Phenacetin is converted to Paracetamol in the body to elicit its action.

Introduction of other radicals into the hydroxyl group of p-aminophenol and into the free amino group of aniline reduces toxicity. It is 4_ - hydroxyacetanilide. It is a white crystalline powder, odourless and soluble in 70 parts of water. It is an analgesic but a weak anti-inflammatory agent.

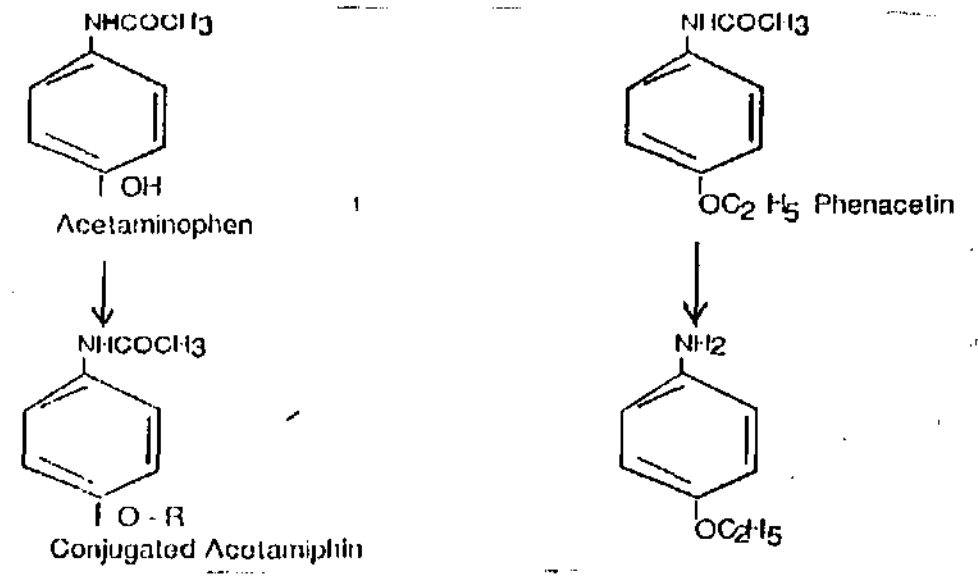
Best result is with alkyl group in the hydroxyl group as in phenacetin and/or an acid group in the amino group of aniline.

2.2.3 Absorption: This is rapid and completed from the gastrointestinal tract. Peak plasma levels is achieved within half-an hour after administration. It diffuses rapidly and uniformly into tissues.

2.2.4 Metabolism: This is by microsomal enzyme system. About 3 per cent of free paracetamol is excreted in the urine. 80% is hydroxylated in the liver.

It is capable of causing methaemoglobinemia due to metabolites probably p-aminophenol which causes red blood cells haemolysis.

Metabolic Profile



R = glucoronate (major) = 60%

= Sulphate (minor) = 35%

or Cystein (3%)

2.2.5 **Pharmacological Action**: Paracetamol is an analgesic (strong) and a weak anti-inflammatory agent. It increases the threshold of pain sensation. It is a substitute for aspirin which has some setbacks.

2.2.6 **Mechanism:** It elicits its pain relief via inhibition of release of prostalglandin in cells which are responsible for pain sensation.

2.2.7 **Overdose:** Ingestion of paracetamol in overdose is very common since it is an over-the-counter (OTC) drug. However, death due to this overdose is comparatively rare. Only 2 of 394 episodes of fatal poisoning in New Zealand between 1975 and 1982, were due to paracetamol and only 28 deaths were reported out of over 11,000 cases of paracetamol poisoning reported in U.S.A. between 1976 and 1985 (Smilkstein et al, 1988).

Hepatic necrosis usually occurs in overdose especially in patients with hepatic injury. Management of overdose (if below twenty minutes of ingestion) is by administration of finely powdered charcoal to arrest further absorption and also supportive measures.

2.2.8 ASSAY METHODS

There are different methods for the quantitative determination of paracetamol in pharmaceutical dosage forms and in biologic fluids. Colorimetric method was the earliest used for paracetamol in plasma (Brodie 1949). In this method the P-aminophenol formed by acid bydrolusis is diazotised and coupled to alpha-naphthol. The azo dye is then measured by spectrophotometric method. This method is however, non-specific and time consuming (Wallce et al, 1973).

Dose uniformity of paracetamol content in formulations by micellar electrokinetic chromatography was reported (altria et al, 1994). In this method, capsules were dissolved in 300ml of H_{20} and 50ml of methanolic 4-hydroxyacetophenone (5g/L; internal standard for Ih, the solution was diluted with water to 500ml and filtered. Analyses were performed on fused-silica capilaries at 40°C with 40mM-$N_{a2}B_4O_7$/125mMSodium lauryl sulfate as the separation electrolyte applied voltages of 12 or 15Kv and detection at 210 or 214nm was done. This was done between seven companies and each one prepared four solutions and analysed each solution in duplicate. Repeatable selectivity and acceptable precision levels were obtained by all seven participants and quantitative results agreed with the label claims of the capsules and those generated by HPLC. The used of an internal standard improved the precision.

Determination of paracetamol by first-derrivations differntial pulse pokarography was reported (Liang et al 1994). According to the method 0.3g of sample was dissolved in 500ml of ethanol before addition of 40ml of 2mM-H_2SO_4 and water

to 11. First-derivative differential pulse polarography was performed with measurement of the peak to trough distance at 0.24V versus Ag/AgCl. The response varied linearly for 0.33-3.3mM-paracetamol and the detection limit was 78nM. Excipients did not interfere. The method was used in analysis of raw pharmaceutical material and tablets, and the results are compared with those by spectrophotometry.

Boushain et al (1996) reported a method of determining paracetamol determination in pharmaceuticals by flow-injection Fourier-transform infra-red-spectrometry. In this method a portion (200mg) of the powdered sample was shaken ultrasonically with 20ml ethanol in CH_2Cl_2 (Solvent A for 20 minutes. The solution was filtered and the filtrate was made up to 25ml.

A portion (400µl)of the resulting solution was injected into a carrier stream (0.97ml/min) of solvent A and FTIR spectrum was recorded. Paracetamol (I) was determined by measuring the peak-height absorbance at 1515cm^{-1} corrected with the baseline at 1900cm^{-1}. The calibration graph was linear for 0.8mg/ml I, the detection limit was 33µg/ml and the RSD (n=5) was 0.2%. the interference from citric acid (if present as anxcipient) was eliminated by using first-derivative measurements between 1518 and 1509cm^{-1} for the quantification of I.

Lau et al (1994) reported by the estimation of paracetamol and its major metabolites in both plasma and urine by a single high-performance liquid chromatography assay. According to this method urine was collected into vessels containing CHCL3 and portions (500µl) were mixed with 50µl of 30% $HCLO_4$ and centrifuged. Portions of the plasma (≤30µl) and urine (3µl) samples were analysed by HPLC on a column (10cm x 8mm i.d.) of Nova-Pak C_{18} (5µm) with a similar Guard-Pak precolumn, 0.lM-KH_2PO_4/acetic acid/propan-2-0l (1000:1:750) as mobile phase (1.5ml/min) and detection at 254nm. The

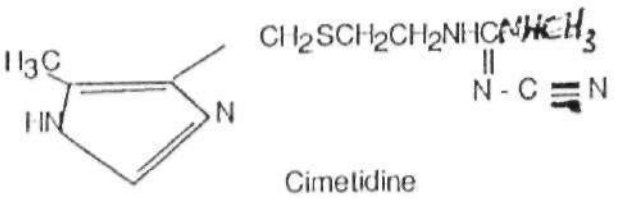
were analysed by HPLC on a column (10cm x 8mm i.d.) of Nova-Pak C_{18} (5µm) with a similar Guard-Pak precolumn, 0.lM-KH_2PO_4/acetic acid/propan-2-0l (1000:1:750) as mobile phase (1.5ml/min) and detection at 254nm. The calibration graphs for paracetamol (I) and its glucoronide, sulphate, cysteine and mercapturate metabolites in urine were linear (ranges given; 5-500µg/m/ for I), the detection limits were 1-2ng (for I) and 5ng (for metabilites) and the RSD were 1.7% for I and ≤ 16.3% for I metabolites. The calibration graphs for I and its glucuronide, sulphate and cysteine metabolites in plasma were linear (ranges given; 5-25µg/ml for I),the detection limits were similar to those in urine, the RSD for I were≤3.3% and the recoveries of I were ≥97.7%.

A microwave-induced rapid hydrolysis of paracetamol and its conjugates in urine for emergency toxicological screen was reported by Dasgupta et al (1993). According to this method, paracetamol was detected by boiling with HCl i.e hydrolysis to 4 - aminophenol which reacted with O-cresol and NH_4OH to form a distinctive indophenol blue colour. The absorbance of this was measured at 620nm. The hydrolysis was still incompletre after 10 minutes, so that hydrolysis was the time-limiting step in the procedure. Paracetamol could be rapidly hydrolysed by microwave irradiation for 2 minutes at 120 watts. Results agreed with those by conventional hydrolysis for 20 minutes.

2.3 CIMETIDINE

2.3.1 Chemistry

Cimetidine contains an imidazole ring and is chemically related to Histamine. Chemically cimetidine is N__ - cyano - N - methyl - N_ - [2 -(5-methylimidazol-4-yl)methylthioethyl] guanidine.



Cimetidine

Formula: $C_{10}H_{16}N_6S$

Molecular weight is 252.34

Melting point is 141 - 142 °C

Solubility: Cimetidine is soluble in alcohol, slightly soluble in water, very slightly soluble in chloroform and insoluble in ether.

Cimetidine hydrochloride is freely soluble in water, soluble in alcohol, very slightly soluble in chloroform and practically insoluble in ether.

It has a low protein binding capacity.

Cimetidine is a white to off-white crystalline powder, has a bitter taste and a characteristic odour.

2.3.2 **Pharmacokinetics**

Cimetidine is rapidly absorbed after oral administration and peak levels occur in 45-90 minutes.

The half-life of Cimetidine (Tagamet$^{(R)}$, SK & F) is approximately 2 hours. Both oral and parenteral (Intravenous or intramuscular) administration provide comparable periods of therapeutically effective blood levels; blood concentrations (60 - 70% bioavailable) remain above that required to provide 80% inhibition of basal gastric acid secretion for 4 - 5 hours following a dose of 300 mg.

Cimetidine crosses placental barrier and it is excreted into breast milk, the concentrations of which are believed to be higher than those in the plasma.

The principal route of excretion is the urine. Following parenteral administration, most of the drug is excreted as the parent compound; following oral administration, the drug is more extensively metabolized, the sulphoxide being the major metabolite. Following a single oral dose, 48% of the drug is recovered

from the urine after 24 hours as the parent compound. Following IV or IM administration, approximately 75% of the drug is recovered from the urine after 24 hours as the parent compound.

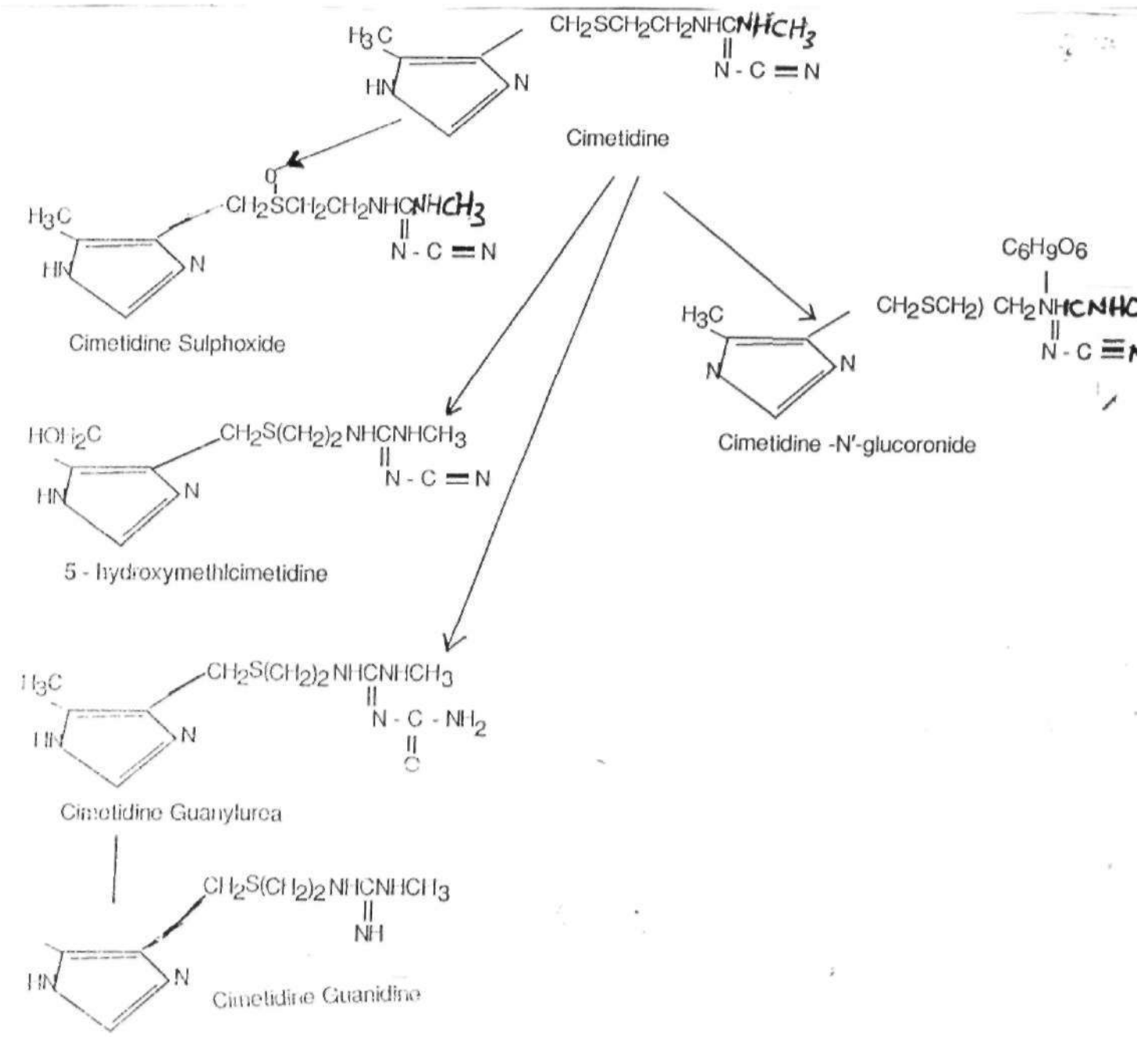


Fig. 2.32. Metabolic profile of cimetidine in man (Mitchell et al, 1982).

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2.3.3 Chemistry and Structural Activity Relationship

Cimetidine is a histamine H_2 receptor antagonist and chemical manipulations of its structure leads to several active drugs with varying structural activity relationship.

Side Chain

Separation of the ring from the N - group should be by a four carbon chain. This gives maximum antagonistic activity. Lower number of carbon in this chain lowers the activity.

N-group

For maximal antagonistic activity, the terminal N-group should be polar, non-basic substituent.

2.3.4 Pharmacological Action and Mechanism

Cimetidine competitively inhibits the action of histamine at the histamine H_2 - receptors of the parietal cells and thus is a histamine H_2 - receptor antagonist. *Thus, it regulates pH of the stomach.*

Cimetidine is not an anticholinergic agent. Cimetidine also inhibits gastric acid secretion stimulated by food, histamine, pentagastrin, caffeine and insulin.
Cimetidine (Tagamet®, SK & F) is indicated in the conditions where inhibition of gastric acid secretion is beneficial. Such cases are duodenal ulcer, gastric ulcer, oesophageal reflux, selected cases of dyspepsia and pathological hypersecretory states like *Zollinger - Ellison syndrome, systemic mastocytosis, multiple* endocrine adenomas, healing of intractable ulcers followings use of Tagamet® (SK &F).

Administration can be oral; parenteral (intravenous or intramuscular) or nasogastric.
Maximum dose is 2.4g daily individual doses.

Use of Cimetidine is contraindicated in patients hypersensitive to it and those with malignant disease.

It interacts with so many drugs, some consequences of which are hazardous while others are of unclear clinical significance.

These interactions occur through three mechanisms;

1. Since cimetidine alters pH of the stomach, the stability of acid labile drugs is increased and presumably also their bioavailability.
2. Since the principal route of excretion of cimetidine is renal via renal tubular secretion, competition with other basic drugs and compounds renally secreted occurs e.g. Triamitrene and creatinine.
3. Being an imidazole containing compound, cimetidine inhibits metabolism of compounds via cytochrome mixed-function oxidase system.

2.3.5 *Overdose and Management*

Toxic doses in animal studies were associated with respiratory failure and tachycardia. In human, this may be controlled by assisted respiration and the administration of a β-blocker.
Human experience with gross overdosage is limited. However, in the few cases which have been reported, doses up to 10 grammes have not been associated with any untoward effect.

2.4 DRUG INTERACTIONS

2.4.1 CHLOROQUINE

Chloroquine possesses a direct relaxant action on isolated guinea pig smooth muscle to prevent histamine-induced contractions and to block anaphylactic contractions of tracheal muscles (Agarwal et al, 1963).

This forms the cornerstone of all chloroquine interactions in which absorption of the affected drugs is tampered with. From the above context, chloroquine is understood to be an anticholinergic drug i.e it counteracts the parasympathetic activity of the gut. The result is a decreased gastrointestinal motility with a decrease in rate of absorbtion of drugs on concurrently administered resulting in reduced blood levels which may precipitate therapeutic failure (Cupit et al, 1980) e.g. ampicillin, and paracetamol in acute pains. Some chloroquine interactions are listed below.

2.4.1.1 Chloroquine and Ampicillin

When these two drugs are administered concurrently, the absorption of ampicillin is reduced, but that of becampicillin is not affected.

The absorption of ampicillin was found to decrease by one third (ie from 29 to 19% as
measured by its excretion in the urine) when 1g chloroquine and 1g of oral ampicillin were given to seven normal subjects (Ali, 1993).

Since these two drugs have almost the same Tmax (oral single dose) of about 2-3 hours, the most reasonable way to bypass this obstacle is to space their administration by about 2hours. During this interval, Chloroquine might have started declining due to elimination so that not much of the chloroquine is available to interact with the ampicillin. In this way the rapeutic failure can be avoided. However the best way to avoid this interaction is to give an alternative

antibiotic drug e.g. becampicillin or give lower doses of chloroquine. In this way, the effect of chloroquine on ampicillin absorption will be minimised.

2.4.1.2 **Chloroquine and Anticholinesterase**

Occasionally, some neuromuscular blocking effects are seen with chloroquine. These may be expected to worsen the symptoms of myasthenia gravis and hence oppose or shadow the effects of drugs (anticholinesterases) used in its treatment. This neuromuscular blocking effect has been seen to be additive with the effect of conventional neuromuscular blockers. One patient developed a myasthenic syndrome within a week of starting 300mg chloroquine daily which was controllable with endrophonium and disappeared within a few days of stopping the chloroquine (Robert et al 1992).

2.4.1.3 **Chloroquine and Antidiarrhoels:**

The absorption of chloroquine can be reduced by about 30% by the concurrent use of Kaolin.

Six normal subjects were given 1g chloroquine and 1g Kaolin after an overnight fast. The Kaolin reduced the Area Under the Curve (AUC) by 28.6%.

In vitro studies using some segments of rat intestine have shown that the absorption of chloroquine and pyrimethamine decreased by 46.5 and 49.9% respectively by kaolin (Mc Elnay et al 1995).

2.4.1.4 **Chloroquine and Antacids:**

The absorption of chloroquine can be reduced by about 20% by concurrent use of magnesium trisilicate. In vitro studies suggest that pyrimethamine may possibly be similarly affected (Mc Elnay et al 1991).

Six normal subjects were given 1g chloroquine with 1g magnesium trisilicate after an
overnight fast. The antacid reduced the AUC of the chloroquine by 18.2%.

Related in-vitro studies using segments of rat intestine showed that the absorption of chloroquine and pyrimethamine were decreased as follows:
Magnesium trisilicate caused absorption decréase of 31.3 and 37.5% to chloroquine and pyrimethamine respectively while calcium carbonate caused a decrease of 52.8 and 31.5% to chloroquine and pyrimethamine absorption. respectively (Mc Elnay, 1991).

In both cases of kaolin and antacids, chloroquine was absorbed onto them thereby reducing the amount available for absorption by the gut. Same applies to pyrimethamine. When this happens, it is equivalent to a decrease in the dose given. This means even the subsequent doses given will not supplement this decrease in the amount absorbed since both drugs are given in multiple doses. Eventually, a therapeutic failure results which might be associated with 'chloroquine resistance' which is becoming more rampant.

Thus, such factors of drug interactions apart from patience compliance must be thoroughly put to mind before one concludes chloroquine resistance.

2.4.1.5 **Chloroquine and Cholestyramine:**

Chlorestyramine reduces the absorption of chloroquine but the clinical importance of this is uncertain. However, it can possibly cause "apparent chloroquine resistance" since the amount of chloroquine absorbed is also reduced. This is consistent with the way cholestylamire interacts with others drugs by binding to

them in the gut. Separating the dosages by a few hours is effective in reducing or possibly avoiding this interaction.

4g cholestyramire reduced the absorption of 10mg/kg chloroquine by 30% in five children aged 6 - 13 years (Gendrel et al, 1989).

2.4.1.6 **Chloroquine and Cimetidine:**

Cimetidine reduces the metabolism and the loss of chloroquine from the body. 400mg cimetidine daily for four days approximately halved (from 0.49 to 0.23 dl/kg) the clearance of a single dose of chloroquine (600mg base) in ten normal subjects.

The elimination half-life was prolonged from 3.11 to 4.62 days (Etter, 1991)
Since cimetidine inhibits the liver microsomal enzyme activity, any drug metabolized via this route will have its metabolism and consequently loss from the body decreased. This is the case with chloroquine. It is prudent to be alert for any sign of chloroquine toxicity during concurrent administration of both drugs.

2.4.1.7 **Chloroquine and Digitalis:**

The blood levels of digoxin was found to be markedly increased in two elderly patients while they were treated with chloroquine. A similar increase has been seen with chloroquine in dogs.

Two women 65 and 68 years who have been taking digoxin (0.25 mg daily) for 2 - 3 years for heart arrhythmias were concurrently treated with chloroquine (250mg twice daily) for rheumatoid arthritis. When the chloroquine was withdrawn, the serum digoxin levels of both women fell by 70 - 75% (3.0 to 0.7n mol/litre and 3.1 to 0.9nmol/litre). Neither showed any evidence of intoxication

during concurrent use and one of them claimed an improvement in her heart rhythm regularity (Mc Elnay, 1985).

The reason for this apparent interaction is not clear. However, it would seem prudent to check on the effects of adding or withdrawing chloroquine in any patient already on digoxin.

2.4.1.8 **Chloroquine and Metronidazole:**

Acute dystonia (facial grimacing, coarse tremors, etc) was reported in a patient on metronidazole when given a single dose of chloroquine.

In a study carried out by Achumba a patient was given a 7 day course of metronidazole 400mg three times daily, following a laparoscopic investigation.

This patient developed acute dystonic reactions on day six within ten minutes of being given a single dose of chloroquine (5ml of the sulphate, equivalent to 200mg base). The patient had been on chloroquine before, without problems. The symptoms subsided within fifteen minutes of giving 5mg diazepam intravenously. Although extrapyramidal reactions of chloroquine appear to be rare, however, it is suggested that sulphadoxine/pyrimethamine (Fansidar®) should be used for prophylaxis where the patient is already on metronidazole.

2.4.1.9 **Chloroquine and Proguanil:**

An extensive study was carried out in 628 men in Belize after observing that mouth ulcers appear to be common amongst those taking antimalarial prophylactically. The volunteers were divided into two groups. One group (control) was placed on 200mg proguanil alone daily while the other was on 200mg proguanil daily plus chloroquine 150-300 mg base weekly (Drysdale 1990).

24% of those on proguanil daily, developed mouth ulcers while 37% of the second group developed ulcers. Incidence of diarrhoea was also increased from 63-82% among those with ulcers. Reason for this chloroquine-proguanil interaction is not understood. However, these two drugs should not be given *together unnecessarily for prophylaxis against P . falciparum malaria.*

2.4.1.10 **Chloroquine and Oral Contraceptives:**

Serum levels of the combined oral contraceptives are neither decreased by chloroquine nor was their reliability affected.

In a pharmacokinetic study in two groups of women (12 and 7) on low dose oral contraceptives (ethinloestradiol and norethisterone) showed that the prophylactic use of chloroquine phosphate, 500mg once a week for four weeks, caused a slight increase in blood levels of the oestrogen (AUC + 15%) but there was nothing to suggest that the normal effects of the contraceptives were changed in anyway. Chloroquine blood levels remained unaltered.

2.4.1.11 **Chloroquine and Penicillamine:**

Seidoman et al (1991), found out that penicillamine serum levels are increased by chloroquine.

Patients already on penicillamine were given chloroquine and it was found that toxicity due to penicillamine was increased in some cases. A pharmacokinetic study involving patients with rheumatoid arthritis on 250mg penicillamine daily revealed that single doses of chloroquine phosphate (250mg) increased the AUC by 34% and raised the peak serum levels by about 55% (Seidoman et al 1991).
If these two drugs must be used concurrently, then evidence of penicillamine toxicity should be monitored.

2.4.2.2 **Paracetamol with Propranolol**

Propranolol reduces hepatic blood flow, thus decreasing the formation of toxic acetaminophen (paracetamol) metabolites in patients with paracetamol overdose.

Clinical Significance: This interaction is based primarily on animal and theoretical considerations with little clinical information available. This is a favourable interaction.

2.4.2.3 **Paracetamol with Oral Anticoagulants**

Prothrombin time of the anticoagulants is increased by paracetamol. Mechanism of this interaction is not established.

Clinical significance: 2.6g paracetamol per day for two weeks given to patients on various anticoagulants resulted in a mean increase of 3.7 seconds in the prothrombin time when compared with placebo (Antlitz et al, 1968).

2.4.2.4 **Paracetamol with Food**

Paracetamol, when taken with food, has its absorption delayed. Mechanism for this may be due to the delayed entry of the drug into the intestine or it may be due to a delay in a tablet disintegration and dissolution (McGilveray, 1972).

Pectin found in the high carbohydrate meal may retard absorption by adsorption as well as through increased viscosity, and slow distribution throughout gastric fluids (Jaffe et al, 1971).

Clinical significance: Reports of the administration of 1g paracetamol with food in four adults led to a considerable reduction in the rate of absorption with little

change in the total (extent) amount absorbed. High protein, high lipid or balanced meal has little inhibitory effect on rate of paracetamol absorption.

Management: For rapid analgesia, paracetamol should not be taken with high carbohydrate meal.

2.4.2.5 **Paracetamol with Metoclopramide**

Metoclopramide increases the absorption rate of paracetamol by stimulating gastric emptying (Nimmo et al, 1973).

Clinical significance: Only rate of paracetamol absorption is increased, the extent of absorption is not affected.

Management: No precaution is necessary when these two drugs are concurrently administered.

2.4.2.6 **Paracetamol with Propantheline**

Propantheline acts directly on the smooth muscles of the gut thereby delaying gastric emptying. The rate of paracetamol absorption when the two are concurrently administered (Nimmo et al,1973) is decreased.

2.4.3 **CIMETIDINE DRUG INTERACTIONS**

2.4.3.1 **Cimetidine with Metronidazole**

Metronidazole (flagyl) [R)] serum concentrations are increased by concomitant administration of cimetidine. Cimetidine reduces the metabolic clearance of metronidazole.

Clinical significance: Pretreatment with cimetidine 800mg daily for six days in six healthy subjects, increased the half-life of an IV dose of metronidazole from 6.2hours to 7.9 hours and reduced its clearance by 29 per cent. Some patients

may develop adverse effects (vertigo or dizziness or peripheral neuropathy) from increased metronidazole serum concentrations when cimetidine is concurrently administered with it.

Management: Patients placed on metronidazole and cimetidine should be monitored for evidence of metronidazole toxicity (Phillip et al., 1989)

2.4.3.2 **General:**

Cimetidine decreases metabolism of warfarin type anticoagulants, phenytoin, propranolol, chlordiazepoxide, diazepam, certain tricyclic antipressants, lidocaine and theophylline. This leads to delayed elimination and increased blood levels of these drugs.

Management: With warfarin-type anticoagulants, monitoring of prothrombin time is recommended. With theophylline, monitoring is recommended regardless of concomitant drug therapy.

Dosage of the drugs mentioned above and other similarly metabolised drugs, particularly those with low therapeutic ration or in patients with renal and/or hepatic impairment, require adjustment when starting or stopping concomitantly administered cimetidine (Tagamet ® SK & F) to maintain therapeutic levels.

2.4.3.3 **Cimetidine with Paracetamol**

Ahmed et al. (1992) recently showed the effect of cimetidine on the LD_{50} of paracetamol in mice. Cimetidine administered intraperitonially protected the mice from liver injury.

Clinical significance: 250, 500, 750 and 1000 mg/kg of paracetamol were administered to mice. One hour later, an aqeous solution of cimetidine was administered intraperitonially. Control group received paracetamol only. The LD_{50} of paracetamol increased from 392 to 550mg/kg by the cimetidine. Cimetidine inhibited the formation of toxic metabolites of paracetamol which are responsible for hepatic toxicity.

Management: Cimetidine could be used clinically in patients intoxicated with paracetamol.

2.4.3.4 **Cimetidine with Praziquantel**

Treatment of experimental S . masoni with praziquantel alone and combined with cimetidine was carried out by Abeid et al (1994). Swiss albino mice infected with S.mansoni (100 cercariae/mouse) were used (seven week post infection). These mice were divided into two groups. First group received praziquantel 100 and 500 mg/kg for two weeks. The second group received cimetidine 50, 100, 200mg/kg for two weeks i.e. in addition to the mentioned doses of praziquantel.

Best regimen was with cimetidine and praziquantel concurrently at 100 mg/kg where the total worm load was decreased by 84.61 per cent.

AIMS AND OBJECTIVE OF THE STUDY

Malaria is already a medical household disease especially in developing countries. Chloroquine is still one of the leading drugs in the treatment of this ailment though some cases of chloroquine resistance have been reported.

Chloroquine, being an anticholinergic agent(Agarwal et al, 1963) tampers with the gut motility and gastric emptying in particular. Since this is certain to occur, it is of interest to investigate the effects of chloroquine on the absorption of other drugs with which it is frequently prescribed. This study therefore becomes essential because most patients go to hospital due to multiple ailments - malaria inclusive.

This study was therefore designed to determined whether or not the absorption of other drugs will be altered in the presence of chloroquine. A comparison is therefore designed: Chloroquine versus Paracetamol and Cimetidine versus Paracetamol. Cimetidine was chosen because it has been established to decrease the absorption rate of other drugs (Garba 1996). On the other hand Paracetamol was chosen to find out this absorption effects because it has been established to be completely absorbed from the gut in about 30minutes (Goodman, 1990).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Drugs

i. Paracetamol: Panadol®
Manufacturer: Sterling Health (Nig.) Plc
Batch Number: N15
Expiry Date: November, 2000
Manufacturing Date: November, 1997
Strength 500mg

ii. Cimetidine: Tagamet®
Manufacturer: SK & F (Nig.) Plc
Batch Number: 296460
Expiry Date: June 1990
Manufacturing Date: June 1994
Strength: 200mg

iii. Chloroquine: Nivaquine®
Manufacturer: May and Baker Nig. Plc
Batch Number: 3157/82/681
Expiry Date: 09/99
Manufacturing Date: 09/94
Strength: 200mg

3.2 Materials

3.2.1 Glass ware

-Extraction tubes
-Pipettes 0.1ml, 1ml, 5ml, 10ml, pyrex, England
-Measuring cylinders: 50ml 100ml, pyrex, England
-Conical flasks : 50ml, 25ml, pyrex, England

-Volumetric flasks : 25ml, 50ml, 250ml, pyrex, England

-Beakers:

-Sample bottles

-Stirring rod

-Plastic screw-caps

-*Burette*

3.2.2 **Equipment**

-Centrifuge Gallenkamp, England

-Pippetteman, Gilson France

-Auto-vortex mixer, Stuart, England

-Hot Air Oven BS size one, Gallenkamp, England

-Flask shaker, Gallenkamp, England

-*Electronic, Balance, Mettler AE 240*

-Disintegration Rate study Apparatus, Erweka England.

-Dissolution-Rate study Apparatus, Erweka, England

-Melting point Apparatus

-U.V. Spectrophotometer SP8-10 UV Spectrophotometer

3.3 **Reagents**

-Methanol, May and Baker, England

-Ethylacetate, May and Baker, England

-Distilled water

-Paracetamol Powder, May and Baker, England

-Hydrochloric Acid, May and Baker, England

-Potassium Dichromate, May & Baker, England

-Sodium Hydroxide, May & Baker, England

-Chloroform, Analar

-Anhydrous glacial acetic acid, Analar

-Sulphuric acid, May & Baker, England

-Barium Chloride solution

-Perchloric acid, May & Baker, England

PREPARATION OF STANDARD SAMPLES

0.1M PERCHLORIC ACID

250ml of 0.1M perchloric acid was prepared according to the method described in International Pharmacopeae (1988) volume 1, general method of analysis. To 225ml glacial acetic acid, 2.05ml of 70% w/w perchloric acid was added, mixed and to the content, 8 ml of acetic anhydride was added and thoroughly mixed. This was cooled and sufficient glacial acetic acid was added to make 250ml. This was allowed to stand for 24 hours.

The solution was then standardised with potassium hydrogen phthalate (previously dried at 120 °C for 2 hours) dissolved in acetic acid. This was kept to be used later.

0.1. SODIUM HYDROXIDE

0.1M solution of sodium hydroxide was prepared by dissolving 4.5g of sodium hydroxide pellets in sufficient distilled water to produce 1000ml solution.

POTASSIUM IODO BISMUTHATE/ACETIC ACID .

This solution used for the identification test for cimetidine tablet was prepared by dissolving 4g of potassium hydroxide in 2 ml of water. The solution was then added to another solution containing 0.085g of bismuth oxynitrate dissolved in 4ml of water and 1ml of glacial acetic acid.

DISSOLUTION/DISINTEGRATION MEDIUM

0.1M hydrochloric acid was used as the disintegration and dissolution medium. Its preparation was as follows:

34.4ml concentrated hydrochloric acid was diluted with distilled water to make up to 4000ml (4L).

3.4 IN-VITRO STUDIES

3.4.1 IDENTIFICATION TESTS

3.4.1.1 Paracetamol:

Twenty tablets were weighed crushed and powdered. A quantity of the powder corresponding to 0.5g paracetamol was extracted with acetone, filtered and the filtrate evaporated to dryness. This was dried at 105°C. The residue complied with the following tests (British Pharmacopoeia 1993).

a) 0.1g of the residue was boiled with 1ml of hydrochloric acid for 3 minutes, 10ml of distilled water was added and cooled. No precipitate was observed. 0.05ml of 0.0167M potassium dichromate was added, a violet colour was observed. This did not turn red.

3.4.1.2 Chloroquine Sulphate

A quantity of the powdered tablets corresponding to 0.1g of chloroquine sulphate was dissolved in a mixture of 10ml of water (distilled) and 1ml of 2M hydrochloric acid. It is then filtered, 1ml of barium chloride was added to the filtrate.

3.4.1.3 Cimetidine

A quantity of the powdered cimetidine tablets equivalent to 20mg was dissolved in 10ml of water. Few drops of potassium iodobismuthate/acetic acid were added.

3.4.2 ASSAY

3.4.2.1 Paracetamol tablets:

Twenty tablets were weighed and powdered. A quantity of the powder equivalent to 0.15g Paracetamol was added to 50ml of 0.1M sodium hydroxide. It was diluted to 100ml with water, shaken for fifteen minutes and sufficient water was added to produce to 200ml. It was mixed and filtered. 10ml of this solution was diluted with water to 100ml. 10ml of this new solution was added to 10 ml of 0.1M sodium hydroxide and diluted to 100ml with water and the absorbance of this new solution was measured immediately at 257nm. The content of Paracetamol was calculated taking 715 as the value of A(1%, 1cm) at the maximum at 257nm.

3.4.2.2 Chloroquine Sulphate Tablets

20 tablets were weighed and powdered. A quantity of the powder equivalent to 0.5g of chloroquine sulphate was dissolved in 20ml of 1M sodium hydroxide and extracted with four 25ml quantities of chloroform. These extracts were combined and evaporated to a volume of about 10ml. 40ml of anhydrous acetic acid was added and this was titrated against the prepared perchloric acid. Crystal violet was added as an indicator. Each ml of 0.1M perchloric acid VS is equivalent to 20.90mg of chloroquine sulphate. Content of the chloroquine sulphate was calculated at the end point.

3.4.2.3 Cimetidine

20 tablets were weighed and powdered. A powder equivalent to 200mg cimetidine was dissolved in acetic acid. A drop of crystal violet was added as indicator. This was titrated against perchloric acid. The content of cimetidine was calculated at the end point.

All assays for the three drug samples were repeated three times and mean drug content calculated.

3.4.3 DISTINTEGRATION RATE TEST.

This was performed for all the drugs -paracetamol, cimetidine and chloroquine sulphate individually six tablets were placed in six tubes (one tablet per tube) of the tablet disintegration apparatus. The apparatus was operated in accordance with Appendix XII procedure of B.P. (1993) to determine the the disintegration time of the tablet as follows:

The assembly of tubes was suspended in a beaker containing the disintegration medium (0.1MHCl) maintained at 37°C. The tubes were made to move up and down in the medium to ensure a constant agitation. The time taken for all the particles of the disintegrated tablets to pass freely through the mesh at the lower end of the tubes is usually considered to be the disintegration rate time.

The procedure was repeated. Results are presented in chapter 4.

3.4.4 Dissolution Rate Test

The rotary basket method described in B.P.(1993), using 0.1M HCl as the dissolution medium was adopted.

One tablet of paracetamol was placed in the basket and the assembly was placed in the flask containing 1000ml of 0.1M HCl, warmed and maintained between 36.5 °C and 37.5°C using an electric heater. The stirrer was set at 100 r.p.m and ran for 45 minutes. Half way into the basket medium, sample of the medium was withdrawn, filtered and assayed using U.V. Spectrophotometer at wavelength of 257nm, the procedure was repeated thrice and average determined.

Same procedure was done for each drug tablet (cimetidine and chloroquine sulphate) and readings were taken at and 344nm respectively.

For chloroquine sulphate A (1%, 1cm) at 344 nm was 450

For Paracetamol A (1%, 1cm) at 257nm was 715.

A (1%, 1cm) is the absorbance of a one percent (1%) solution of a drug in a 1 cm cell.

$$\% \text{ sample released} = \frac{A \text{ (sample)} \times 100}{\text{Conc (sample)} \times A \text{ (1\% 1cm)}}$$

3.4.5 CONSTRUCTION AND VALIDATION OF CALIBRATION CURVE

3.4.5.1 Preparation of Paracetamol Stock Solution

100mg of pure paracetamol powder was dissolved in methanol in a volumetric flask. The volume was made to 100ml with sufficient methanol. This was labelled as SS.

100ml SS contains 100,000µg paracetamol

∴ Xml SS will contain 10µg paracetamol

$$= \frac{100\text{ml} \times 10\mu\text{g}}{100{,}000\mu\text{g}}$$

= 0.01 ml SS contains 10µg paracetamol

This volumetric flask was stoppered and kept in a refrigerator.

Sufficient saliva was collected in a 50ml beaker after rinsing the mouth adequately with water. Using a 10 ml pipette, 2 ml saliva was transferred into each of the previously washed and dried quick fit centrifuge tubes (12).

5ml ethylacetate was added to each of the first two tubes. They were stoppered and mixed for 2 minutes using a rotamixer. They were then centrifuged at 2500 r.p.m for 5 minutes. The upper layer was removed using a pasteur pipette and put in two sample bottles, one for each sample. These two sample bottles were labelled blank and they were kept in a refrigerator.

0.02 ml of SS were added to each of the next two tubes, 5 ml of ethylacetate was added to each of them and same procedure was conducted.

Increasing volumes of SS were added to the rest of the tubes in pairs as indicated, same procedure was carried out for all the tubes.

Table 3.4.5.1 Volume of Stock Solution of Paracetamol (SS) Spiked into Saliva Samples and the Concentration for the Calibration Curve.

SAMPLE SERIAL NO.	VOL. OF SS SPIKED TO 2ML SALIVA (ML)	CONC. OF PARACETAMOL OBTAINED IN SALIVA SAMPLE (µg/ml)
1	0	0.00
2	0	0.00
3	0.02	10.00
4	0.02	10.00
5	0.04	20.00
6	0.04	20.00
7	0.06	30.00
8	0.06	30.00
9	0.08	40.00
10	0.08	40.00
11	0.10	50.00
12	0.10	50.00

The volumes of SS to be spiked into 2 ml saliva to obtain the desired concentrations (10, 20, 30 µg/ml etc) were calculated from the following equation.

$C_1 V_1 = C_2 V_2$

C_1 = concentration of SS (1000µg/ml)

V_1 = volume of SS to be spiked (unknown)

C_2 = concentration ranges of paracetamol in the saliva samples (10, 20, 30 etc)

V_2 = volume of saliva (2ml)

The upper layers (ethylacetate) removed from each sample had their absorbance measured individually at 262nm using 1cm silica cuvettes and a double beam SP8-10u.v. spectrophotometer. The absorbance for the blank saliva samples were then subtracted from those containing the stock solution (SS). The calibration curve was plotted using mean absorbance (Y-axis) against corresponding concentrations of paracetamol (X-axis).

3.4.5.2 Validation of the Calibration Curve.

In order to ensure that this constructed curve can be used to determine several concentrations of paracetamol in the subsequent invivo phase of the work, the following procedure was carried out.

Sufficient quantity of saliva was collected in a clean beaker. Using an autopipette, 2ml saliva was put in each of 8 clean and dried centrifuge tubes. Two were left as blanks while the remaining six were spiked with different volumes of SS giving different concentrations (5,10,15,20,25 and 30 µg/ml saliva).

Table 3.4.5.2: Volumes of Paracetamol Stock Solution (SS) spiked into saliva samples

and the concentrations obtained

SAMPLE SERIAL NO.	VOL. OF SS SPIKED TO 2ML SALIVA (ml)	CONC. OF PARACETAMOL OBTAINED IN SALIVA SAMPLE (µg/ml))
1	0	0
2	0	0
3	0.01	5
4	0.02	10
5	0.03	15
6	0.04	20
7	0.05	25
8	0.06	30

Each sample was then extracted with ethylacetate and the ethylacetate layer removed, was analysed using SP 8-10 U.V. spectrophotometer.

Absorbance readings obtained were then converted directly to the corresponding concentrations of paracetamol using the constructed calibration curve. Actual concentrations were compared with the estimated ones. Results are in chapter four.

3.6: *INVIVO PHARMACOKINETIC STUDIES*

3.6.1 Protocol of Study

Volunteers for the study were healthy and non-smoking. They were abstained from all types of drug taking, alcohol inclusive for 1 week before commencement. The study will be in four phases. For each phase, volunteers will have to fast overnight and abstain from eating throughout course of the phase.

Phase I: Six volunteers were given 1g of paracetamol tablets with plenty of water. Saliva samples were collected from each volunteer. 2ml saliva sample was put into centrifuge tubes. They were extracted using the extraction method adopted and analysed using SP 8-10 U.V. spectrophotometer

Phase II: A wash-out period of one week was allowed before commencement of this phase. This phase involved concurrent administration of 1g paracetamol and 400mg cimetidine to the volunteers with plenty of water. Saliva samples were collected and treated as above.

Phase III: A wash-out period of one week was allowed to elapse. Then 400mg cimetidine was administered to each volunter. One hour later, 1g paracetamol was administered to each volunteer. Saliva samples were collected from each volunteer, extracted and analysed as above.

Phase IV: A wash-out period of one week was allowed before this phase starts, and 0.8g of chloroquine sulphate was administered to each volunter. One and half hours was allowed and 1g of paracetamol was administered to each volunteer. Saliva samples were collected, extracted, and analysed as above.

3.6.2 **Extraction Method and Analysis**

Bello's (1990) method was adopted for the extraction and analysis of the paracetamol. 2ml saliva was placed in each tube and 5 ml ethylacetate was added and mixed (vortex mixer) for 1 minute and centrifuged at 2500 rpm for 5 minutes. The ethylacetate (upper layer) was removed using pasteur pipette and absorbance measured at 262nm using 1cm silica cuvettes and a double beam SP8-10 U.V. spectrophotometer.

All other saliva samples were treated as such and absorbance recorded.

3.6.3 Data Handling

The values for the concentration of paracetamol in saliva samples were plotted against time on a logarithmic scale. The method of residuals was then used to determine the following pharmacokinetic parameters, using saliva concentration-time curves as base data: Lag time, Absorption and elimination half-lives ($t_{½}ab$ and $t_{½}el$ respectively), absorption and elimination constants (Kab and Kel respectively).

The remaining pharmacokinetic parameters were determined as follows:

(i) AUC (from time 0 to 6 hour) was calculated using the triangular - trapezoidal method

(ii) AUC (from time 0 to infinity - $AUC_{0-\infty}$)

was calculated as follow:

$AUC\ (6-\infty) = AUC_{(0-6hr)} + AUC_{(6hr-\infty)}$

$$AUC\ (0-\infty) = \frac{\text{Conc. at 6 hour}}{\text{Kel}}$$

(iii) Volume of distribution (Vd) = $\frac{FD}{AUC.Kel}$

(iv) Clearance (Cl) = Vd.Kel = $\frac{0.693 \times Vd}{t_{½}\ el}$

AUC = Area under the salivary conc. time curve

D= Dose of paracetamol ingested.

The mean pharmacokinetic parameters for the study were then calculated from the results obtained in the six subjects.

This was done for all the 4 phases of the study.

The mean pharmacokinetic parameters were analysed statistically using the student t-test of paired data.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 QUALITY CONTROL ASSESSMENT

The quality control assessment carried out for paracetamol chloroquine and cimetidine.

Conformed with B.P. (1993) requirement (table 4.1.1). The identification tests are based on colour reaction. Paracetamol gave a violet colour with 0.0167M potassium dichromate solution and a melting point of about 168-170°C. Chloroquine gave a white precipate on addition of barium chloride solution Cimetidine gave an orange precipitate on addition of potassium iodobismuthate/acetic acid solution and when ignited under a lead nitrate paper, the vapour evolved, darkened the paper (WHO, 1991).

To be assured of accurate administration of doses during the study, test for weight uniformity was performed. Mean weights for paracetamol, chloroquine and cimetidine tablets were found out to be 0.589g, 0.190g and 0.272g. The maximum percentage deviations of a tablet individual weight were, 1.01, 0.29 and 1.84% for paracetamol, chloroquine and cimetidine respectively. All the tablet samples have passed the test.

Table 4.1 **QUALITY CONTROL ASSESSMENT RESULTS OF PARACETAMOL CHLOROQUINE AND CIMETIDINE SAMPLES**

QUALITY ASSESSMENT	PARACETAMOL	CHLOROQUINE	CIMETIDINE	REMARK
1. Identification Test	POSITIVE	POSITIVE	POSITIVE	PASS
2. Weight Uniformity(g) Mean (± M.P.D.)	0.589(1.01)	0.190 (0.29)	0.272(1.84)	PASS
3. FRIABILITY Mean (±SEM) %Friability after 100 falls	0.520	0.291	0.450	PASS
4.ASSAY FOR CONTENT Mean (± S.E.M) % Content	99.90(0.522)	99.50(0.10)	100.5(0.06)	PASS
5. DISINTEGRATION RATE, Mean (± S.E.M) (minutes)	3.38(0.422)	6.02(0.270)	5.13(0.362)	PASS
6. DISSOLUTION RATE Mean (±S.E.M) % released after 45 minutes	88(0.513)	85(0.721)	89(0.320)	PASS

Key:

S.E.M = Standard Error of mean

M.P.D. = Maximum percentage deviation of tablet weight from the mean weight

B.P.(1993) states that for tablets of average weight of 250mg or more to pass the test, not more than 2 of the individual weight deviates from mean weight by more than 5% and, none deviates by more than twice that percentage. From this, the samples passed the uniformity of weight test.

Friability test, though less important as a quality assessment in bioanalytical study, was performed with a friabilator. The tablets were subjected to self

abrasion as the cylinder section rotates, and shock as they fall 6 inches on each turn of the friabilator. This was to verify the ability of the tablets to resist shock and frictional forces that most frequently cause tablets to chip, cap or break during handling. Weight loss after 100 falls in 4 minutes (friability) for paracetamol, cimetidine and chloroquine were 0.520g, 0.450g and 0.291 percentages respectively. This test is considered satisfactory when the sample exhibits a weight loss of less than 0.8% (Gunsel et al, 1970).

The B.P. (1993) requirement to pass the test for assay for content of active ingredient is 95-105% of the stated amount. All the three samples passed this criterium.

Disintegration rate tests were satisfactory (3.38, 6.02 and 5.13 minutes for paracetamol, chloroquine and cimetidine respectively). 15 minutes or less is the period specified by B.P. (1993) for a tablet to disintegrate. This test ensures that the tablets disintegrate within a reasonable time in the G.I.T even though not all the physiologic conditions are fully simulated (Gunsel et al, 1970).

The official requirement to pass dissolution test is 70% (or above) of the stated active ingredient to be released after 45 minutes. All the tablet samples passed this test. 0.1M HCl was used as the medium for both disintegration and dissolution rate studies.

4.2 CONSTRUCTION AND VALIDATION OF CALIBRATION CURVE

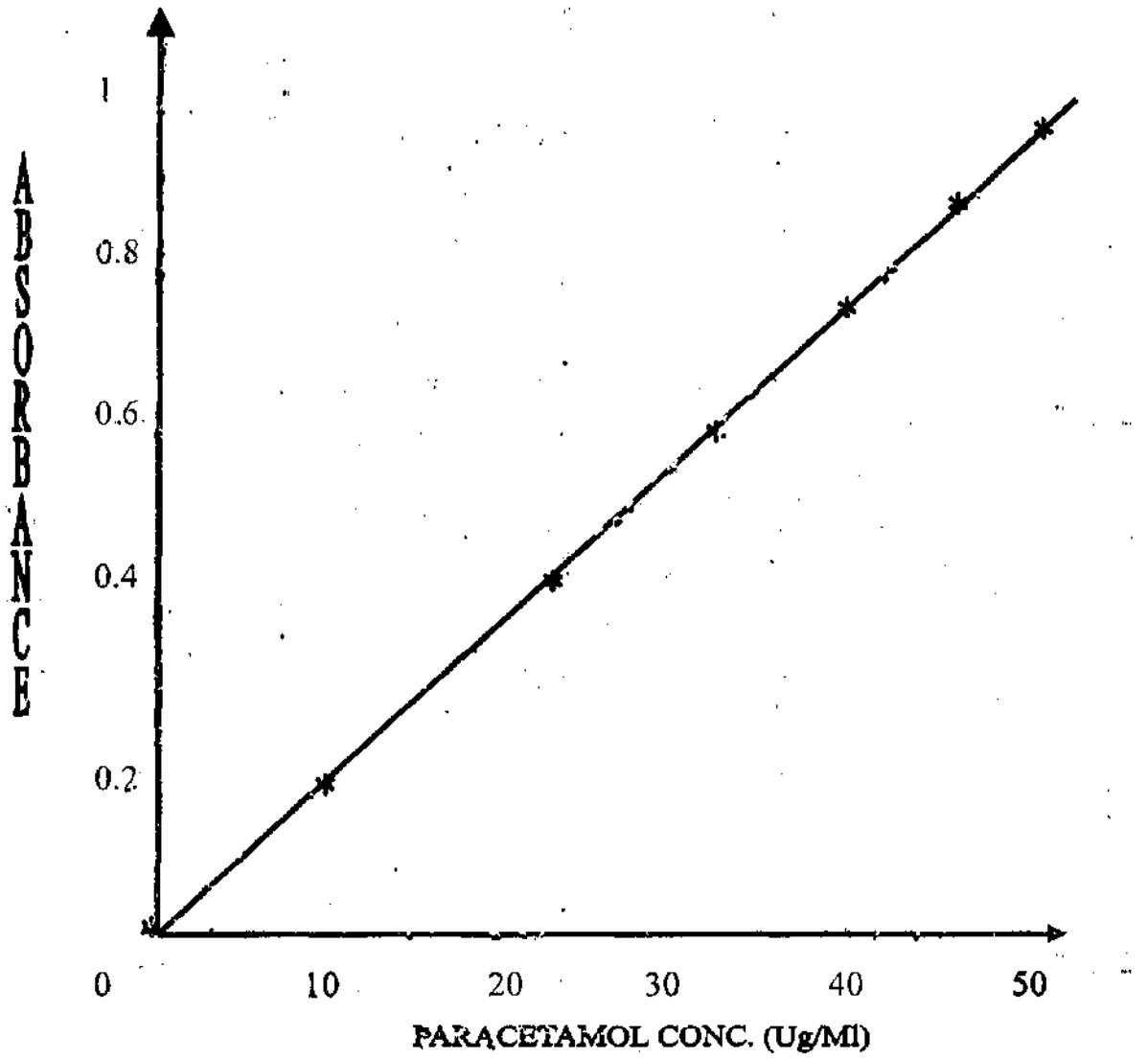
4.2.1 *Calibration Curve*

Linear calibration curve, with a good correlation coefficient (r = 0.9888) of paracetamol standard powder in ethyl acetate using SP8-10 U.V. spectrophotometer (λ max = 262 nm) is shown in figure 4.2.1., Table 4.2.1 shows the data obtained for this calibration curve.

Table 4.2.1: Mean data obtained for the construction of Calibration Curve

CONC. OF PCM/ML OF SALIVA (X)	TOTAL MEAN ABSORBANCE (Y)	STANDARD DEVIATION (SD)	ABSORBANCE MINUS BLANK (Y)
0.00 µg/ml	0.000		
10.00 µg/ml	0.210	0.06101	0.210
20.00 µg/ml	0.480	0.02700	0.480
30.00 µ g/ml	0.670	0.06340	0.670
40.00 µ g/ml	0.900	0.05216	0.900
50.00 µ g/ml	1.000	0.04101	1.181

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4.2.1 Calibration Curve for the Analysis of PCM Concentrations in Saliva

4.2.2 **Validation of the Calibration Curve**

Table 4.2.1. shows the data obtained for the validation of the calibration curve. These results compare well with those obtained by Bala (1998).

Table 4.2.2. Data obtained for validation of the Calibration Curve.

SAMPLE SERIAL NO.	**VOL. OF STOCK SOLUTION ADDED TO 2 ml SALIVA (ml)**	**ABSORBANCE**	**EXPECTED CONC. (A) ug/ml**	**ESTIMATED CONC. (B) FROM CURVE (Ug/ml**
1	**0**	0	**0**	0
2	**0**	0	**0**	0
3	**0.01**	0.101	**5**	5.1
4	0.02	0.198	**10**	9.4
5	0.03	0.350	**15'**	16.0
6	**0.04**	0.462	**20**	20.3
7	0.05	0.560	**25**	25.3
8	0.06	0:672	**30**	30:0

Due to the recovery losses and the variability encountered in measurements in biological fluids, construction of a standard (calibration) curve becomes essential in such bioassays. In analyzing a drug or a metabolite, the same medium e.g. saliva, plasma etc in which the bioassay is going to be carried out, must be used to construct the calibration curve.

Such a curve consists of a line depicting a relationship between an independent variable X (usually drug concentration) and a dependent one Y (resulting from

X). The results obtained for the validation, showed a good correlation between the spiked concentrations and the ones estimated from the calibration curve. The results compare well with those obtained by Bukhari (1996). Calculated relative recoveries are presented in table 4.2.2.2. The range of the values is an indication for accuracy in quantitative determination of paracetamol in saliva samples in a concentration range of the calibration curve. The relative recovery is the closeness of the actual concentration to that obtained from the curve.

Table 4.2.2.2. Percent Recovery of Analytical Method as Calculated after reference to the Calibration Curve (% relative recovery)

SPIKED CONC. (µg/ml	ESTIMATED CONC. FROM CALIBRATION CURVE (µg/ml)	PERCENT RELATIVE RECOVERY
5	5.10	102
10	9.40	94.00
15	16.00	106.00
20	20.30	101.00
25	25.60	102.00
30	30.00	100.00

4.3 IN-VIVO PHARMACOKINETIC STUDIES

The pharmacokinetic parameters of a single oral dose of paracetamol (1g) were studied under the influence of cimetidine using two study protocols i.e. delayed administration of paracetamol and concomitant administration of both drugs.

These parameters of paracetamol were also investigated when chloroquine was given one and a half hours after chloroquine.

Table 4.3.1 shows the mean salivary concentration - time data for paracetamol alone i.e. the control, for the influence of cimetidine on paracetamol (concomitant and delayed administration of paracetamol) and also that of chloroquine (delayed administration).

Six healthy volunteers were employed for each of the four phases of the study.

4.3.1 SINGLE DOSE SALIVARY LEVELS OF PARACETAMOL

The salivary levels of paracetamol alone was studied in six healthy volunteers in fasting state. The table below shows the mean salivary levels of paracetamol following 1g paracetamol tablets.

Table 4.3.1(i) Mean Salivary Concentration - time data in 6 healthy volunteers following oral administration of 1g of paracetamol (Control) in fasting state.

TIME (HOUR)	PARACETAMOL CONCENTRATION µg/ml Mean (± S.E.M)
0.00	0.00
0.25	10.00 ± 0.47
0.50	20.00 ± 1.12
1.00	18.00 ± 0.73
2.00	17.50 ± 0.50
3.00	13.00 ± 0.26
4.00	10.00 ± 0.14
5.00	8.50 ± 0.11
6.00	4.60 ± 0.04

Table 4.3.1(ii) Mean Salivary concentration-time data in 6 healthy volunteers following oral administration of 1g of paracetamol and 400mg of cimetidine (concurrent and delayed adm)

TIME (HOUR)	PARACETAMOL CONCENTRATION *µg/ml MEAN ± S.E.M*	
	CONCURRENT ADM.	DELAYED ADM.
0.00	0.00	0.00
0.25	10.40 ± 0.55	7.20 ± 0.20
0.50	18.00 ± 1.08	9.00 ± 0.37
1.00	15.00 ± 0.71	11.50 ± 0.44
2.00	16.00 ± 0.51	11.00 ± 0.40
3.00	12.00 ± 0.23	10.30 ± 0.35
4.00	11.00 ± 0.12	9.40 ± 0.32
5.00	9.00 ± 0.10	9.00 ± 0.21
6.00	6.00 ± 0.05	7.60 ± 0.16

Table 4.3.1(iii)Mean Salivary Concentration - time data in 6 healthy volunteers following oral administration of 1g of paracetamol one and half hours after a dose of chloroquine sulphate 0.8g in fasting state

TIME (HOUR	DELAYED TREATMENT Paracetamol conc. µg/ml Mean (± S.E.M)
0.00	0.00
0.25	8.20 ± 0.23
0.50	13.00 ± 0.39
1.00	12.00 ± 0.48
2.00	11.20 ± 0.42
3.00	9.20 ± 0.38
4.00	7.30 ± 0.27
5.00	5.60 ± 0.194
6.00	4.00 ± 0.130

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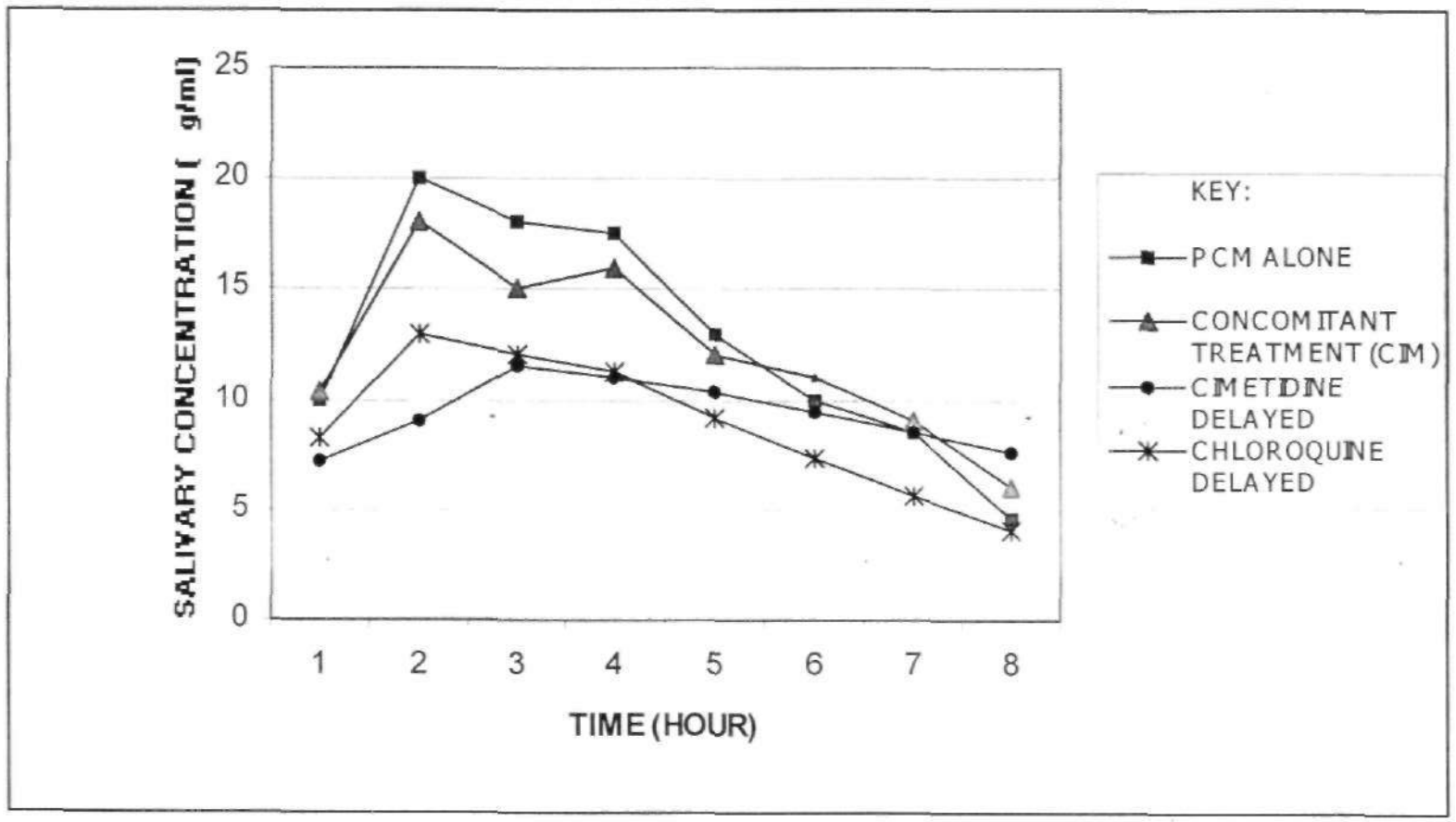


FIGURE 4.3.1 Mean Salivary Concentration – time curves for PCM alone (Control) and PCM in the other three study protocols

Table 4.3.1(iv)**Mean salivary pharmacokinetic parameters of paracetamol following 1g paracetamol in six healthy individuals.**

S/N	PARAMETER (UNIT)	MEAN ±S.E.M
1	C_{max} (µg/ml)	20.00 ±2.90
2	T_{max} (hr)	0.50 ±0.12
3	$T_{½ab}$ (hr)	0.270 ±0.03
4	K_{ab} (hr^{-1})	2.566 ±0.23
5	$T_{½el}$ (hr)	2.50 ±0.08
6	K_{el} (hr^{-1})	0.2772 ±0.13
7	V_d (L)	51.535 ±2.90
8	$Clearance_t$ (L/hr)	14.280 ±1.24
9	Lag time (hr)	0.15 ±0.03
10	$AUC_{(0-6hr)}$(µg.hr/ml)	70.00 ±4.31

The Cmax i.e. peak salivary concentration, was found to be 20µg/ml. This agreed with the one reported (20.0µg/ml) in USP (1986) using saliva samples. However, this value is lower than the 30.00 µg/ml reported by Garba et al, (1997) using plasma levels. Bello (1990) reported a Cmax of 15.29µg/ml and Bala (1998) reported a Cmax of 22.96 µg/ml both using saliva levels. These two values are close to the Cmax in this study. Tmax i.e. the time to reach maximum concentration, was 0.50hour which is lower than that of Bukhari (1996) and Bala (1998) which were 0.88hour and 0.8438 hour respectively. However, it is within the range reported by Ameer et al (1977) and most literatures e.g. Goodman et al (1990), which is 0.6 -1hour.

The absorption half life t ½ab was found to be 0,30 hour with the corresponding absorption rate constant (Kab) of 2.31 hour $^{-1}$. These are in agreement with those of Bukhari (1996) i.e. 0.3 hour and 2.66 hour $^{-1}$. Lag time i.e. the time taken before appreciable amount of the drug is detected in the plasma was 0.15 hour which is exactly the one reported by Garba et al (1997) and close to the one reported by Bala (1998) i.e. 0.140 hour.

Elimination half life (t½el) was found to be 2.5hour while the elimination rate constant was 0.2772. These closely resemble the t ½el reported by Bello (1990) and Bukhari (1996) i.e. 2.4 hour was 2.69 hour respectively. Both used saliva levels. Volume of distribution (Vd) was 51.535. While the clearance was 14.28L/hr. Bello (1990) reported a Vd of 43.012L and a clearance of 12.07L/hr using saliva levels. The clearance in this study and that of Bello (1990) was lower than the 18.186 L/hr reported by Bala (1998). The area under the curve (AUC) from time O to six hours was 70 µg.hr/ml and it is close to the 77.497 µg.hr/ml reported by Bello (1990). However, that reported by Bukhari (1996) i.e. 90.750 µg.hr/ml is higher and that of Bala (1998) i.e. 57.7367 µg.hr/ml is lower than the one in this study. AUC from time zero to infinity was 98.00 µg.hr/ml and it is less than the 128.98 µg.hr/ml reported by Bukhari (1996).

There is considerable variation in the values of pharmacokinetic parameters of drugs reported in the literature. The extensive studies (Cummings 1967, Thomas 1993) on paracetamol pharmacokinetics revealed that there are individual variations in absorption and disposition of the drug in subjects given the same dose. These variations have been clearly demonstrated in this study and those of Bello (1990), Bukhari (1996) and Bala (1998). These individual variations may be attributed to individual differences in the rate of gastric emptying (Heading et al, 1973). Physical and environmental factors such as sex, age, body weight, smoking, diet

and disease also affect metabolism and response of a drug and ultimately affect the drug's pharmacokinetics. For example deficiency in protein (can be present in any of the volunteers) results in unavailability of source materials for formation of metabolic enzymes. The rate of the drug metabolism will ultimately be decreased. The resultant higher amount of the drug will definitely affect the pharmacokinetics of the drug especially clearance, Vd, AUC, $t_{½}$ el and Kel (Booyd et al, 1969). Spices and oils inflame the gastrointestinal membrane thereby increasing permeability to drugs i.e. more of the drug will be absorbed. This means both rate and extent of absorption will be affected and hence t½ab, Kab and AUC will also be affected. (Milne 1965). The environment around a volunteer or patient or experimental animal prior to, during or after drug administration has also been shown in some circumstance to have significant impact on drug absorption (Frederick 1972).

4.3.2 INFLUENCE OF CIMETIDINE AND CHLOROQUINE ON THE SALIVARY PHARMACOKINETICS OF PARACETAMOL

Table 4.3.2(i) Influence of cimetidine on the salivary pharmacokinetics of paracetamol in six healthy volunteers (concomitant adm.) (± S.E.M)

PARAMETER (UNIT)	CONTROL (PCM ALONE)	CONCOMITANT TREATMENT	CHANGE(%)	P-VALUE
C_{max} (µg/ml)	20.00 (2.90)	18.00 (2.26)	10.0	P>0.100
t_{max} (hr)	0.50(0.12)	0.50 (0.08)		"
$t_{½ab}$(hr)	0.270(0.03)	0.272 (0.04)	0.74	"
k_{ab}(hr^{-1})	2.566(0.23)	2.547 (0.070)	-0.74	
$t_{½el}$(hr)	2.50(0.08)	2.60 (0.03)	4.00	"
k_{el} (hr^{-1})	0.2772 (0.13)	0.2665 (0.02)	-3.86	"
V_d (L)	51.535(2.90)	51.406 (3.01)	-0.25	"
C_l (L/hr)	14.280(1.24)	13.69 (1.30)	-4.13	"
Lag time (hr)	0.150(0.03)	0.166 (0.01)	10.66	"
AUC $_{0\text{-}6h}$ (µg.hr/ml)	70.0(4.31)	73.00 (3.92)	4.28	"
AUC $_{0\text{-}\infty}$ (µg.hr/ml)	98.0	100.39 (4.38)	2.44	"

S.E.M - STANDARD ERROR OF MEAN.

Table 4.3.2(ii) Influence of cimetidine on the salivary pharmacokinetics of paracetamol in six healthy volunteers (Delayed administration of paracetamol). (± S.E.M)

PARAMETER (UNIT)	CONTROL	DELAYED TREATMENT	CHANGE(%)	P-VALUE
C_{max} (µg/ml)	20.00	11.50 (2.24)	-42.50	P<0.05
t_{max} (hr)	0.50	1.00 (0.18)	100.00	"
$t_{½ab}$(hr)	0.27	0.90 (0.09)	233.00	"
$k_{ab}(hr^{-1})$	2.566	0.77 (0.27)	-69.99	"
$t_{½el}$(hr)	2.50	3.00 (0.02)	20.00	"
$k_{el}(hr^{-1})$	0.2772	0.231(0.008)	-16.66	"
V_d (L)	51.535	47.600 (3.12)	-7.64	P>0.10
Cl (L/hr)	14.280	10.995 (1.70)	-23.00	P<0.05
Lag time (hr)	0.150	0.216 (0.03)	44.00	P<0.05
AUC $_{0\text{-}6h}$ (µg.hr/ml)	70.0	90.945(5.18)	29.92	"
AUC $_{0\text{-}\infty}$ (µg.hr/ml)	98.0	484.64(3.14)	494.5	"

Administration of paracetamol an hour after cimetidine brought about significant changes in paracetamol pharmacokinetic parameters (Table 4.3.2.2)

Lag time increased by 44 percent while Bala (1998) reported an increase of 66.71% AUC $_{0\text{-}6hr}$ and AUC $_{0\text{-}\infty}$ increased by 29.92% and 494.5%, respectively.

Bala (1998) reported an increase of 18.70% in the AUC $_{0\text{-}6hr}$. Cmax decreased by 42.50% while Bala (1998) reported a decrease of 36.50%. Tmax increased significantly by 100% ($P<0.05$) while that of Bala (1998) was 62.95%. T½ab significantly ($P<0.05$) increased by 233% with the corresponding significant ($P<0.05$) decrease of 69.99% in the Kab. Bala (1998) reported an increase of 56.48% and a decrease of 32.40% in T½ab and Kab respectively. T½el increased by 20.00% with a corresponding decrease of 16.60% in the Kel. The increase in the T½el (26%) tallies with the observed increase in the AUC $_{0\text{-}hr}$ (29.92%). $AUC_{0-\infty}$ increased significantly ($P<0.05$) by 494.5% while Vd insignificantly ($P>0.10$) decreased by 7.64%. Clearance was reduced by 23.00% and Bala (1998) reported a reduction of 15.33%.

Cimetidine being an imidazole - containing compound, inhibits metabolism of compounds via cytochrome mixed function oxidase system. The net effect will be more of the inhibited drug in the body. This has been indicated in this study by the increase in the AUC. Only metabolised form of the drug (paracetamol) is cleared, and since this metabolism is inhited to some extent, the clearance of paracetamol will be decreased. This was shown in this study. The reduction was 23%. This metabolic inhibition of cimetidine was also illustrated by the increase (26%) in T½el. T½el is the time taken to reduce the plasma drug level by half, and since the metabolites to be eliminated were reduced (due to the metabolic inhibition), definitely this elimination had to be slowed down with a corresponding increase in the T½el. This was also illustrated by the decrease in the elimination rate constant Kel (16.66%).

Cimetidine, being an antihistamine inhibits gastric acid secretion thereby raising pH.

Paracetamol being a weak acid will be dissociated more in the acid and since this is now less in the presence of Cimetidine, paracetamol will have more of it in the

undissociated form which will not be readily absorbed. This effect was coupled with the inhibitory effect of cimetidine on the rate of gastric emptying (major effect) resulting in a very significant decrease (42.50%) in the Cmax i.e. maximum plasma drug concentration. This inhibitory effect on the rate of gastric emptying is not through anticholinergic effect, since cimetidine does not act directly on the smooth muscle (Goodman 1990). Therefore another mechanism of action through which cimetidine decreases rate of gastric emptying should be investigated in further studies. This effect on the rate of gastric emptying brought a change in the rate of absorption of paracetamol as indicated by the decreased Cmax, increased T½ab (time taken for half the administered dose of the drug to be absorbed), increased Tmax i.e. time to reach maximum concentration in the plasma and also the decrease in the absorption rate constant (Kab).

Even though Kwanashie et al (1992) showed that cimetidine at higher concentrations (500 to 1000 µg/ml) caused relaxation of the guinea pig ileum still this does not explain the mechanism for the decreased gastric emptying rate. This is because the usual doses of cimetidine do not give the plasma concentrations attributable to the observed relaxation of the ileum.

The reported lack of effect of cimetidine on paracetamol kinetics by Slattery et al. (1989) must be due to the protocol adopted. If the two drugs were concurrently administered, then the changes in the paracetamol kinetics would be insignificant as indicated in the second phase of this study (concurrent administration). This was so because for any interaction between two or more drugs to occur, an appreciable concentration of the two must be in the plasma at the same time. When the two drugs (cimetidine and paracetamol) were administered concurrently, paracetamol absorption was almost complete and so was its metabolism before cimetidine reached its maximum concentration in the plasma. The Tmax for paracetamol and cimetidine are 0.50hr and 1-2 hrs, respectively.

By the time cimetidine reached Cmax, paracetamol level to be acted upon by cimetidine was very insignificant to bring about any profound changes in the kinetics of paracetamol. To affect these kinetics of paracetamol, paracetamol was given one hour after cimetidine.

Cimetidine undergoes renal tubular secretion via the renal cationic transport system. This will lead to competition with other basic compounds renally secreted e.g. triamterene, etc (Samogyi, 1989). So these drugs will have more of these drugs in the plasma since cimetidine is being excreted at their expense.

Heading et al (1973) showed that paracetamol absorption is related to gastric emptying and this was confirmed by the studies of Nimmo et al (1973) and Ayuba (1998) who showed that paracetamol absorption was decreased by propantheline - a drug which slows down gastric motility and consequently gastric emptying. This effect was seen in this study via the reduced Cmax and the increased T½ab and Tmax. Similar observations were reported by Garba et al (1997).

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Table 4.3.2(iii) Influence of choloquine on the salivary pharmacokinetics of paracetamol in six healthy volunteers (delayed administration of paracetamol) (± S.E.M.)

PARAMETER (UNIT)	CONTROL	CHLOROQUINE DELAYED	CHANGE(%)	P-VALUE
C_{max} (µg/ml)	20.00	15.00 (2.60)	-25.00	P<0.05
t_{max} (hr)	0.50	1.00 (0.17)	100.00	"
$t_{½ab}$(hr)	0.27	0.82 (0.60)	203.00	"
k_{ab}(hr $^{-1}$)	2.566	0.845 (0.35)	-67.06	"
$t_{½el}$(hr)	2.50	2.40 (0.25)	-4.00	P>0.10
k_{el}(hr $^{-1}$)	0.2772	0.2828(0.03)	2.02	"
V_d (L)	51.535	53.041(2.80)	2.92	"
C_l (L/hr)	14.280	15.00 (1.62)	5.04	P>0.10
Lag time (hr)	0.150	0.192 (0.02)	28.00	P<0.05
AUC $_{0-6h}$ (µg.hr/ml)	70.0	66.66(4.21)	-4.77	p>0.10
AUC $_{0-\infty}$ (µg.hr/ml)	98.0	302.406	208.57	P<0.05

When chloroquine was given one and half hours before paracetamol, the kinetics of paracetamol were affected Concurrent administration was by passed because the Tmax for chloroquine is even higher (2-3hrs) than that of cimetidine (1-2hr). The insignificant changes observed with concurrent administration of cimetidine and paracetamol would not even manifest in concurrent administration of chloroquine and paracetamol.

Cmax of paracetamol significantly (P<0.05) changed from 20.0µg/ml to 15.0µg/ml (25% decrease). Tmax significantly (P<0.05) increased by 100%.

T½ab increased significantly (P<0.05) by 203% with a corresponding significant (P<0.05) decrease of 67% in the Kab. T½el was insignificantly (P>0.1) decreased by 4% with a corresponding insignificant (P>0.1) increase of 2.02% in the Kel (elimination rate constant). Vd (apparent volume of distribution) insignificantly (P>0.1) increased by 2.92% while clearance insignificantly (P>0.10) increased by 5.04%. Lag time significantly (P<0.05) increased by 28%. The AUC from zero to six hours increased by 4.77% while that from zero to infinity increased significantly by 208.57%.

Table 4.3.2(iv) Comparison of the Influences of CQ and Cimetidine on the Pharmacokinetics of Paracetamol

PARAMETER (UNIT)	CIMETIDINE CONCOMITANT TREATMENT	DELAYED TREATMENT		CONTROL (PCM ALONE)
		CIMETIDINE TREATED	CHLOROQUINE TREATED	
Cmax (µg/ml)	18.00	11.50	15.00	20.00
Tmax (hr)	0.50	1.00	1.00	0.50
T½ab(hr)	0.272	0.90	0.82	0.27
Kab(hr^{-1})	2.547	0.77	0.845	2.566
T½el(hr)	2.600	3.00	2.4	2.50
Kel(hr^{-1})	0.2665	0.231	0.2828	0.2772
Vd (L)	51.406	47.60	53.041	51.535
Cl (L/hr)	13.69	10.995	15.00	14.280
Lag time (hr)	0.166	0.216	0.192	0.150
AUC_{0-6hr} (µg.hr/ml)	73.00	90.945	66.66	70.00
$AUC_{0-\infty}$ (µg.hr/ml)	100.39	484.64	302.406	98.00

Table 4.3.2(v) Comparison of the Influences of CQ and Cimetidine on the Pharamacokinetics of Paracetamol

PARAMETER (UNIT)	DELAYED TREATMENT		CHANGE %	P- VALUE
	CIMETIDINE TREATED	CHLOROQUINE TREATED		
C_{max} (µg/ml))	11.500	15.000	30.43	P<0.05
t_{max} (hr)	1.000	1.000	0.00	-
$t_{½ab}$(hr)	0.900	0.820	-8.88	P>0.10
$k_{ab}(hr^{-1})$	0.770	0.8450	9.74	"
$t_{½el}$(hr)	3.000	2.400	-20.00	"
$k_{el}(hr^{-1})$	0.231	0.2828	22.42	"
V_d (L)	47.600	53.041	11.43	"
C_l (L/hr)	10.995	15.000	36.42	P<0.05
Lag time (hr)	0.216	0.192	-11.11	p>0.10
AUC $_{0\text{-}6hr}$ (µg.hr/ml)	90.945	66.660	-26.70	"
AUC $_{0\text{-}\infty)}$ (µg.hr/ml))	484.64	302.406	-37.60	P<0.05

Analysing these results, it becomes clear that the extent of decrease in the absorption of paracetamol brought by chloroquine was between that brought by concomitant and delayed administration of paracetamol and cimetidine. This is evident in the absorption half life. Concomitant administration of cimetidine and paracetamol brought 0.74% increase in the T½ab while delayed treatment brought 233% increase and chloroquine increased the T½ab by 203%. This decrease in

Table 4.3.2(v) Comparison of the Influences of CQ and Cimetidine on the Pharamacokinetics of Paracetamol

PARAMETER (UNIT)	DELAYED TREATMENT		CHANGE %	P- VALUE
	CIMETIDINE TREATED	CHLOROQUINE TREATED		
C_{max} (µg/ml))	11.500	15.000	30.43	P<0.05
t_{max} (hr)	1.000	1.000	0.00	-
$t_{½ab}$(hr)	0.900	0.820	-8.88	P>0.10
$k_{ab}(hr^{-1})$	0.770	0.8450	9.74	"
$t_{½el}$(hr)	3.000	2.400	-20.00	"
$k_{el}(hr^{-1})$	0.231	0.2828	22.42	" "
V_d (L)	47.600	53.041	11.43	
C_l (L/hr)	10.995	15.000	36.42	P<0.05
Lag time (hr)	0.216	0.192	-11.11	p>0.10
AUC $_{0\text{-}6hr}$ (µg.hr/ml)	90.945	66.660	-26.70	"
AUC $_{0\text{-}\infty)}$ (µg.hr/ml))	484.64	302.406	-37.60	P<0.05

Analysing these results, it becomes clear that the extent of decrease in the absorption of paracetamol brought by chloroquine was between that brought by concomitant and delayed administration of paracetamol and cimetidine. This is evident in the absorption half life. Concomitant administration of cimetidine and paracetamol brought 0.74% increase in the T½ab while delayed treatment brought 233% increase and chloroquine increased the T½ab by 203%. This decrease in

absorption of paracetamol by chloroquine was effected through the anticholinergic effect of chloroquine on the smooth muscle of the gut (Goodman 1990). This consequently affected (decreased) the gastric emptying rate and paracetamol absorption, being dependent on gastric emptying, had its absorption rate reduced. This reduction in absorption rate of paracetamol by chloroquine was elaborated by the increase in the lagtime i.e. from 0.15hr to 0.192hr (28% increase). The Cmax in the delayed treatment (cimetidine later paracetamol) would have been expected to be higher than that in chloroquine treatment (15.00 (µg/ml)). However, the Cmax in chloroquine treatment was higher (15.00 (µg/ml)) than the delayed (Cimitidine later paracemol) treatment (11.50µg/ml)) because even though cimetidine is known to be a metabolic inhibitor while chloroquine is not, cimetidine reduced the paracetamol absorption to a greater extent than chloroquine did. The metabolic inhibition of cimetidine was evident via the significant increase in the elimination half life (T½el) of paracetamol which was increased by 20% in the delayed treatment. Chloroquine brought just 4% decrease in this T½el showing that chloroquine is not a metabolic inhibitor. The Area under the curve from zero to six hours was 66.66µg/ml (4.77% decrease), while delayed (Cimitidine later paracetamol) treatment brought AUC_{0-6hr} of 90.945 µg/ml (29.92% increase) indicating metabolic inhibition of paracetamol in the latter case. The time (Tmax) to reach maximum plasma concentration (Cmax) was 1hr (100% increase) in both delayed Cimetidine later paracetamol treatment and chloroquine treatment but the Cmax were 11.50 µg/ml and 15.00µg/ml respectively. This showed that even though their Tmax are close but cimetidine brought more conspicuous reduction in the absorption of paracetamol resulting in a lower Cmax than that brought by chloroquine treatment.

4.4 DISCUSSION

The quality of all the drug tablets used in the study was analysed and found out to conform with B.P. (1993) standards. Chloroquine was extracted with chloroform and assayed using non-aqeous titration, cimetidine was also assayed through this method while paracetamol was assayed spectrophotometrically.

The method of Bello method (1990) of paracetamol extraction with ethyl acetate and U.V. analysis has minimal interference from U.V. absorbing endogenous materials. The calibration curve constructed obeyed Beer's law over the concentration range used. The correlation coefficient (r) was within the accepted range. This curve was further validated since it was used for the analysis of samples containing unknown quantities of the drugs and relative recoveries was satisfactory.

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The doses used in the study are the ones used clinically and in most pharmacokinetic works involving drugs as cited in the literature. It was observed that concentrations of drugs in the saliva are mostly proportional to the concentrations in plasma. It was then suggested that in therapeutic drugs monitoring and in pharmacokinetic studies in general, saliva might be substituted for plasma (Danhofand et al, 1978) due to its simplicity of collection.

In this study, paracetamol was shown to be well absorbed after oral administration. Values of all pharmacokinetic parameters determined were within those reported in the literature. These parameters were not affected significantly when paracetamol and cimetidine were administered concurrently. This was due to the long Tmax (2-3hr) of cimetidine as compared to that of paracetamol (about

30 mins). By the time cimetidine reached appreciable plasma concentration to affect absorption of paracetamol, absorption of paracetamol was almost complete.

When Cimetidine was given 1hour before paracetamol, enough time was allowed for cimetidine drug to be in appreciable amount in the plasma to affect paracetamol absorption. The kinetics of paracetamol were significantly altered.

Chloroquine (Tmax = 2 - 3hr) given one and half hours before paracetamol, affected paracetamol absorption significantly although not as much as that by cimetidine delayed treatment. The concurrent administration of chloroquine and paracetamol was looked over because in this case even the insignificant changes brought by Cim/pcm concurrent administration would not even manifest. This is due to the long Tmax (2-3hr) of CQ. So before CQ reaches Cmax to interact with paracetamol, absorption of paracetamol would have been completed. Application of pharmacokinetic parameters derived from single dose studies in a small number of healthy subjects to individual patients is limited (Garba, 1992). In patients, different pathological studies which affect drug disposition, must be recognised. Kinetic parameters derived from healthy individuals, may differ widely from those observed in patients with impairement of one or more major organ systems (Williams et al, 1980). Moreover, parameters may change not only between healthy and ill individuals, but also in the same individual with age (Gladtke et al, 1979).

CONCLUSION

The study illustrated no effect on the pharmacokinetics of paracetamol when it was concurrently administered with cimetidine. Profound effect on the kinetics were obvious when cimetidine was administered one hour before paracetamol. Moreover, chloroquine (one and half hour delayed administration) affected kinetics of paracetamol almost in the same ways as that of cimetidine. While

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cimetidine affected (reduced) both absorption and metabolism of paracetamol, chloroquine affected (reduced) only its absorption and related parameters. i.e. lag time, AUC, T½ab, Kab, Cmax to almost the same extent.

The reduced absorption of paracetamol brought by cimetidine was due to indirect modification of gastric emptying since cimetidine is not an anticholinergic agent. Chloroquine being an anticholinergic drug acted directly on the smooth muscle to slow down gastric emptying. This resulted in a decrease in paracetamol absorption.

The decrease in absorption brought by cimetidine and chloroquine may have clinical implications when each is administered with other drugs having similar Tmax as the affecting drug (cimetidine or chloroquine). This interaction is also clinically important even if the drug to be administered with either cimetidine or CQ does not have the same Tmax as the affecting drugs (Cim and CQ). If they are administered in multiple therapy, then there will be time when the two drugs will be in appreciable concentration to bring about this interaction. Tmax, i.e time to reach maximum plasma concentration, is a very important tool in designing a pharmacokinetic study protocol.

Caution should be borne in mind when interpreting data obtained from healthy volunteers and correlating it to patients. Results vary significantly from healthy subjects to patients in a recent study (Garba, 1992). Paracetamol clearance was found to be 343.6 ± 8.00 (healthy) while it almost tripled this amount (840.6 ± 10.068) in the patients. T½ab was also, 0.200hr (healthy) and more than thrice this (0.716hr in the patients).

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APPENDIX 1

Weight Variation Test for Paracetamol, Chloroquine and Cimetidine Tablets B.P. 1988

Uncoated tablets:

20 tablets should be selected at random and the average weight determined. For tablets that have average weight of 250mg or more not more than two of the individual weight should deviate from the average weight by more than 5% and none deviates by more than twice that percentage.

Tablets No	Paracetamol(g)	Chloroquine(g)	Cimetidine(g)
1.	0.5922	0.1930	0.275
2.	0.5920	0.1916	0.267
3.	0.5965	0.1841	0.270
4.	0.5845	0.1905	0.263
5.	0.5950	0.1840	0.280
6.	0.5870	0.1908	0.270
7.	0.5880	0.1901	0.274
8.	0.5850	0.1901	0.270
9.	0.5820	0.1904	0.265
10.	0.5990	0.1901	0.280
11.	0.5770	0.1931	0.270
12.	0.2950	0.1926	0.270
13.	0.5910	0.1911	0.272
14.	0.5970	0.1917	0.270
15.	0.5850	0.1840	0.280
16.	0.5850	0.1911	0.280
17.	0.5860	0.1970	0.278
18.	0.5900	0.1900	0.280
19.	0.5930	0.1900	0.265
20.	0.5950	0.1900	0.271
	11.7952	3.800	5.450
mean:	0.5897	0.190	0.2725

APPENDIX 2

Data Obtained For The Construction Of Calibration Curve for paracetamol

CONC. OF DRUG/ml	ABSORBANCE Expt. I	II	III	IV	V
0.00					
10.00	0.223;0.148	0.224;0226	0.225;0227	0.198;0.220	0.213;0.196
20.00	0.524;0.493	0.515;0.512	0.478;0.476	0.483;0483	0.426;0.41
30.00	0.644;0.642	0.71;0.707	0.732;0.732	0.668;0666	0.691;0.508
40.00	0.956;0.901	0.811;0.808	0.805;0.803	1.114;1.111	0.829;0862
50.00	1.285;1.284	1.231;1.232	1.273;1.279	1.262;1.268	0.886;0.810

APPENDIX 3

Paracetamol saliva concentrations in 6 healthy volunteers following oral administration of 1g PCM in fasting state

	Paracetamol Concentration (μg/ml)					
	Volunteer No.					
Time(hr)	**I**	**II**	**III**	**IV**	**V**	**VI**
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	20.40	6.28	11.40	6.80	9.06	6.06
0.50	43.92	14.84	12.25	13.00	20.10	12.89
1.00	30.50	18.10	9.00	24.00	11.80	14.60
2.00	22.00	12.50	15.60	28.50	10.00	16.40
3.00	14.44	11.10	12.90	19.90	8.60	11.06
4.00	11.00	9.84	9.10	12.16	8.20	9.70
5.00	9.84	8.49	7.57	8.40	7.70	9.00
6.00	4.05	3.50	5.00	4.05	5.00	6.00

APPENDIX 4

Paracetamol saliva concentration in 6 healthy volunteers following oral dose of 1g PCM concurrently with 400mg cimetidine.

	Paracetamol Concentration (μg/ml) Volunteer No.					
Time(hr)	**I**	**II**	**III**	**IV**	**V**	**VI**
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	20.51	6.30	11.80	7.02	9.20	7.57
0.50	40.00	12.80	14.50	12.50	18.90	9.30
1.00	26.80	15.50	8.55	22.20	11.30	6.10
2.00	21.05	12.00	14.61	24.20	10.93	13.21
3.00	15.16	10.50	11.80	18.30	8.00	8.24
4.00	13.50	11.90	10.80	12.90	8.00	8.90
5.00	10.30	9.56	8.74	9.50	7.40	8.50
6.00	6.20	4.60	6.16	6.00	6.02	7.02

APPENDIX 5

Particulars o subjects used for the study.

S/No	Code Name	Age Years	Weighed (kg)	Sex	Remark
1	DA	25	58	Male	"
2	AD	28	56	"	"
3	HL	28	59	"	"
4	MM	27	62	"	"
5	HM	30	60	"	"
6	AI	36	62	"	"

APPENDIX 6

Paracetamol saliva concentrations in 6 healthy volunters following oral dose of 1g paracetamol an hour after cimetidine 400mg in fasting state.

	Paracetamol Concentration (µg/ml)					
	Volunteer No.					
Time(hr)	**I**	**II**	**III**	**IV**	**V**	**VI**
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	13.16	5.30	7.50	6.05	6.00	5.10
0.50	19.50	7.20	6.80	7.00	8.50	5.00
1.00	23.17	13.20	5.50	15.30	7.03	4.80
2.00	21.55	12.90	5.15	14.60	6.90	4.63
3.00	20.00	11..88	4.85	14.10	6.35	4.35
4.00	18.24	10.20	4.31	13.40	6.12	4.13
5.00	15.82	9.55	4.08	12.75	6.00	3.00
6.00	13.68	8.76	3.51	11.62	5.37	2.66

APPENDIX 7

Paracetamol saliva concentrations in 6 healthy volunteers following oral dose of 1g paracetamol one and half hours after 0.8g chloroquine sulphate in fasting state.

	Paracetamol Concentration (µg/ml)					
	Volunteer No.					
Time(hr)	**I**	**II**	**III**	**IV**	**V**	**VI**
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	13.88	6.02	8.09	7.14	8.07	6.00
0.50	22.37	10.91	11.52	10.37	13.28	9.55
1.00	18.59	11.40	8.92	12.94	12.15	8.00
2.00	16.86	10.86	7.64	11.59	10.62	9.63
3.00	13.49	8.80	6.92	9.60	8.80	7.59
4.00	10.97	7.35	5.33	7.52	6.10	6.53
5.00	8.62	6.13	3.78	4.76	5.50	4.81
6.00	6.79	4.34	1.99	2.85	4.06	3.97