COMPARISON OF THE EFFECTS OF CIMETIDINE AND METHYLDOPA ON THE PHARMACOKINETICS OF PARACETAMOL IN HUMAN

BY

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

DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY,

FACULTY OF PHARMACEUTICAL SCIENCE,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

MARCH 2009

DECLARATION

I, Nurudeen Sani Ibrahim, hereby declare that the work reported in this thesis was carried out by me under the supervision of Professor Magaji Garba and Dr (Mrs.) M. T. Odunola. It has not been submitted anywhere for the purpose of a degree award. The information derived from literature has been duly acknowledged in the text and a list of reference provided.

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CERTIFICATION

This thesis entitled “COMPARISON OF THE EFFECTS OF CIMETIDINE AND METHYLDOPA ON THE PHARMACOKINETICS OF PARACETAMOL IN HUMANS” by NURU-DEEN SANI IBRAHIM, meets the regulations governing the award of MASTER OF SCIENCE (PHARMACEUTICAL CHEMISTRY) of AHMADU BELLO UNIVERSITY and is approved for its contribution to sciences knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late father

MAL MUHAMMAD SANI IBRAHIM

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My thanks also go to my, friend and Dahiru Magaji Zarewa, whom we struggled together to get to this point.

Thanks to members of my family and above all to Almighty Allah for sparing my life all through.

ABSTRACT

Comparison of the effects of cimetidine and methyldopa were studied in 5 phases: via phase I, ingestion of paracetamol 1g alone, phase II, administration of paracetamol 1g and cimetidine 400mg concurrently, phase III administration of cimetidine 400mg followed by paracetamol 1g after an hour, phase IV, ingestion of paracetamol 1g and methyldopa 250mg concomitantly, phase V, administration of methyldopa 250mg followed by paracetamol 1g an hour after. The protocol involves 8 healthy male volunteers, non-smokers and non-alcoholic who fast overnight. Saliva samples were usually collected after ingestion of the drugs for different phases at an interval of 0.25, 0.5, 1, 2, 3, 4, 5 hours for extraction and analysis. Analytical method was used to determine paracetamol concentration using ultraviolent spectroscopic meter. The method was validated for both; within day and between day processions with extract recovery of 96%. The pharmacokinetic parameters for the five phases were determined using a log transfer data, these are Cmax, Tmax, Kβ, Kα, t1/2α, t1/2α, t1/2 ,Cl, and Vd. and AUC curves were used to generate pharmacokinetic parameters. The data obtained were compared, with values of $P > 0.05$ considered to be statistically significant. When cimetidine and paracetamol were concomitantly administered no significant change was observed ($P < 0.10$) with values obtained when paracetamol was administered alone (control), on the other hand when paracetamol was administered an hour after cimetidine significant changes of pharmacokinetic parameters of paracetamol

were recorded and compared to the value of the control phase. In this phase Paracetamol administration was analyzed by an hour after cimetidine ingestion. There are so many changes observed compared to the phase one parameters obtained this is as a result of possible drug interaction sequence to the inhibition of Paracetamol a absorption by cimetidine it could be seen that there is considerable reduction in the figures of Cmax and Kab by 36.45 % and 47.05% respectively ($P< 0.05$) which clearly indicates that there is a reduction in the absorption of Paracetamol. Tmax, $t_{½\alpha}$and lag time had significantly increased by 52.40% 52.5% and 43.78% respectively ($P<0.050$). It was also observed that there are insignificant changes in the pharmacokinetic parameters of paracetamol when paracetamol was administered with methyldopa irrespective of time interval leading to a suggestion that there is no influence in the pharmacokinetics of paracetamol by methyldopa. The findings indicate that on comparison, cimetidine may influence the pharmacokinetics of paracetamol depending on time of administration of the two drugs. While no change of pharmacokinetic parameters observed when methyldopa was administered with paracetamol irrespective of time interval.

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ABBREVIATIONS

α	=	Alpha
β	=	Beta
BNF	=	British National Formulary
^{o}C	=	Degree centigrade
HCL	=	Hydrochloric Acid
UV	=	Ultra Violet
Log	=	Logarism
rpm	=	Revolution Per minutes
Mwt	=	Molecular weight
Sem	=	Standard error of mean
Ug	=	Micrograms
Mg	=	Milligram
Ln	=	Natural log
L	=	Liter

SD	=	Standard deviation
MTD	=	Methyldopa
CMT	=	Cimetidine
PCM	=	Paractamol
Nm	=	Nanometer
Cv	=	Coefficient of Variation

APPENDIX 1

Phase I

Saliva concentration of eight Volunteers following oral administration of 1g paracetamol tablet in the fasting state (concentration =ug/ml)

	Volunteers								
Time(hrs)									Mean Sem
0.00									0.00 + 0.000
0.25									11.67 +0.538
0.50									17.95 +0.124
1.00									36.08 +2.345

2.00	[illegible]	[illegible]	[illegible]	30.33 +4.562
3.00	[illegible]	[illegible]	[illegible]	21.63 +0.321
4.00	[illegible]	[illegible]	[illegible]	15.42 +0.981
5.00	[illegible]	[illegible]	[illegible]	10.92 +1.043

SEM = Standard error mean

APPENDIX 2

Phase II

Saliva concentration of eight Volunteers following oral administration of 1g paracetamol tablet and Cimetidine tablet 400mg (concurrently) in the fasting state (concentration =ug/ml)

	Volunteers								
Time(hrs)									Mean: Sem
0.00									0.00 + 0.000
0.25									10.21 +0.542
0.50									21.33 +0.891

1.00	33.45 +1.790
2.00	24.70 +2.453
3.00	17.58 +0.543
4.00	12.50 +3.212
5.00	7.50+ 1.012

SEM = Standard error mean

APPENDIX 3

Phase III

Saliva concentration of eight Volunteers following oral administration of 400mg cimetidine followed by 1g paracetamol tablet an hour later(delayed) in the fasting state (concentration =ug/ml)

	Volunteers								
Time(hrs)									Mean Sem
0.00									0.00 + 0.000
0.25									8.17+ 1.098

0.50	[illegible]	[illegible]	[illegible]	15.58+2.076
1.00	[illegible]	[illegible]	[illegible]	23.33+0.543
2.00	[illegible]	[illegible]	[illegible]	17.45+0.432
3.00	[illegible]	[illegible]	[illegible]	11.25+0.021
4.00	[illegible]	[illegible]	[illegible]	8.17+0.431
5.00	[illegible]	[illegible]	[illegible]	5.13+1.042

SEM = Standard error mean

APPENDIX 4

Phase IV

Saliva concentration of eight Volunteers following oral administration of 1g paracetamol tablet and 250mg methyl dopa tablet (concurrent) in the fasting state (concentration =ug/ml)

	Volunteers								
Time(hrs)									Mean Sem
0.00									0.00 + 0.000
0.25									10.18 +0.43

	2
0.	18.08
50	+1.98
	1
1.	35.58
00	+2.08
	1
2.	28.85
00	+0.12
	3
3.	18.80
00	+0.54
	1
4.	12.70
00	+0.65
	2
5.	
00	8.12+

	3.321

SEM = Standard error mean

APPENDIX 5

Phase V

Saliva concentration of eight Volunteers following oral administration of 250mg methyl dopa tablets and 1g paracetamol tablet (concurrently) in the fasting state (concentration =ug/ml)

Time(hrs)	Volunteers	Mean Sem
0.00		0.00 ± 0.000

0.25	[illegible]	[illegible]	[illegible]	10.08 ±2.012
0.50	[illegible]	[illegible]	[illegible]	18.53 ±0.216
1.00	[illegible]	[illegible]	[illegible]	36.58 ±0.129
2.00	[illegible]	[illegible]	[illegible]	27.08 ±3.121
3.00	[illegible]	[illegible]	[illegible]	20.83 ±2.321
4.00	[illegible]	[illegible]	[illegible]	16.03 ±0.431

5.					9.80±
00					2.321

SEM = Standard error mean

Appendix 6

Mean Paracetamol Concentration in saliva for the five phases.

Time(hrs)	PCM ALONE	PCM+CMTD(C)	CMTD+PCM(D)	PCM+MTD(C)	PCM+MTD(D)
0.25	11.67±0538	10.26±0.542	8.17±1.098	10.18±0.412	10.08±2.012
0.50	17.95±0.12	21.33±0.891	15.58±2.076	18.08±1.981	18.53±0.21

	4				6
1.00	36.08±2.345	33.45±1.790	23.33±0.543	35.58±2.081	36.58±0.129
2.00	30.33±4.562	24.70±2.453	17.45±0.432	28.85±0.123	27.08±3.121
3.00	21.63±0.321	17.58±0.543	11.25±1.021	18.80±0.541	20.83±2.321
4.00	15.42±0.981	12.50±3.212	8.17±0.431	12.70±0.652	16.03±0.431
5.00	10.92±1.043	7.50±1.012	5.13±1042	8.12±3.321	9.80±2.321

Appendix 7

Volunteer's profile

Volunteers no	Weight	Age	Sex`
V1	52	28	Male
V2	49	34	Male
V3	56	28	Male
V4	62	29	Male
V5	63	33	Male
V6	59	36	Male
V7	52	25	Male
V8	55	28	Male
	Mean = 56.00±2.214	Mean = 30.13±1.032	_

CHAPTER ONE

INTRODUCTION

1.0 DRUG INTERACTION

It is evident that in some cases when two or more drugs are administered concurrently or at an interval of time, the expected pharmacological action is not being obtained. Sometimes potentiation of action of one of the drug may be observed. While on the other hand a diminished in action of the other drug occurs in some cases. This could be best explained through the study of possible interaction between the drugs in question. This is termed as drug – drug interaction study. (Pixtirn et al 1995).

There are many ways in which drug administrated by any route may interact in the patient to produce a harmful effect. Some of these interactions occur only when two drugs are administered within hours, days or weeks of each other; and sometimes the interaction take place only after one of the drugs has been taken for several weeks. There are biochemical explanations for some of this interaction, but for others there is as yet no information available about the basic mechanism involved.

1.1.0 GENERAL MECHANISM OF DRUG INTERACTION

The mechanism of drug interaction development can be best explained into 2 ways.

Pharmacokinetics

A). Absorption

b). Distribution

c). Elimination

Pharmacodynamics

a. Enhanced effect produced by 2 drugs acting at the same site

b. The increase effect produced by 2 drugs at different receptors site.

c. Enhanced effect of 2 drugs by one which is devoid of action itself.

d Antagonism of the effect of one drug by another.

1.1.1 PHARMACOKINETICS

Most of drugs interactions are kinetics in origin, one of the most useful pharmacokinetics concepts to have emerged in recent years particular in understanding interaction is that of the area inscribed by the plasma concentration – time curve of a drug. After a single dose, this area (AUC) is a function of the dose, the fraction of the dose entering the general circulation and the drug clearance. This can be expressed as follows:-

$$AUC = D \times F/C$$

Where;

AUC = area under curve

D = dose

C = drug clearance

F = fraction of the dose entering the general circulation

When a drug is given repetitively, accumulation occurs and the plasma concentration increase after each dose until a steady state is reached provided that:

a. Absorption dose not altered.

b. Drug binding to plasma proteins remains constant over a wide concentration range.

c. Elimination rate is not dose dependent.

d. The drug does not induced its metabolism, then

AUC (so) = AUC ($_{MD}$)

AUC ($_{SD}$) = Area under curve during single dose.

AUC ($_{MD}$) = Area under curve during multiple dose.

1.1.2 DRUGS ABSORPTION INFLEUNCE

Many drugs influence gastrointestinal function; the usual result of absorption interaction is a reduction in the rate of absorption or in the total amount of drug absorbed, so that drug effect are reduced or abolished. Interactions that cause therapeutically failure are obviously important but are unlikely to be recognized unless significantly looked for; on the other hand the risk of drug toxicity may be increased if absorption is enhanced. From the practical point of view, it is important

to differentiate between interaction that alter the rate of drug absorption and those that increase or decrease the total amount of drug absorbed since the consequences may be quite different. A change in the rate of absorption of a long – acting drug such as warfarin would probably have little effect or no effect, whereas a change in total amount absorbed may be disastrous. In contrast, if the absorption of a drug with a short biological half life such as procainamide is slowed dawn, therapeutic plasma concentration may never be reached.

The absorption of drug from gastrointestinal tract is a complex process that depends on many physiological and physicochemical factors. This leads to many pathways within which the mechanisms of drugs absorption interaction are proposed.

1.1.3 MECHANISM OF DRUG ABSORPTION INTERACTION

There are many proposed mechanism for drug absorption interaction. The possible ones are as follows:

a). **P^H effect on dissolution and ionization**

Drug induced change in P^H of gastrointestinal fluids may have complex and unpredictable effect on the absorption of other drugs taken at the same time. According to the P^H partition theory, weak organic acids are largely absorbed from the stomach, where as weak bases are absorbed best from the more alkaline contents of the upper small intestine it is sometimes stated that the absorption of weak acid is reduced if they are given with alkaline drug, since less drug would be present in the unionized lipid soluble diffusible state.

b). **Change in gastric emptying and GI motility**

The stomach is not an important site of drug absorption. Basic drugs and compounds absorbed by active transport are not absorbed from the stomach to any extent, and even weakly acid drugs such as aspirin, warfarin, and barbiturates and low molecular weight natural compounds such as ethanol are absorbed much more slowly from the stomach than from the small intestine. Drugs are probably absorbed more rapidly from the upper small intestine than from the stomach, because of the much greater surface area of the intestine. The rate of gastric emptying may therefore limit to the rate of drug absorption and particularly important in the context of interactions since it can be influenced by many drugs. Drug effects can be reduced dramatically or even abolished if gastric emptying is retarded by food. In contrast, absorption is more rapid and toxicity can be greatly increased when drugs are given orally in the same dose in dilute form rather than concentrated solutions, and this effect has been attributed in part to rapid gastric emptying.

c. **Formation of complexes, in – pairs and chelates**

Drugs may interact in the gastrointestinal tract to form complexes, ion pairs and chelates which may be absorbed more rapidly or more slowly than the parent drugs. The absorption of tetracycline is inhibited by the formation of insoluble chalets with metals such as Calcium and iron. Dicoumarol absorption is increased by the formation of more soluble chelates with magnesium hydroxide, and the absorption of quaternary ammonium anti

arrhythmic agent is enhanced by ion – pair formation with salicylate and trichloroacetate. The absorption of drugs may also be reduced by absorption onto kaolin or charcoal or binding to ionic exchange resins.

d. **Interference with active transport**

Drugs that are analogues of naturally occurring purines, pyramidines, sugar and amino acid may be absorbed by small intestinal active transport; and it has been suggested that absorption may be reduced by competition between substrates such as L-Dopa and phenyl amino derived from dietary sources. It is possible for one drug to inhibit enzymes involved in the active transport of another drug and such an interaction has been postulated between chlorpromazine and L-dopa.

e. **Distribution of Lipid micelles**

Interference with micelles formation may limit the solubility of lipids and inhibition of absorption of cholesterol, bile acids and vitamin A by neomycin has been attributed to this mechanism.

f. **Change in portal blood flow**

The splanchnic blood flow may occasionally be a rate limiting factor in drug absorption and this may be of clinical significance because many drugs could have direct effect on the local gastrointestinal blood flow.

g. **Toxic effect on gastrointestinal Mucosa**

Toxic effect on gastrointestinal mucosa may cause mal-absorption syndrome with impaired absorption of other drugs. Neomycin, p-Amino Salicylic acid and

colchicines may cause megaloblastic anemia through interference with vitamin B_{12} absorption.

h. **Change in Volume, composition and viscosity of secretion**

Drugs can also influence the volume and composition of gastrointestinal secretions (including bide) and changes in viscosity may modify drug absorption. It is now recognized that many drugs interact through this mechanism.

I. **Effect on mucosal and bacterial drug metabolism**

Recent findings have shown that many drugs are extensively metabolized by the gastrointestinal mucosa and the gut bacterial flora, and this process might be influenced by the concurrent administration of other drugs.

j. **Change in membrane permeability**

During oral therapy, the intestinal mucosa is exposed intermittently to very high drug concentration that many alter the permeability of the gastrointestinal epitheliums. In this context it is interesting to note that insulin and many polypeptides greatly enhanced the membrane transport of rethidine, isonizid, salicylate and chlorpromazine (drugs normally considered to cross cell membrane by passive diffusion). Thus, intestinal up take of ionized is enhanced by insulin and this effects is antagonized by quabain.

1.1.4 DRUGS DISTRIBUTION INETRACTION

Competition between co-administered drugs at non specific binding sites in the body can result in displacement of one drug by another, with a resulting rise in the free and active fraction of a drug. It is widely believed that this displacement or redistribution phenomenon causes the enhanced clinical effects and toxicity seen when certain drugs and other substances interact in man.

The distribution of drugs is dependent on the following factors:-

- Regional blood flow
- Partition coefficient of drug between blood and tissue
- Binding plasma proteins and tissues macromolecules
- Active transport.

This mechanism of drug interactions can be broadly divided into those occurring at the receptor level or beyond and those occurring by pharmacokinetic process prior to the receptor. In the intact body, more than one mechanism may operate simultaneously.

Many drugs reversibly bound to plasma proteins. Drugs may compete for common binding sites on the binding proteins and when administered together they may displace each other. As a result, there is tendency towards an increased in the free (unbound) plasma concentration. The potential importance of this interaction lies in

the fact that only the unbound (free) fraction of drug in plasma is available for distribution to tissue.

Although in theory this mechanism could produce potent drugs interaction. It has been overemphasized in the past because unwanted extrapolations have been made from in-vivo studies. If a drug A displaced a second drug B from its plasma protein binding site, the increase in the unbound fraction of B will occur in – vivo will always be less than that observed in vitro. The reason for this is that a proportion of displaced drug molecules will diffuse extravascularly into the drugs distribution volume. Consequently the greater the distribution volume, the less will displacement interactions results in clinically important effect. In addition, displacement interaction will only be significant if the displaced drug is itself highly protein bound (more than 90%) otherwise increase in free concentration will be too trivial to be relevant.

The following considerations would helps to determine whether a given interaction was purely re-distributional one involving plasma protein.

- The whole interaction should be mimicked by any drugs with comparable displacement ability.
- Absorptive interaction should be excluded e.g. the interaction should be shown to occur when both drugs are administered parentally or alternatively formed pharmacokinetic analysis of absorption studies.
- Metabolic and excretory rates should be measured. Any changes should be entirely predictable from changes in the concentration of free drug.

If these conditions are not satisfied, some quantitative allowance must be made for the contributions of these and other pharmacokinetic models of interaction.

1.1.5 DRUGS ELIMINATION INTERACTION

The elimination rate of a drug can best be described by its clearance. Changes in renal function can modify a number of pharmacokinetic processes in the body and there by lead to unanticipated drug effects or drug interaction. Most of drugs are eliminated by renal excretion, by metabolism or by a combination of the two processes. Total clearance is equal to the sum of the individual clearance for renal excretion and metabolism.

a. RENAL EXCRETION

Drug loss by the kidneys is determined by the net effect of Glumerular filtration, tubular secretion and tubular re-absorption,

Glumerular filtration is not greatly influenced by other drugs although displacement for protein binding sites may lead to increase concentration of drug in Glumerular fluid and enhance renal elimination. Tubular secretion of acid drugs is mediated by relatively non – specific active transport system and a variety of endogenous and exogenous compounds are potential substrate. Competition for tubular secretion may lead to unpaired renal excretion of drugs whose major route of elimination is via this pathway. Thus probenecid impairs the elimination of penicillin salicylate and indomethacin.

Tubular re-absorption is passive and dependent upon the lipid solubility with which a drug can cross the tubular epithelium and on the concentration gradient between tubular fluid and plasma water. Since many drugs are weak electrolytes, the P^H of the tubular fluid will be a determinant of a drugs re-absorption. Consequently, the clearance of acid drug (e.g. salicylate) will be more rapidly excreted in acid urine. These changes in urine P^H however will only be of practical importance for the elimination of those drugs for which renal excretion plays a significant role.

b. DRUG METABOLISM

Metabolism of drugs generally leads to the formation of polar (lipid – insoluble) derivatives which can undergo renal excretion. Drug metabolites are also usually devoid of pharmacological activity, so that metabolism determines drugs effects.

Many commonly used drugs are oxidized in the hepatic endoplasmic reticulum. The activity of the enzyme systems involved is subject to wide inter-individual variability. Moreover, the activity of this system may be enhanced (induced) or diminished (inhibited) by other drugs or environmental pollutants. Induction of hepatic microsomal oxidizing system leads to increased drug clearance and a reduced steady state level during multiple dose therapy. This may result in therapeutic failure. By contrast, inhibition of hepatic microsomal oxidation leads to reduced drug clearance and increased steady state plasma

levels. Inhibition of microsomal oxidation is therefore liable to precipitate toxicity. The mechanisms involved in inhibition of drug metabolism are unclear. It is important, therefore to realize that a drug which inhibits the metabolism of one oxidized drug does not necessarily inhibit the metabolism of other oxidized drug. There are several factors that could influence metabolism, the most important ones are, genetic physiological factors, pharmacodynamic factors and environmental factors.

1.1.6 PHARMACODYNAMICS

Pharmacodynamic interaction occur when one drug directly alter the molecular, cellular or physiological action of another. Four varieties of pharmacodynamics interactions may be described.

a. **Enhanced effect produced by two drugs acting at the same site**

Such an interaction is observed in patients who received drugs that have weak depolarizing properties e.g. streptomycin in the presence of depolarizing muscle relaxant. This type of interactions is normally predictable, but magnitude of the observed effects is dependent on the relative potencies and dosages of the two drugs and the positions and the slopes of their dose – response curve. Consequently the magnitude of the observed interaction may be equal to or greater than, the sum of their individual effects.

b. **The increased effects produced by two drugs acting at different receptor site**

Such an interaction results in an effect which is greater than the sum of the component effects (i.e. potentiation). Deliberate use is often made of this variety of interaction, so that relatively small (and therefore less toxic) doses of number of different drugs can be administered and adverse reactions avoided. Typical example includes antihypertensive and cytotoxic drug therapy.

c. **Enhanced effect of a drug by one which is devoid of action itself**

Interactions of this type are extremely unusual, but may account for the increased anticoagulant effect of warfarin during therapy with clofibrate or D – thyroxine. These agents although devoid of effect on clotting factor synthesis themselves; possibly increased the affinity of warfarin for its receptor site in their liver.

d. **Antagonism of the effect of one drug by another**

This type of interaction can be caused by several underlying mechanisms. The commonest variety is that produced by competition for the receptor site by two drugs both with affinity for receptor site but only one (the agonist) having receptor stimulant activity (often called intrinsic activity). Example of antagonist's molecule adrenoceptor blocking agent (alpha and beta) are, muscle relaxants, antihistamines and naloxones.

1.1.7 BASIC PHARMACOKINETICS

The term pharmacokinetics refers to the qualitative and quantitative study of fate of a drug in the organism to which it is administered. It involves the action of the organism on the drug. A drug exert its pharmacological effect only when it reaches its site of action, thus it must first reach the general circulation and this requires the crossing of physiological barrier (GIT) for orally administered drug and the process being known as absorption, while its fraction absorbed is known as the absorption coefficient of the drug. The drug enters the portal circulation after absorption, then to the liver where it is metabolized or transformed into metabolite that are more water soluble, hence more easily eliminated, a phenomenon known as hepatic first pass effect. The net effect of absorption and first pass effect determines greatly the bioavailability of a drug. On reaching the systemic circulation, a drug will first interact with erythrocytes and plasma proteins where it undergoes some binding. It is then transported to all the tissues to a different extent and this phase is referred to as distribution phase of drug. After the distribution of drugs into the various tissues, several elimination processes occur. These processes include urinary excretion, billiary excretion and conversion into metabolites by organs like the liver, intestine, lungs, and kidney. These elimination processes sum up what is known as the total clearance of the drug.

To ascertain the pharmacokinetics characteristics of a drug, the different stages of absorption, distributional elimination must be quantified and donated by specific parameters that can be determined mathematically using methods based on plasma

and/or urinary kinetic data obtained after the administration on the compound or different routes. The most important pharmacokinetics parameters that are being considered in drug absorption are:-

- Coefficient of absorption
- First pass effect
- Area under curve
- Half life

These parameters determine to a large extent the pharmacological responses of individual patient.

Pharmacokinetics helps define the frequency of administration while excluding any risk of toxicity. Physiological state of the individual related to his age, sex genetic makeup and morphology are also taken into consideration. In order to estimate the effect on the pharmacokinetic properties of the drug, the pathological state of the patient whether transient or permanent is assed. This clinical pharmacokinetics is referred to as the application of pharmacokinetics therapy to the management of the patient.

1.1.8 Compartmental models

In pharmacokinetics experimental data on drug concentration in plasma are fit into mathematical equations that represent flow of drugs and their metabolites through the discrete compartments of a model system (Paxton 1981).

Even with inter-individual variations certain principles can be generally applied in order to manipulate pharmacokinetics data so as to precisely described drug disposition which is of clinical importance. These principles include the assumption that:-

i. Drug enters the system via a central compartment and is eliminated only from the compartment.

ii. Reversible transfer occurs between central and peripheral compartments.

iii. The exit of drug from all compartments in the system is described by a first order kinetics.

iv. The third assumption is true for most drugs excepts for drugs eliminated by biotransformation where by saturation of metabolite enzymes makes elimination a zero order process.

1.1.9 SINGLE COMPARTMENT MODEL

Here, exchange of drug between the plasma and tissue proceeds rapidly compared with the rates elimination with the whole body considered mathematically as a single compartment. We assume an instantaneous distention after an IV injection of a drug into the models.

This equation then holds true:

$$C_o = D / V_{dc}$$

Where;

C_o = Concentration immediately after injection

D = Dose

V_{dc} = apparent volume of distribution.

After instantaneous distribution, the concentration will fall according to first order kinetics with plot of plasma drug concentration against time on an arithmetic scale; we get an exponential curve, while on a semi – logarithm scale, we get an exponential curve, while on a logarithm scale; we get a straight line.

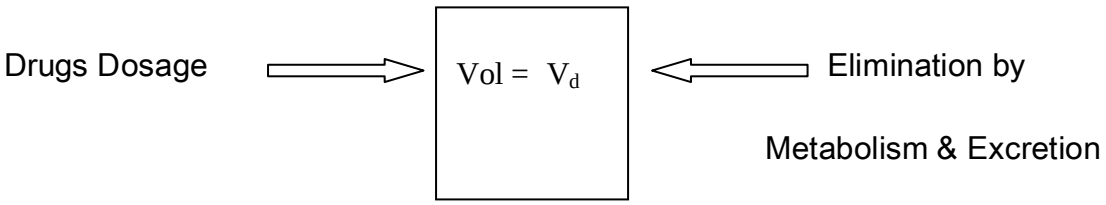


Fig 1: Rapid IV injection into a single compartmental model.

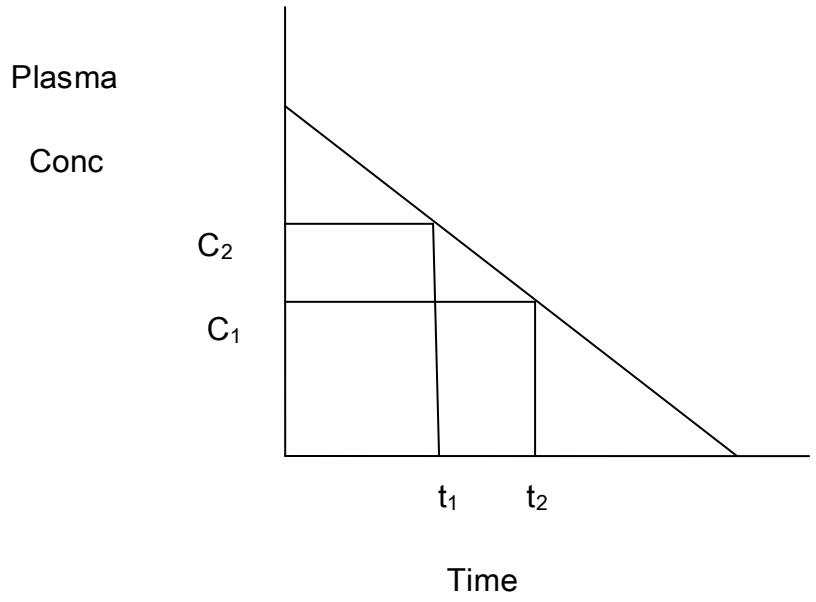


Fig 2: Plots of plasma drug concentration against time for a single Compartmental model on log scale.

The decline in drug concentration may be expressed as;

$$dc/dt = -B.ct$$

Where B = Elimination rate constant

On integration,

$Ct = Coe^{Bt}$ = e = base of natural log

$$\ln Ct = \ln Co - Bt$$

Converting to logarithm to the base 10

$Log\ C_{10} = Log\ C_0$. – B+/2.303

1.2.0 MULTIPLE COMPARTMENTAL MODELS

It is worthy to note that the plasma concentration time curve of a drug is often not a straight line on a semi – log plot for a slow distribution of drug, a single compartmental model cannot be fitted into; hence a peripheral compartment in addition to the central must be considered.

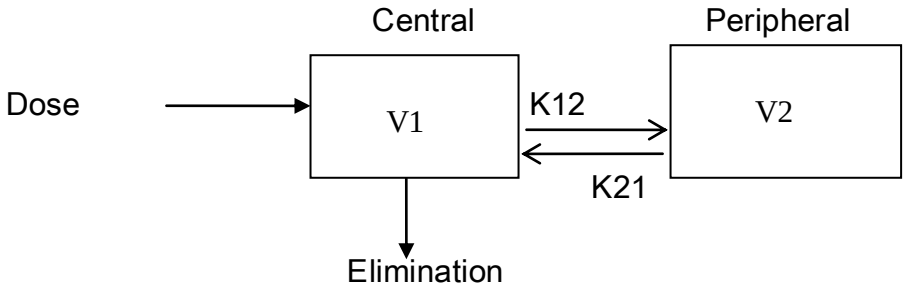


Fig 3: Rapid IV injection into a two compartments

V_1 and V_2 = apparent volume of distribution.

K_{12} and K_{21} = distribution rate constants

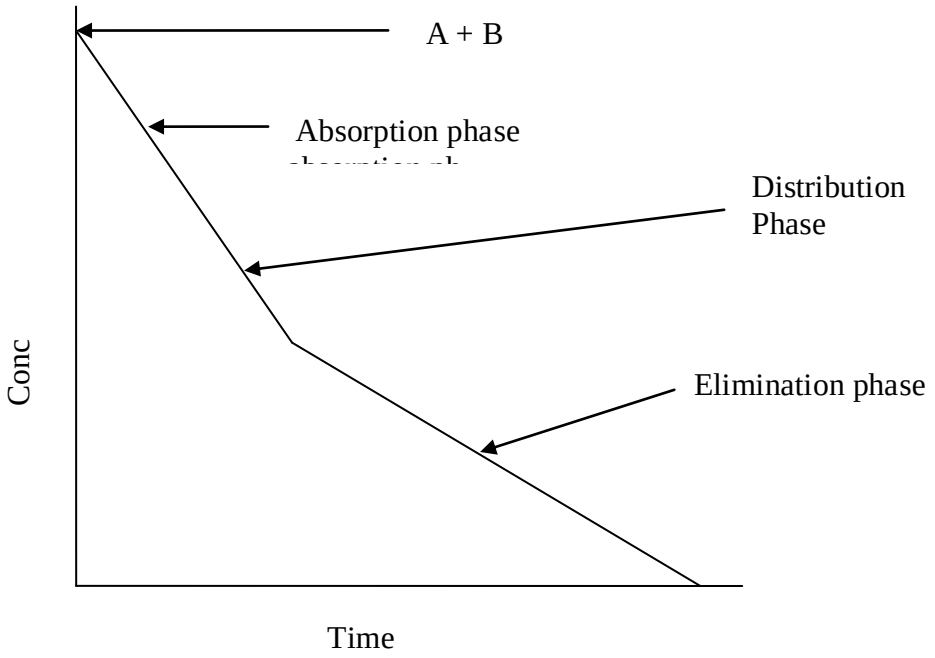


Fig 4: Plot of plasma drug concentration against time for a two compartmental model on a logarithm scale.

This may be represented by the equations below.

$$C_t = Ae^{xt} + Be^{Bt}$$

The coefficient B is the intercepts on the ordinate obtained with extrapolation of the elimination phase.

A + B is the actual intercepts of the concentration curve at T = 0

a and B are the distribution and elimination rate constant.

A two compartment model may be expanded to contain additional compartments which can be described mathematically as the sum of many individuals' exponents' functions as there are relevant compartments.

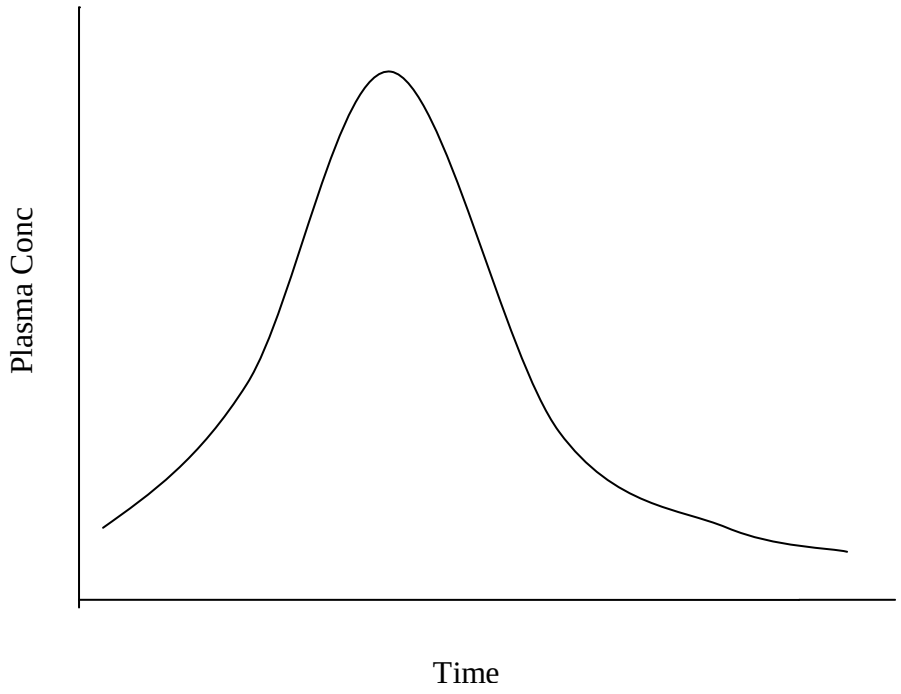


Fig 5: Plot of plasma drug concentration against time for three compartment models on logarithm scale.

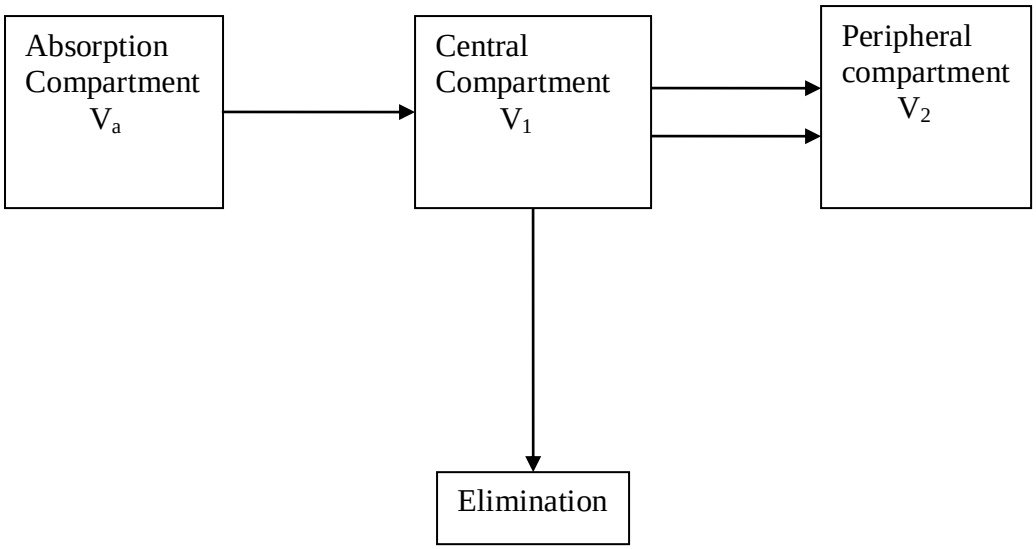


Fig 6: Three Compartment model after a single oral dose

1.2.1 NON LINEAR Pharmacokinetics

The pharmacokinetics of a drug are said to be non linear when one or more of the pharmacokinetics parameters vary with dose, the concentration at a given time.

Non linear can be related to absorption, the hepatic first pass effect, distribution, urinary excretion billiary elimination or metabolism.

The most frequent reasons for non linear kinetics are:-

- Saturation of the hepatic first pass effect
- Saturation of the plasma protein binding site
- Saturation of the process of re-absorption of tubular secretion.
- Saturation of the enzymes system involved in biotransformation.

Examples of non linear Kinetics are;

- Absorption - Clomidine, Griseofulvin, Chlorthiazide
- First pass effect – midazolam, propranolol hydrallazine.
- Tissue Distribution – Quinidine Disopyramide
- Protein Binding – Quinidine, ceftriazone.
- Metabolism – Phenytoin, Theophylline erythromycin probenicid penicillin G.

Non linearity has important pharmacological and or clinical consequences when it occurs at therapeutic levels. The decline in the plasma concentration of a drug whose plasma kinetics are non linear generally occurs in accordance with the Micheali – Menten process (Labanne 1989).

$d_c/d_t = V_m .C/K_m .t$ $\underline{d_c}$ = Elimination rate const

V_m = Maximum velocity of enzyme process

K_m = Micheali-Menten const

1.2.2 PHARMACOKINETICS PARAMETERS

As it has been earlier mentioned, pharmacokinetic parameters determine to a large extent the pharmacological response of individuals. These parameters include the following;

Absorption rate constant (Kα).

This is the rate constant of the entire process of drug transfer into the body through all biological membrane. It has units of reciprocal of time (h^{-1}) it may be calculated from the following.

$$Ka = \frac{0.693}{t_{1/2}}$$

Area under the curve (AUC)

This is the area defined by the axis and the curve of blood or plasma concentration versus time. It may be limited to a specific time or be extrapolated to infinity.

It is the total blood or plasma drug concentration from time to infinity. It measures the quantity of the drug, which has been absorbed and has entered the general circulation. Thus it is a measure of the amount of circulating drug. It has units of mg / h/ml it can be calculated by the trapezoid method where by trapezoid determined according to the formula.

$$\text{Area} = \frac{C_1 + C_2 (t_2 - t_1)}{2}$$

$$AUC^{a}_{0} = AUC^{t} + \frac{C}{B}$$

Where B = Elimination rate constant

Mathematical methods

After striping the curve, it is determined by the formula

$$AUC^{a}_{O} = \frac{A}{a} + \frac{b}{B}$$

Absorption Half life ($t_{½\alpha}$)

This is the time taken for half of the total drug absorbed to be achieved (half of the difference between theoretical and experimental values). It has units of time in hours.

Elimination Rate Constant (Kβ)

This is the rate constant of the process (es) leading to the elimination of the drug from the body. It is the sum of all the individual administration rate constants it has units of reciprocal of time (hr^{-1})

Elimination half life ($t_{1/2}$ β)

This is the time take for a quantity of drug to be reduced by 50%.

This is as a result of elimination whose kinetics is first order.

$$t_{1/2\ \beta} = \frac{0.6793}{K_{\beta}}$$

Where K_β = rate constant

With the first order, process, $t_{1/2\beta}$ is constant and independent of the initial concentration and the administered dose. It may be calculated from elimination phase of concentration time curve on a logarithm scale or from the following equation.

$$t_{1/2}\,\beta = \frac{0.693 \times V_d}{Cl} \quad \text{or} \quad t_{1/2}\,\beta = \frac{0.693}{\beta}$$

Where B = elimination rate constant

Cl = Clearance

V_d = Volume of distribution

It is used to estimate the appropriate dosage interval during maintenance therapy.

ABSORPTION LAG TIME

It begins where extrapolated straight line and that of the residual intersect. It reflects the time taken between the administration of a drug and the time absorption begins. It has unit of time (hr).

TOTAL BODY CLEARANCE

This is the capacity of the organism to eliminate a substance after it has reached the general circulation. It is the total sum of all the individual clearance by the various organs. It reflects the volume of blood completely cleared of the drug per unit time. Organ clearance refers to the volume of blood or plasma completely cleared of a drug by the organ per unit time.

Total clearance = Cl (renal) + Cl (hepatic) + Cl (other organs of metabolism)

Clearance by any organ depends on the blood flow through the organs and the extraction ratios of the drug by the same organ.

$$Cl = \frac{\text{Dose iv}}{AUC} = \frac{\text{f x oral dose}}{AUC_{oral}}$$

Where F = Bioavailability

It has unit of L/hrs

Clearance relates the concentration to the rate of drug elimination.

Rate of elimination = clearance x concentration

VOLUMES OF DISTRIBUTION.

a) Initial volume of distribution

This is the ratio of the administered dose to the plasma drug concentration extrapolated to zero time (after an intravenous or intra – arterial injection).

b) Apparent volume of distribution (v_d)

This is the ratio of the amount of drug in the body to its plasma concentration equilibrium. Volume of distribution (V_d) rates the amount of drug in the body to the concentration of drug in blood plasma. Volume of distribution has unit of L or L/Kg.

$$\text{Initial } V_d = V = \frac{D}{C_O}$$

Where D = Dose administered by rapid IV. Injection

C_0 = Theoretical blood Conc at time O

$$\text{Apparent } V_d = V = V_p = V_t$$

CHAPTER TWO

LITERATURE REVIEW

This research work concerns the modification of one drug by the presence of another whether by direct or indirect means with reference to paracetamol, cimetidine and methyldopa. In general drug – drug interaction may affect absorption, distribution, receptor action, metabolism and excretion. This type of interaction may be beneficial or hazardous. They may vary from person to person and from species to species. They may also be of major clinical significance or of no clinical significance at all.

2.0 PARACETAMOL

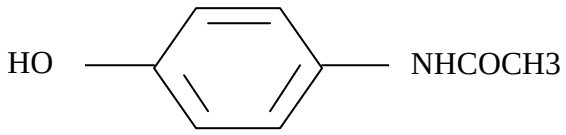
Introduction and history

Acetaminophen (paracetamol) was first used in medicine by Von Miring in 1893; it has gained popularity since 1949, after it was recognized as the major metabolite of acetanilide and phenacetin (Goodman and Gilman, 1990). Paracetamol was introduced in America in 1950 as a compound analgesic containing paracetamol 125mg, aspirin 230mg and caffeine 30mg, but it was withdrawn because of a suspicion that it could cause aggrenulocytosis. The work of Boride and Axel rod in 1950 led to the introduction of paracetamol in United Kingdom, primarily as a prescription product. The prohibition of the use of phenacetin as an active ingredient of medicine in 1979 has made paracetamol to become the most widely accepted alternative to aspirin as an antipyretic and analgesic. Its use has been further

stimulated by the perceived risk of the other previously popular OTC analgesic aspirin, notably its gastrointestinal effect and its associate with Reyes syndrome in children because paracetamol ions are well tolerated, it lacks many of the side effects associated with aspirin.

2.1.0 PHYSIOCHEMICAL PROPERTIES OF PARACETAMOL

Paracetamol is a Para-aminophenol derivative with the chemical name of 4 – hydroxyacetamide N (4 – hydroxphenoyl) acetamide; N-acetyl-p-aminophenol. Below is the structural formula.



paracetamol

Fig 7: Structure of paracetamol

a). Molecular formula: $C_6H_9N0_2$

b). Description: white odorless crystalline powder with a bitter test.

c). Melting point: 169 – 172^oC

d). pka: 9.5 (Weak acid)

e). Molecular weight: 151.2

f). P^H: 6 (saturated aqueous solar)

g). Stability: stable with half life of over 20 yrs (stability decreases in acid or alkaline condition)

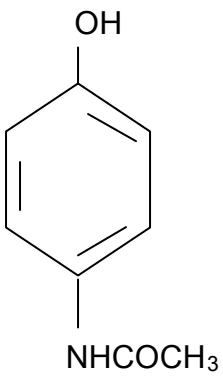
h). Solubility

Table 1.0 Solubility of paracetamol

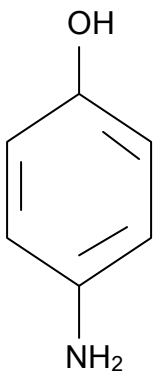
S/N	Soluble	Sparingly soluble	Very springy soluble	In soluble
1.	In boil water (1:20)	Water	Chloroform	Benzene
2.	Acetone	Ether	-	-
3.	Alkaline hydroxide	-	-	-
4.	95% Ethanol (1:10)	-	-	-

2.1.1 STRUCTURAL ACTIVITY RELATIONSHIP

According to wiliest 1982, the structural activity relationship depends on the saturation of amine (Primary, Secondary or tertiary). The presence of primary amine indicates strong antipyretic analgesic action, but it is too toxic to serve as a drug. In general any type of substitution on the amino group that reduces its basicity, results also in a lowering of its physiological activity (including toxic effect). Acetylation is one type of substitution that accomplishes this effect (Willelte 1982).



I

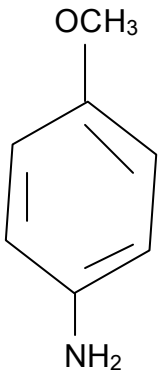


II

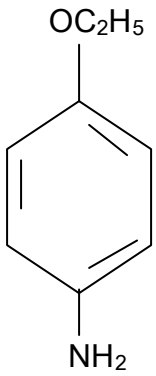
Fig 8: Acetylation of paracetamol

Etherification of the phenecitic – OH group produces stronger analgesic action but too toxic to use due to the free amino group, example, amisidine and phenetidine which are the methyl and ethyl

ethers respectively



Anisidine



Phenitidine

Fig 9: structure of Anisidine and Phenitidine

Etherification of the OH – group with an acetyl moiety produced analgesic which has the same activity and disadvantage as the free phenol e.g. p-acetoxy acetanilide (Wilette 1982).

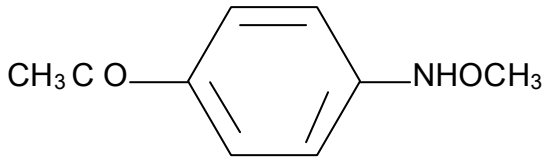


Fig 10: Structure of p-acetoxyacetanilide

However, the salicyl ester exhibits a diminished toxicity and an increased antipyretic activity.

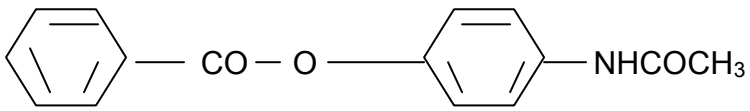
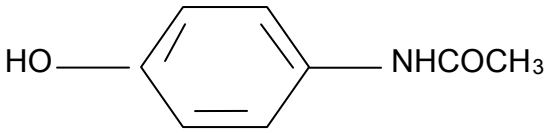


Fig 11: Structure of Salicyl ester (Phenetsal)

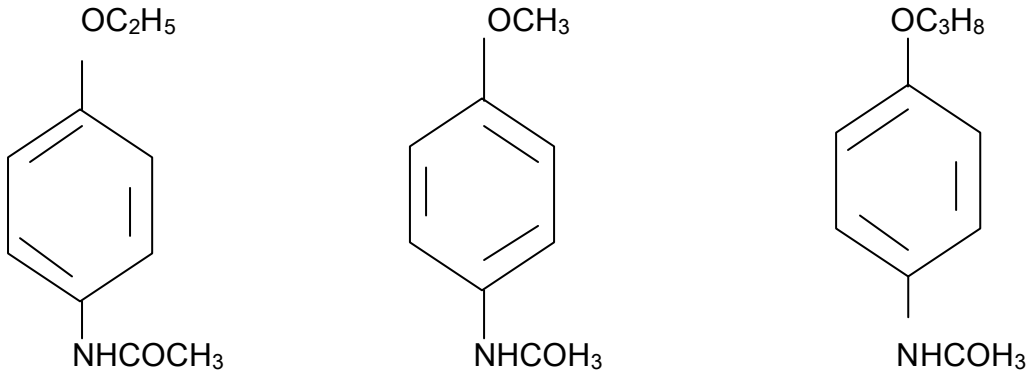
Acetylation of the amino group with free OH group produces the best p-aminophenol e.g. paracetamol (Willet 1982).



Paracetamol

Among the alkyl esters of the N-acetyl – p – aminophenol derivatives, the ethyl ether was found to be the best and is now the official phenacetin.

The methyl and propyl homologs were undesirable from the start – point of causing emesis, salivation, dieresis and other reaction (willette, 1982).



Ethyl ether Methyl ether Propyl ether

Fig 12: Structure of ethyl ether, methyl ether and propyl ether

2.1.2 PHARMACOKINETICS

ABSORPTION

In normal doses, paracetamol is well absorbed and peak blood level of the parent drug occur within one hour (Presott et – al, 1971) paracetamol is a week acid with pka of 9.5 it is largely unionized in both the stomach and the small intestine and should be well absorbed from both sides (Schanker et – al 1957). In humans, however the absorption of paracetamol is very slow in the stomach and the primary site for the absorption resides in the intestine.

The rate of absorption has been reported to be directly related to the rate of gastric emptying (Heading et – al 1973). Paracetamol is mainly absorbed from the small intestine, therefore the rate of gastric emptying (Sample, 1973 and weigklel, 1958). Heading et – al (1973) found that peak plasma concentration after oral administration of 1.5g paracetamol to 14 patients varied from 7.4 to 37.0 mg/ml and the time taken to reach these levels ranged from 30 to 180 min, suggesting that this variation might be due to individual difference in the rate of gastric emptying.

The presence of food in the stomach has been reported to reduce the rate of absorption of paracetamol (Heading et – al 1973 Jaffe et – al 1771). This is probably due largely to inhibitory effect of food on gastric emptying (Welling, 1977).

Certain specific dietary components can significantly alter the absorption pattern of orally administered paracetamol (Jaffe et – al 1971) it was reported (Pottage et – al

1974) that alterations in gastric P^H have no appreciable effect on paracetamol abortion. Although paracetamol is rapidly absorbed unchanged from gastrointestinal tract, it is incompletely available to systemic circulation after oral administration a variable proportion being lost through first pass – metabolism (Chio, 1975, Perucca and Richens 1979).

DISTRIBUTION

Paracetamol is relatively well distributed throughout the body (Flower et – al 1988) it was demonstrated that paracetamol is well distributed evenly in all tissues with the exception of fat in dogs (Gwilt et – al, 1963). In human, usual analgesic doses produces total serum concentrations of 5 – 20mg/ml.

In man, the ratio between the whole blood and plasma concentration of paracetamol is close to unity (Gwilt, et – al 1963). Binding of the drug to plasma proteins is variable. In humans plasma protein binding is negligible with plasma, concentrations which correspond to the therapeutic dose.

However 15 – 20% of paracetamol is protein bound at concentrations of about 280mg/ml, corresponding to the levels observed after human overdose (Gazzard et – al 1973). Paracetamol has been found in human saliva in concentrations which correlate well with plasma levels (Gynn and Bastain 1973). This observation has led to the suggestion that in therapeutic drug monitoring, or in pharmacokinetics studies

in general saliva might be substituted for plasma (Danlof and Breimer, 1978). Paracetamol is also secreted in breast milk (Bannet, 1983, Findlay 1981) though its presence is probably without clinical significance.

METABOLISM

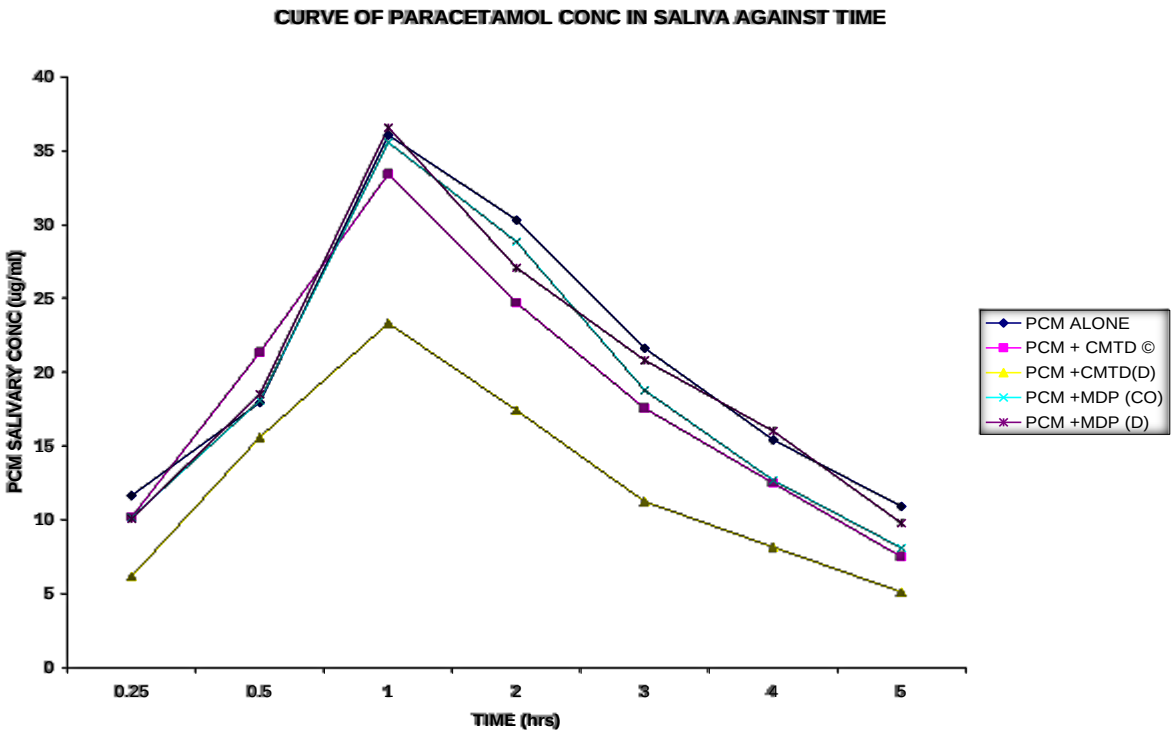
Paracetamol is metabolized via variety of pathways. Fig 13 illustrates the metabolic pathways of paracetamol. It normally undergoes glucuronidation (major) and sulphation (Minor) to the corresponding metabolites (Ameer and Greenblatt, 1977).

The conjugates are pharmacologically inactive and comprise 95% of the total excretion metabolite. The alternative cytochrome p450 dependant glutathione (GSH) conjugation accounts for the remaining 5% (Correi, 1995). In newborn infants, glucuronide formation of paracetamol may be delayed because the enzymes synthesizing system are not completely developed, perhaps leading to enhanced toxicity (Vest and Streiff, 1959).

When paracetamol intake exceeds therapeutic doses the glucuronidation and sulphation pathway are saturated and the cryotron p450 – dependant pathway becomes increasingly important. Little or no hepatotoxicity results as long as glutathione is available for conjugation. However, with time hepatic glutathione is depleted faster than it can be regenerated, and accumulation of a reactive and toxic metabolite occurs (Correia 1995).

Consistent with this view, the effects of various pretreatments that alter the availability or glutathione also altered hepatic necrosis by practiced. (Mitchell and Jallow, 1974).

Depletion of glutathione by diethylmaleate-pretreatment dramatically potentiate paracetamol induced hepatic neurosis (Mitchell et – al 1973)


CURVE OF PARACETAMOL CONC IN SALIVA AGAINST TIME
PCM SALIVARY CONC (ug/ml)
40
35
30
25
20
15
10
5
0
0.25
0.5
1
2
3
4
5
TIME (hrs)
PCM ALONE
PCM + CMTD ©
PCM +CMTD(D)
PCM +MDP (CO)
PCM +MDP (D)

EXCRETION

Paracetamol is excreted in the urine primarily as inactive glucuronate and Sulphate conjugation. Cummings et al (1967) examined the excretion of paracetamol in man; they found that uncharged drug together with Sulphate and glucuronide conjugates are excreted in the urine.

Studies have shown that after oral administration of 1g dose of paracetamol, the average recovery of drug in all forms was 52% mostly as glucoronide and sulphate which are nontoxic (levy and Yamada 1971).

When paracetamol is taken in normal doses, the urinary excretion is as follows: Uncharged (3%) glucuronide conjugate (55%) sulphate conjugate (30%) and mercapturic acid conjugated (8%) (Thomas 1993). In an overdose without liver damage, there is an increased percentage in the sulphation pathway and increased metabolic activation of paracetamol. Glucuronidation is not saturated, but consumption of glutathione by the therapeutic acid conjugation pathway prevents detoxification and excretion of reactive intermediate metabolite N-acetyl –P-benzoquinone imines (Thomas (1993).

2.1.3 Mode of action of paracetamol

The analgesic effect of paracetamol is readily demonstrated against pain of moderate intensity such as occurs with head ache, neuralgia, rheumatism, lumbago

and dysmenorrhea. But intensive pain or visceral pain produced by smooth muscle spasm is not relieved (Woodbury, 1975).

The mechanism of analgesic action has not been fully determined (USP, 1986). Acetaminophen may act by inhibiting prostaglandin synthesis in the central nervous system (CNS) and though a peripheral action by blocking pain – impulse generation. The peripheral action may also be due to inhibition of the synthesis actions of other substances which sensitized pain receptors to mechanical or chemical stimulation (USP 1986).

The antipyretic effect of paracetamol in man has been recognized from the time it was first used in medicine. (USP,1986). Paracetamol is equivalent to aspirin as an analgesic and antipyretic agent (Styrct and Sugarman, 1990). This, it is probably the best substitute for aspirin as an antipyretic drug but not as an anti inflammatory compound. The drug is useful in mild to moderate pain such as headache myalgia postpartum pain, etc.

2.1.4 OVERDOSES/TOXICITY

Because it lacks many undesirable effects produced by aspirin is gaining favour as the “common household analgesic” (Tyle 1986). However, there is also growing concern that increasing house hold availability and public lack of recognition of paracetamol toxicity will produce a new health hazard (Sultan and Sotka, 1973;

Goulding, 1973). Plasma load of paracetamol in thirty patients who have taken overdoses of the drug was studied (Baillie and Leonard, 1974). Seventeen patients developed hepatic necrosis of the drug was found to be impaired in patients with hepatic injury and there was a significant correlation half – life in the plasma. This half – life was considered to be the most reliable early prognosis of liver damage. Such damage is to be expected if the half life exceeds 4 hours (Tyle, 1986, Clark et – al., 1973). Chronic excessive use of paracetamol (greater than 5g per day) for several weeks can produce hepatotoxicity, which is potentiated by chronic alcohol consumption (Baker et – al, 1977; Mc Chain et – al, 1980). In adult, symptoms of acute toxicity may occur following the ingestion of 10 – 15g of paracetamol (Poud and Wright, `1970). A simple oral ingestion of 15 – 25g is seriously hepatotoxicity and potentially fatal (Koch- Wester, 19786). Ambier and Alexander, 1977; Mc Junking et – al., 1976).

The progression of symptoms with acute paracetamol poisoning include; vomiting within a few hours, anorexia, and nausea and stomach pain within 24 hours; evidence of hepatotoxicity in 2 – 4 days with jaundice and death at any time in 2 – 7 days (Goodman and Gilman, 1980; Poud and Wright, 1980). In addition, kidney damage, disturbances in clotting mechanisms; metabolic acidosis, hypoglycemia and myocardial necrosis may occur (Sutton and Soyka, 1973; Goodman and Gilman, 1980). In man, the dose required to produce hepatic damage cannot accurately be estimated from the history of patients taking overdose as the dose taken is often not

accurately reported (Thomas, 1993). When used as directed, paracetamol rarely causes severe toxicity. Side effects associated with paracetamol may include hypoglycemia, jaundice and hematological disorders like neutropenia leucopenia, pancytopenia, and allergic reaction such as skin eruptions, urticaria and erythematous skin reactions.

Some animal studies were performed to establish whether paracetamol, like its precursor, phenacetin and acetanilide, might cause analgesic nephropathy after repeated use, but no evidence of this was found (Thomas, 1993). Hepatic necrosis after long exposure was reported in cat (Elder, 1964), and in rats toxic doses cause early death due to respiratory failure, however deaths occurring between 1-7 days were caused by hepatic necrosis (Thomas, 1993).

Management of paracetamol poisoning

Emergency first aid treatment of paracetamol should include emesis with syrup of ipecac (Krenzelok et – al 1977). Activated charcoal will reduce paracetamol absorption significantly but is most effective if given immediately after emesis within the first 3 minutes following paracetamol ingestion (Levy al Houston 1976). Fluid and electrolyte therapy may be required in the early stage of poisoning, if nausea and vomiting has been a feature.

Occasionally, IV glucose is needed to prevent hypoglycemia. IV sodium bicarbonate is often but there is no evidence that this effect outcome. In addition to supportive therapy, measures that have proved extremely useful are the provision of sulfhydryl groups to neutralize the toxic metabolites (Payan and Katzug, 1995).

2.1.5 PARACETAMOL – DRUG INTERACTION

There are few reports of pharmacokinetic drug interactions involving paracetamol in the literature and in most cases occur at the site of metabolism and gastrointestinal tract (GIT) absorption (Randal and Selitto 1985).

Anticholinegic may delay the onset of the response to acetaminophen, but the clinical importance of this effect is not established. The mechanism is by slowing gastric emptying thus, reducing the rate of paracetamol absorption from the intestine. (Lokken and Skjelbred, 1980). 1.5g of paracetamol was given orally to six patients with and without pretreatment with propanthaline (Probanthine) 30mg IV; the rate of absorption of paracetamol was considerably slower in the presence of propanthaline than in its absence (Boxil, et – al, 1958, Nimmo 1973).

Other agents with antichohinergic activity also probably delay paracetamol absorption (Heading et – al, 1973. Nimmo et al 1973 Prescott 1974). This interaction is likely to be clinically important in a patient receiving paracetamol several times daily, because extent of absorption is not affected (Hastens and Horn, 1989). In

contrast, the rate of paracetamol absorption has been shown to be hasten by metoclopramide with the extent of absorption unaffected (Nimmo et – al 1973, Nimmo 1973).

Patient who clinically received barbiturate may experience a somewhat reduced effect from therapeutic doses of paracetamol and perhaps increased toxic symptoms from paracetamol overdoses. (Jones, 1976).

In a study (Perucca and Richer 1979) the fate of paracetamol (Orally and iv) was compared in healthy subjects and epileptic patients receiving chronic anticonvulsant (Barbiturates, primidone, carbamazepine, phenytoin) therapy, results indicated a lower and paracetamol bioavailability and shorter serum half life of paracetamol following its intravenous Administration in epileptic patients on anticonvulsants. Phenobarbitone has been shown to increase the hepatotoxicity and neprotoxicity of paracetamol over dose in rat (Hasten and Horn, 1989), but evidence from humans is limited to isolated case reports (Boyer and Rouff, 1971).

It was reported that large doses of activated charcoal may reduce serum acetaminophen concentrations. Activated charcoal in large doses (5 – 10g) considerably reduces the gastrointestinal absorption of acetaminophen, however, the effect of smaller doses of activated charcoal, such as that found in antidiarrheals and antiflatulants on acetaminophen absorption is not established (Philip and John 1989).

Cholestyramine inhibits the gastrointestinal absorption of acetaminophen and as such markedly reduces plasma concentrations of acetaminophen and reduces acetaminophen therapeutic response. In four healthy subjects, concurrent administration of Cholestyramine 12g orally markedly reduced plasma level of acetaminophen 2.0g orally (Dordoni, et – al 1973).

Preliminary evidence indicates that acetaminophen may reduce diazepam bioavailability, but the clinical importance of this effect is questionable. Acetaminophen (3.0g/day for 5 days) somewhat reduced the 96 hours urinary excretion of diazepam and its metabolites in four subjects following a single 10mg dose of diazepam (Mulkey et – al 1978).

Food containing high content carbohydrates may delay the absorption of acetaminophen. This may be due to delayed entry of the drug into the intestine or to delay in tablet disintegration and dissolution (Mc Gilveray and Malhdet, 1972, Nimmon, et – al 19732). In a study (Jaffe, et – al 1971) high carbohydrate test meals (Crackers, Jelly and dates) significantly delayed absorption of acetaminophen as measured by urinary excretion. For rapid analgesia, Acetaminophen should not be taken with meals, especially meals high in carbohydrate (Philip and John, 1989).

It is proposed that metoclopramide increase the absorption rate of acetaminophen by stimulating gastric emptying. Metoclopramide may hasten the onset of the effect

of acetaminophen slightly but the clinical importance of this is doubtful. Although the absorption rate of acetaminophen has shown to be increased by metoclopramide in healthy subjects (Moxil et – al 1985; Nimmon, 1973).

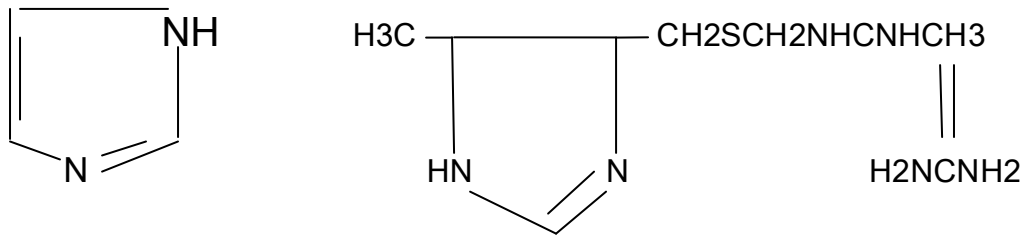
CIMETIDINE

2.1.6 INTRODUCTION AND CHEMISTRY:

Cimetidine is one of the drugs which have specific antagonistic activity on histamine H_2 – receptors. Its principal action is on parietal cell histamine H_2 – receptors. Cimetidine is the first histamine H_2 – receptor antagonist. With its safety profile, cimetidine has become extensively used in the treatment of ulcer disease and acid related disorders. The drug was introduced into market in 1976, became available in the United Kingdom (UK) in November 1976 and in the United States of America (USA) in August 1977, and by the end of 1978, it was available for clinic use in more than 90 countries (Duncan and Parsons, 1980) its popularly known with a brand name of Tagamet from Glaxowellcome.

2.1.7 STRUCTURE

Chemically Cimetidine is a substituted imidazole compound with a chemical nomenclature – 2 – cyna – methyl 3 – [2 – (5 – methylimidazole – 4 – ylmethylthio) ethyl] guanide (B. P. 2002) and a graphic structural formula below.



Imidazole Ring Cimetidine

Fig 14: Structure of Imidazole Ring and Cimetidine

Cimetidine is a weak base with a high degree of water solubility. These properties affect many of the drugs pharmacokinetic characteristics. The solubility of cimetidine in water is greatly increased with the addition of dilute acid which protonates the imidazole ring.

2.1.8 PHYSICAL PROPERTIES

1. Appearance - White to off-white crystalline powder
2. Odour - Odourless or with a faint odour.
3. PH – value - PH of 5.0mg/ml in carbon dioxide
   Free water is 8.0-9.5 (Basic)
4. Solubility - Slightly soluble in water, very soluble in
   Methanol and practically insoluble in
   Dichloromethane and ether.

5.	Stability	-	stable for 48 hours at room temperature when added to commonly Used i.v solutions.

2.1.9 CHEMICAL PROPERTIES

1.	Mol formula	-	$C_{1O}H_{16}N_6S$
2.	Mol weight	-	252.34
3.	Melting point	-	141 – 143^0C
4.	Loss on drying	-	when dried to constant weight at 100^0C – 105^0C, loses not more than 0.5% of its weight.

2.2.0 PHARMACOKINETICS:-

Cimetidine is rapidly absorbed from the gastrointestinal tract (GIT) and is approximately 60 – 70% bioavailable after oral administration. When Cimetidine is administered orally in a tablet or in liquid form in the fasting state, the blood/plasma concentration – time profile is discontinuous, 2 “peak” are observed, the first at about I hour ad the second after about 3 hours (Somogyi and Gugler, 1983).

Cimetidine distributes widely and extensively throughout the majority of body fluids, organs and tissue in man. There is extensive uptake of Cimetidine into selected

organs e.g. (kidney and lung) and tissues (Schentag et al 1981). However, the amount distributed depends upon the skeletal muscle uptakes of Cimetidine.

Cimetidine distributes into the cerebrospinal fluid (C.S.F) at a ratio of 0.1 to 0.2 compared with plasma. Higher ratio has been observed in patients with liver disease (Schentage et al, 1981). The mean saliva to plasma ratio is 0.2 (Somogyi and Gugler, 1983).

Cimetidine is eliminated from the body by renal, metabolic and billiary process. However, the principal, route is by renal elimination (Taylor et al, 1978; Grahnen et al1979; Walkenteins et al 1978). Between and 50 and 80% of the dose administered i.v is recovered in urine as unchanged Cimetidine. This fraction is less after oral administration, but is independent of the amount of the dose (Somogyi and Gugler, 1983). In ulcer patients, 40% is recovered unchanged in urine after oral administration. The high urinary excretion of Cimetidine coupled with low plasma concentrations results in a high renal clearance of the drug (Somogyi and Gugler 1983). Billiary excretion of Cimetidine accounts for only 2% of the dose (Gugler et al, 1981). Thus, the elimination of Cimetidine into bile is negligible and of no clinical significance.

Cimetidine is metabolized to 3 known products namely – Cimetidine sulphoxide, hydroxymethyl/Cimetidine and guanylurea Cimetidine, although the later may be formed non – enzymatically and may an in-vitro degradation product (Taylor et al (1978).

Elimination of Cimetidine is accelerated by an average of 15% in the presence of Phenobarbitone, due to induction of its metabolism (Somogyi et al 1981). Elimination half life of Cimetidine is approximately 2 hours in healthy adults with normal kidney and hepatic function (Somogyi and Gugler, 1983). It increases in renal impairments, hepatic impairments and in the elderly. Cimetidine is not significantly removed by haemodialysis or peritoneal dialysis (Drug facts and comparisons, 1989b).

2.2.1 MECHANISM OF ACTION:-

Cimetidine competitively and selectively inhibits the action of the histamine H2 – receptor of the parietal cells. Thus it inhibits the secretion of gastric acid stimulated by histamine, pentagastrin, acetylcholine, insulin, caffeine, food and other secretory substances.

2.2.2 USES

Cimetidine is used in conditions where inhibition of gastric acid may be beneficial such as in the following:-

- Short term treatment of active duodenal ulcer
- Maintenance therapy for duodenal ulcer patients at reduced dosage after healing of active ulcer.
- Pathological hypersecretory conditions such as in Zollinger – Ellison syndrome, systematic mastosytosis multiple endocrines.

- Unlabelled uses: - Oral or I.V Cimetidine, 60 – 90 minutes before anesthesia has been used to prevent aspirations pneumonitis.
- Cimetidine has been used in the prophylaxis of stress – induced ulcers and of acute upper gastrointestinal bleeding, in gastro esophageal reflux, tinea capitis, herpes virus infection and hirsute women (Drug facts and comparisons 1989b).

2.2.3 ADMINISTRATION AND DOSAGE

Cimetidine is available for oral use as tablets containing 200mg, 400mg or 800 mg and as a liquid containing 200 mg/5ml.

For treatment of active duodenal or benighin gastric ulcers, pathological hyper secretary conditions, dosage is 800mg at bed time or 400mg twice daily.

A dose of 400mg may be given at bedtime for prevention of recurrence of duodenal ulcers. For hospitalized patients with pathological hyper secretary conditions or intractable ulcers, or patients unable to take oral medication, parenteral form may be used. Usual dose is 300mg I.M or I.V every 6 to 8 hours. Dosage may be increased if necessary but not exceeding 2400mg.

2.2.4 ADVERSE EFFECT OF CIMETIDINE

Cimetidine is among the safest drugs currently available. Most adverse effects of Cimetidine are of minor nature and are usually promptly reversible upon stopping treatment.

Side effects of Cimetidine include altered bowel habit, intestinal nephritis, acute pancreatic, tiredness and hypersensitivity. Reversible liver damage and hematological disorders are reported. Cardiac arrhythmias and arrest following I.V bolus may occur. Gynaecomastia is also an occasional problem with Cimetidine and reversible impotence has also been reported.

CNS adverse effects associated with Cimetidine include dizziness, headache, confusional states, hallucinations and delirium. The apparent causes of Cimetidine included CNS toxicity appear to be due to the prescribing of large doses of Cimetidine in patients who have a reduced ability to eliminate the drug (Somogyi and Gugler, 1983).

2.2.5 CIMETIDINE DRUG INTERACTION

Interactions with Cimetidine occur primarily through 3 different mechanisms:-

The ability of Cimetidine to raise gastric P^H may have influence on oral drug bioavailability. Change in P^H may also increase the stability and presumably the oral bioavailability of acid-labile drugs (Mayersohn, 1979).

Cimetidine, like other substituted imidazoles inhibits various microsomal drug metabolizing enzymes in animals as human liver (Wilkinson et al, 1974). Thus in man Cimetidine interacts with various clinically important drugs (Somogyi and

Gugler, 1982; Klotz and Reiman 1984) which are extensively metabolized in the liver.

Due to the ability of Cimetidine to undergo renal tubular secretion via the renal cationic transport system, competition with other basic compounds renally secreted may occur as in the case of triamtene (Somogyi et al, 1989) Chlopropamide (MOcda et al 1994) Zidovudine (Fletcher et al 1995) and creatinine (Zeimniok et al 1986).

2.2.6 EFFECTS OF CIMETIDINE ON ABSORPTION OF OTHER DRUGS:

In 1978, Fairfax et al concluded that increased absorption of acid – labile drugs can occur in some patients taking Cimetidine. Cole et al 1980 concluded that chronic treatment with Cimetidine reduced the absorption of tetracycline from a capsule formulation, but had no effect when tetracycline is given in solution form. This measured by urinary excretion.

Cimetidine has been shown to slightly increase serum salicylates concentrations and bioavailability following administration of enteric – coated aspirin (Paton et al 1983, Willoughby et al 1983).

In a study by Rogers et al, 1980 where six healthy subjects received 400mg of Cimetidine every 6 hours, and on the 6th 500mg ampicillin given and assessed by the peak day plasma concentration (Cmax), the time of its occurrence (Tmax), the AUC and the terminal half life, Cimetidine induced no alterations in ampicillin disposition.

In the same study with Cotrimaxazole – no significant differences in the pharmacokinetics of sulphamethoxazole or trimethoprin were observed, although plasma concentration of the later tended to be higher under Cimetidine treatment.

When 400mg of Cimetidine was given 2 hours prior to administration of ketoconazole, the AUC of ketoconazole was reduced by 65% over the control, but was increased by 52% when ketoconazole was given in an acid solution, again 2 hours after Cimetidine (Vander meer et al, 1980).

Cimetidine increases the urinary excretion of Zidovudine decreasing the fraction of Zidovudine, hence converted to metabolites. It was concluded that Cimetidine presumably inhibits the renal clearance of Zidovudine by competing for renal tubular secretion.

Pharmacokinetics studies have consistently shown that Cimetidine reduces theophyline plasma clearance, increase theophyline half life and increase plasma theophyline levels (hanstern and horn (1989d).

Ahmad et al 1992 reported the effect of Cimetidine on the LD_{50} of paracetamol in mice. The LD_{50} of paracetamol was increased by the administration of Cimetidine. Thus Cimetidine protects liver injury induced by paracetamol and could be used clinically in patients intoxicated with paracetamol.

2.2.7 CLINICAL SIGNIFICANCE OF CIMETIDINE DRUG INTERACTION.

Drug interaction caused by Cimetidine has stimulated the interest of clinicians and pharmacist due to the large number of drugs affected. However, much more has been made of this than is probably justified, given that most such interactions are of purely pharmacokinetic interest (Howden, 1993). Before the effect of Cimetidine interaction can be regarded as significant, it is suggested that the change caused by the interaction should be between 20 – 25% (Upton et al 1982).

It has been shown that some of the effects of Cimetidine interactions are not significant at clinical level. However, many of those which are potentially harmful only occur in a small proportion of patients. (Howden 1993).

Clinically important interactions do occur between Cimetidine and certain other drugs which extensively metabolized in the liver and a narrow therapeutic index. Example a decrease in elimination of benzodiazepines during Cimetidine administration may not necessitate dosage changes in most patients, since benzodiazepine have a wide therapeutic concentration rage. However, Cimetidine-induced inhibition of metabolism of, for example anticoagulants can have serious consequences (Somogyi and Gugler, 1982). Patients at increased risk from Cimetidine-drug interactions include the elderly and those with impaired renal or liver function.

PRECAUTIONS

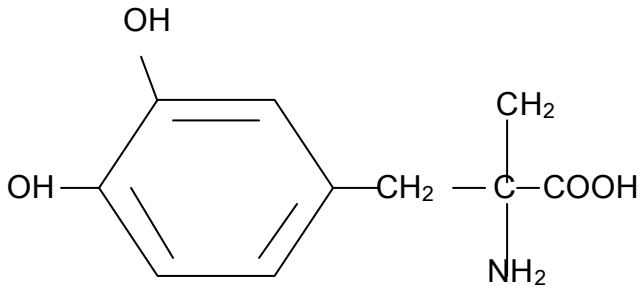
Reduce dose in renal and hepatic impairment, avoid I.V injection in high dosage and in cardiovascular impairment, pregnancy, breast feeding.

METHYLDOPA

2.2.8 Introduction

Methyldopa is an adrenergic neuron-blocking agent. It is the alpha methyl congener of the catecholamine precursor, 3, 4, dihydroxy phenyl alanine (Dopa). Methyldopa appeared to induce neither release of norepinephrine from storage sites and to interfere with the release in response to stimuli, probably through the action decarboxylated metabolites of methyldopa methyldopamine and methyl norephineprine.

2.2.9 **Structure**



1(-)-3-(3,4 Dihydroxy phenyl) -2- methylalanine

Fig 15: structure of methyldopa

2.3.0 Physical properties

Colour: - white to yellowish

Texture: - Fine powder

Ordourless - oudourless,

Test - Testless

Stability - relatively stable in both light and air.

Solubility - Sparingly soluble in alcohol, practically Insoluble in water

2.3.1 Chemical properties

Mol formular - C_{10} H_{18} N O_4

Melting point - above 29^0C

Mol weight - 211.22

2.3.2 Administration and Doses

Methyldopa comes in a form of tablet normally in strength of 250mg. The usual dose is between 500mg to 2g daily in divided doses. Maintenance dose is between 1-1.5g in divided doses.

2.3.3 Uses

Methyldopa is used in the treatment of severe to moderate hypertension.

It is also used in the treatment of primary or essential hypertension.

2.3.4 Mechanism of action

Methyldopa mechanism of antihypertensive actions is currently thought to involve stimulation of central alpha-adrenergic receptors by a metabolite, alpha-methylnorepinephrine, thus inhibiting sympathetic outflow to the heart, kidneys and peripheral vasculature. Reduced peripheral resistance and plasma rennin activity levels may also contribute to its effect.

2.3.5 Bioavailability

Oral absorption is incomplete and excretion is via urine and faeces..

2.3.6 Drug interactions

- Concurrent use with the following drugs is not recommended: Monoamine oxidase (MAO) inhibitors, levodopa and methotrimepvazine.

- Concurrent use with the following drugs requires careful monitoring: alcohol, antihypertensives, general anaestetics and norepinephrine.

2.3.7 Overdosage

The major effects that are observed are dizziness, drowsiness, hypertension, tachycardia or bradycardia, hypothermia, and extra-pyramidal signs.

Treat overdosage by emesis or gastric lavage, if appropriate, and IV 1-2mg benztropine if extrapyramidal sings are present.

2.3.8 Main side/Adverse effects

The main side effects are drowsiness, edema, dry mouth and headache.

2.3.9 Precautions

- Evidence of hemolytic anemia is indication for discontinuing the drug.
- Positive coomb's tests may indicate an incompatible cross-match when transfusion is required and an indirect coomb's test should be carried out.
- Safety for use in pregnancy and lactation has not been established.
- Caution if any kind of surgery or dialysis is required.

2.4.0 AIMS AND OBJECTIVES OF THE STUDY

The aim and objective of this study are as follows:

1 To determine pharmacokinetics of paracetamol when administered alone and in combination with cimetidine and methyldopa concurrently and at delay period.

2 To evaluate the effect of cimetidine and methyldopa on the pharmacokinetics of paracetamol when administered at the same time and at time interval of an hour.

3 To compare the effect of cimetidine and methyldopa both on concurrent and delay administration.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1.0 Glass ware

- 10ml extraction tubes, Pyrex, England
- 20ml burette, Pyrex, England
- Crucible
- Glass weighing pan
- Conical flasks, 250ml, 100ml, 50ml, 500ml, 25ml, and 1000ml
- Round bottom flask, Pyrex, England
- Glass funnel and filter papers (whatman)
- Measuring cylinders, 5ml, 10ml, 50ml, and 100ml.
- Pipettes, 0.02ml, 0.1ml, 1ml,5ml and 10ml
- Testubes,10ml and 20ml
- Syringe and needle,2ml, 5ml and 20ml
- Reflux condenser, Pyrex, England

3.1.1 Equipment

- Centrifuge, junior, Gallenkamp, Gilson, England
- Hot air oven BS size one Gallenkamp, England
- Electronic balance, Metller, AE 240
- Disintegration rate study apparatus, Erweka, Germany

- Dissolution rate study apparatus, Erweka, Germany
- Auto-vortex mixer, Stuart, England
- SP8-100UV spectrophotometer, Pye unicam
- Hot plate, B212, Bibby, UK
- Water bath, Gallenkamp, England
- Friabilator, Erweka, Germany
- Melting point apparatus, Gellenkamp, England
- HPLC priming syringe, Hamilton, England

3.1.2 Reagents and standard samples

Chemicals

- Methanol, Analar, BDH Chemicals England
- Sodium hydroxide pellet May and Baker, England
- Potassium hydroxide, May and Baker
- Bismuth oxynitrate, Analar, BDH Chemicals England
- Ferric chloride, May and Baker, England
- Distilled and filtered water
- Acetic anhydride, May and Baker, England
- Lead nitrate, May and Baker, England
- Crystal violet, Harris reagent, England
- Glacial acetic acid Harris, England
- Hydrochloric acid Analar, BDH Chemicals, England

- Acetone, May and Baker, England

Standard sample

The standard sample of paracetamol was obtained from SAA PHARMACEUTICAL COMPANY, Kano while that of cimetidine and methyldopa were obtained from Smithkline Beecham (Nig) Ltd and

Bond Chemicals Nig Ltd, respectively

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3.1.3 Drug Sample

The following is the information of the tablet sample used in the practical:

Paracetamol Tablets

SOURCE: Purchase from Imperial Pharmacy, Kano

BRAND NAME: Panadol ®

MANUFACTURER: Sterling Products Nig Ltd

MANU DATE 10, 2007

EXP DATE: 10, 2010

STRENTH: 500mg per tablet

BATCH NO: 186D

Methyl Dopa Tablet

SOURCE: Purchase from Imperial Pharmacy, Kano

BRAND NAME: Bondamet ®

MANUFACTURER: Bond Chemicals Nig Ltd

MANUF DATE: 12, 2006

EXP DATE: 12, 2009

STRENGTH: 250mg per tablet

BATCH NO: VS231D

Cimetidine Tablet

SOURCE: Purchase from Imperial Pharmacy, Kano

MANUFACTURER: Smithkline Beecham Nig Ltd

BRAND NAME: Tagamet®

MANU DATE: 09, 2006

EXP DATE: 09, 2009

STRENGTH: 200mg per tablet

BATCH NO: 325478

3.1.4 METHODOLOGY

3.1.5 In- vitro study

The research work was divided into two segments, the in vitro study and the in vivo study. The in vitro study was used to determine the quality control of the tablets which on having a satisfactory result will allow for the in vivo study to commence. The quality control tests carried out were identification test, uniformity, assay, dissolution test, and disintegration and friability tests. These tests were conducted according to the BP 2002 specifications.

3.1.6 Construction of calibration curve

i) **Preparation of paracetamol stock solution**

Calibration curve was constructed using the data generated from the result. The stock solution was prepared using standard paractamol powder, and a concentration of 5mg/ml was prepared by dissolving 100mg of the powder in 100ml of Methanol thereafter withdrawing 5mls of the solution which is equivalent to 5mg/5ml and using same and made a serial dilution to prepare 10ug/ml, 20ug/ml, 30ug/ml, 40ug/ml and 50ug/ml.

Sufficient quantity of blank (drug-free) saliva was collected with the aid of chewing a piece of semi solid paraffin. The collected saliva sample was pooled and then distributed into twelve in a 10ml extraction (Centrifuge) tubes each containing 2mls, using an auto pipette. Two out of the twelve tubes were kept different to serve as

banks (without drugs) while the remaining ten were paired and spiked with paracetamol concentrations in methanol of 10µg/ml, 20µg/ml, 30µg/ml 40µg/ml and 50µg/ml respectively (using microcline Hamilton syringe).

To each of the twelve samples 5ml of ethyl acetate was added, the extraction centrifuges were well stoopered. The mixture in the tube was vortex-mixed for 1 minute and centrifuged at 2500rpm for 5 minutes. The upper layer (ethyl acetate) was removed from each of the tube using a pasture pipette.

3.1.8 Analytical Method

The analytical method used was adopted and modified from Garba M. 1992. The absorbance for each tube corresponding to the concentration of paracetamol was measure at 262nm using 1cm silica curette, using a double bean sp 8-100 UV spectrophotometer.

The mean absorbance for each set of concentration was calculated. And using the result the calibration curve was constructed by plotting a mean absorbance obtained against the corresponding concentration of paracetamol (10µg-50µg/ml) in saliva samples and correlation coefficient calculated.

3.1.9 Validation of the calibration curve

The calibration curve constructed was validated by randomly spiking a set of six (6). Two milliliter (2mls) blank saliva samples with different known concentrations of paracetamol. Each sample was then extracted with 5ml ethyl acetate and the

absorbance corresponding to the various spiked concentration obtained by UV measurement. The absorbance data obtained were then converted to the corresponding concentrations of paracetamol using the calibration curve also the concentration obtained were then compared with the actual spiked concentrations and correlation calculated.

The residue obtained was dried at 105^0C and then 0.1g of same was boiled with 1ml of hydrochloric acid for 3 minutes 10ml distilled water was added and cooled. (No precipitate was produced). 0.05ml of 0.137M Potassium dichromate was added, a violet color was produced slowly which did not turn red. Melting point of 169^0c was determined.

3.2.0 QUALITY CONTROL TEST

Uniformity of weight

The method used was in accordance with BP2002. Twenty tablets of paracetamol were selected at random and their average weight determined. The tablets were then weighed individually and the percentage weight deviation of each tablet from the mean (average) weight was calculated. The same procedure was repeated for tablet cimetidine. The result obtained was recorded accordingly (see chapter four).

Assay of paracetamol tablet

Six tablets were randomly selected for the assay, each was weighted, powered and kept separately, and 0.15g of each was taken and subjected to the following test:

50ml of 0.1M sodium hydroxide was added to 0.15g of the powdered paracetamol and then diluted with 100ml of water, it was shaken for 15 minutes and sufficient water was added to produce 200ml, the solution then filtered and 10ml of 0.1M sodium hydroxide was added to 10mls of the filtrate and made up to 100ml with distill water. The absorbance of the resulting solution was measured at the wavelength of 257nm and the percentage content for each tablet was determined.

Disintegration test

The disintegration test was carried out in accordance with the BP 2002 specification, as seven tablets of paracetamol were picked at random. The Eureka disintegration apparatus was used. Each tablet was placed in the tube of the basket; the assembly of the tubes was suspended in a beaker containing the disintegration medium. (Distilled water) maintained at $37^0C \pm 1^0C$ by an electrically heated water bath. The assembly of the tube was made to move up and dawn in the medium so that the tablets were constantly agitated. The time taken for all the particles of the disintegrated tablets to pass freely through the mesh at the lower end of the tubes was noted for each.

Dissolution test

The Rotary Basket method was used as described in the BP 2002. One tablet of paracetamol was placed in the basket and the assembly was placed in the flask containing 1000ml of 0.1M hydrochloric acid, it was warmed and maintained between 36.5 and 27.^{0}c by an electronic heater. The stirrer was set at 100rpm and the apparatus was operated for 45minutes the sample of the dissolution medium was then withdrawn, filtered and assayed using a UV spectrophotometer at wavelength of 257nm. Using the same procedure, six different tablets were subjected to the same test. The percentage of paracetamol released from the dosage form after 45 minutes was calculated. Using the formula;

$$\text{Percentage released} = \frac{\text{Amount of sample released}}{\text{Content of standard pc m tablet}}$$

Friability test

Ten tablets were weighed and put into a friabilitor (Eurweka) which was set to rotate at 25rpm for 4 minutes, at the end of 100 revolutions; the tablets were removed and weighed again. The two weights were compared and the percentage loss was determined.

$$\text{Percentage friability} = \frac{\text{(Initial weight) – (Current Weight)}}{\text{Initial weight}}$$

3.2.1 Extraction Method

The extraction method was adopted and modified from Garba M. 1992; with percentage recovery of 96%.

2mls of saliva sample was placed in 10ml centrifuge tube using auto-pipette. 5mls of ethyl acetate was then added. The centrifuge tube was stopped with plastic screw-caps and was shaken vigorously for one minute with a Rota mixer, and centrifuge for five minutes at 2500rpm. The ethyl acetate layer (upper layer) was removed with Pasteur pipette and kept at -4^{o}C for analysis.

3.2.2 IN-VIVO PHARMACOKINETIC STUDIES

The last segment of the practical involves the participation of eight healthy male volunteers aged between 28 to 32 years, weighted between 55 to 64kg. The volunteers were assessed clinically fit for the study and were made sure that all of them were free from taking any drug for the last two week, before the commencement of, and during the study. They were all non smokers and non alcohol consumer. The volunteers were fully briefed on the study and were informed on their role in the practical.

The crossover study was divided into five phases and was approved by the ethical committee of Murtala Mohamed special Hospital, Kano.

Protocol

The protocol of study was designed to have a wash – out period of 2 weeks between the phases of the study.

- The first phase of the study involved the ingestion of 1g dose of paracetamol tablet with about 50ml of water after overnight fasting. Saliva sample of 4ml were then collected prior to ingestion of drug and thereafter at the interval of 0.25, 0.50, 1.0, 2.0, 3.0, 4.0 and 5.0 hours with the aid of chewing semi solid paraffin. The saliva samples were then store at -4^{o}C pending analysis.

- The second phase involved the concomitant ingestion of two tablets of paracetamol 500mg and 400mg tablet of Cimetidine after overnight fasting, and the saliva samples were collected at the interval of time similar to that of phase one, same were treated and stored for analysis as mentioned above.

- The third phase was ingestion of 400mg tablet of Cimetidine followed by two tablets of paracetamol 500mg one hour later (delayed) saliva samples were collected at the ingestion time (zero hour) and the times mentioned above, thereafter treated and kept for analysis.

- The fourth phase involved the concomitant administration of two tablet of paracetamol 500mg and a tablet of 250mg tablet of methyldopa after overnight fasting saliva samples were later collected at 0 to 5hrs as usual.
- The last phase involves ingestion of one tablet of methyldopa followed by two tablets of paracetamol 500mg an hour later. Saliva samples were later collected immediately and at the other times as mentioned above.

3.2.3 Sample Handling

Sample collected were extracted using extraction procedure mention above and absorbance was measured at λmax 270nm.

3.2.4 Analysis of sample

Absorbance was converted to concentration and using the calibration curve pharmacokinetics parameters were generated by residual method and AUC was determined by trapezoid rule. All pharmacokinetic data, obtained were compared and the statistically significance of the difference determined using the student t = test of paired data, Values of $p < 0.05$ considered insignificant and $p > 0.05$ considered insignificant.

CHAPTER FOUR

4.0 RESULTS

4.1.0 Quality Control Assessments

4.1.1 Uniformity Test

Tab 1.2: Summary of the uniformity contents of the six tablets of paracetamol

NO	% CONTENTS	COMMENT
1	98.0	Satisfactory
2	99.7	√
3	100.5	√
4	99.3	√
5	102.5	√
6	101.1	√

Mean ± Sem 100.92 ± 0.970

4.1.2 DISSOLUTION TEST

Table 1.3 Mean dissolution profile for four brands of six paracetamol tablets.

Table NO	TIME(MIN) RELEASED	% RELEASED			
		I	II	III	IV
1	45	95	92	94	95
2	45	96	95	93	93
3	45	92	87	88	90
4	45	85	90	88	94
5	45	87	99	98	88
6	45	90	92	92	87

.

4.1.3 Disintegration test

Table 1.4 RESULT OF DISINTEGRATION TEST

TABLET NO.	DISINTEGRATION TIME (MINS)	COMMENT
1	4.5	Satisfactory
2	4.3	,,
3	3.3	,,
4	3.8	,,
5	4.7	,,

Mean 4.12 Sem ± 0.631

4.1.4 FRIABILITY TEST

Table 1.5 Results for friability test

Drug	Cimetidine	Paracetamol
No of Tablet	10	10

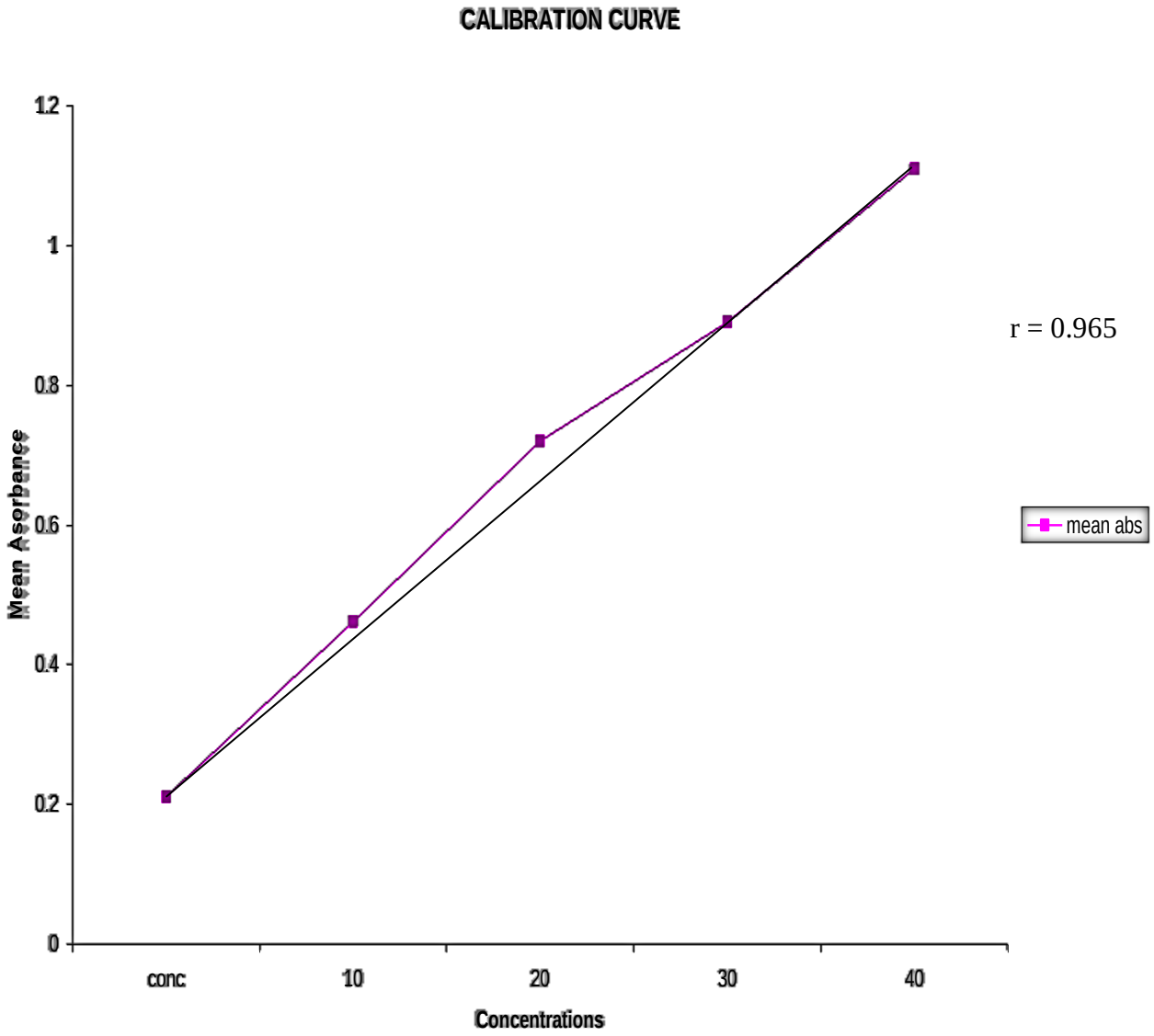
Weight before	4.017g	5.891g
Weight after	3.9937g	5.8504g
Percentage loss	0.58%	0.69%

4.1.5 Calibration Curve Data

Table 1.6 Results for calibration curve

Conc.(ug/ml)	Mean Absorbance(nm)
0.00	0.000±0.000
10.00	0.245±0.0655
20.00	0.459±0.0351
30.00	0.754±0.0945
40.00	0.924±0.0552
50.00	1.123±0.0126

Fig 16: Calibration Curve of mean absorbance against concentration



4.1.6 Validation of calibration curve

Table 1.7 Validation of the calibration curves

s/n	Spiked Conc	Absorbance (nm)	Estimated conc from the graph(ug/ml)	Remark
1.	0.00	0.00	0.00	Good Correlation
2.	5.00	0.558	4.80	” “
3.	15.00	0.660	15.50	” “
4.	25.00	0.991	23.80	” “
5.	35.00	0.559	33.80	” “
6.	45.00	0.662	45.10	” “
7.	55.00	1.000	54.10	” “

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4.1.7 In–vivo pharmacokinetics

Table 1.8 Results for pharmacokinetics studies for the five phases are as follows

Mean paracetamol concentration in saliva (ug/ml)

Time (h)	PCM Alone (1g)	PCM+CMT Concomitant Administration	PCM + CMT Delayed Administration	PCM +MTD Concomitant Administration	PCM +MTD Delayed administration
0.25	11.65	10.21	8.17	10.18	10.08
0.50	17.95	22.33	15.58	18.08	18.53
1.00	36.08	33.45	23.33	35.58	36.58
2.00	30.33	24.70	17.45	28.85	27.08
3.00	21.50	17.58	11.25	18.80	20.83
4.00	15.42	12.50	8.17	12.70	16.03
5.00	10.92	7.50	5.13	8.12	9.80

Fig 17: curve of paracetamol concentration in saliva against time

4.1.9 Comparism of pharmacokinetic parameters

Table 2.0: Comparism of mean pharmacokinetics parameters of phase I and II values ±SEM

S/N	Pharmacokinetics parameters	Phase I PCM Alone	Phase II PCM+CMT (Concur)	o/o change	P-values
1.	$t_{½\alpha}$ (hr)	0.3096	0.3142	0.02	P>0.05
2.	K_{α} (hr^{-1})	2.4013	2.3712	0.05	P> ”
3.	C_{max} (ug/ml)	36.08	33.45	0.52	P> ”
4.	t_{max} (hr)	1.00	0.980	1.00	P> ”
5.	$t_{½\beta}$ (hr)	1.698	1.665	0.35	P> ”
6.	K_{β} (hr^{-1})	0.680	0.6650	0.00	P> ”
7.	Lag time (hr)	0.1560	1.520	0.06	P> ”
8.	Vd (L)	30.2011	28.841	5.30	P> ”
9.	Cl (L/hr)	10.9610	11.650	0.02	P> ”
10.	AUC_{0-5} (ug/hr/ml)	140.140	138.58	1.10	p> ”

Table 2.1 Comparism of mean pharmacokinetic parameters of phase I and III ± SEM.

		Phase I	Phase III		
	Pharmacokinetics	PCM alone	CMD+PCM	o/o charge	P-values
S/N	parameters		Delayed		
1.	$t_{½\alpha}$ (hr)	0.3096	0.5020	52.21	P< 0.05
2.	K_{α} (hr^{-1})	2.4013	1.3712	42.26	P< "
3.	C_{max} (ug/ml)	36.080	23.33	35.34	P< "
4.	t_{max} (hr)	1.000	1.324	52.40	P< "
5.	$t_{½\beta}$ (hr)	1.698	1.272	23.33	P< "
6.	K_{β} (hr^{-1})	0.6801	0.3767	44.61	P< "
7.	Lag time (hr)	0.1560	0.2243	43.78	P< 0.05
8.	Vd (L)	30.2011	40.783	35.04	P< "
9.	Cl (L/hr)	10.961	13.541	23.54	P< "
10.	AUC_{0-5} (ug/hr/ml)	140.140	81.149	42.09	P< "

Table 2.2 Comparism of mean pharmacokinetics parameters of phase I and IV ± SEM

S/N	Pharmacokinetics parameters	Phase I Paracetamol Alone	Phase IV PCM+ MTD(Concur)	o/o change	P-values
1	$t_{½\alpha}$ (hr)	0.3096	0.3103	0.02	P>0.05
2	K_{α} (hr^{-1})	2.4013	2.389	0.05	P> ”
3	C_{max} (ug/ml)	36.08	35.58	0.52	P> ”
4	t_{max} (hr)	1.00	1.010	1.00	P> ”
5	$t_{½\beta}$ (hr)	1.698	1.704	0.35	P> ”
6	K_{β} (hr^{-1})	0.6801	0.6779	0.00	P> ”
7	Lag time (hr)	0.1560	1.550	0.06	P> ”
8	Vd (L)	30.2011	31.721	0.53	P> ”
9	Cl (L/hr)	10.9610	11.689	0.02	P> ”
10	AUC_{0-5} (ug/hr/ml)	140.140	138.58	1.10	p> ”

Table 2.3 Comparism of mean pharmacokinetics parameters of phase I and V±SEM

S/N	Pharmacokinetics Parameters	PCM alone	MTD+PCM (DELAYED)	o/o change	P-values
1.	$t_{½\alpha}$ (hr)	0.3096	0.3089	0.00	P>0.05
2.	K_{α} (hr^{-1})	2.4013	2.3990	0.10	P> ”
3.	C_{max} (ug/ml)	36.080	36.58	0.02	P> ”
4.	t_{max} (hr)	1.000	1.000	0.00	P> ”
5.	$t_{½\beta}$ (hr)	1.698	1.867	1.00	P> ”
6.	K_{β} (hr^{-1})	0.6801	0.7023	3.26	P> ”
7.	Lag time (hr)	0.1560	0.1640	5.13	P> ”
8.	Vd (L)	30.2011	32.012	6.00	P> ”
9.	Cl (L/hr)	10.961	10.550	3.75	P> ”
10.	AUC_{0-5} (ug/hr/ml)	140.140	139.96	0.01	P> ”

CHAPTER FIVE

5.0 Discussion

There have been so many research conducted to prove that some drugs after oral administration appears in saliva equally like in plasma, paracetamol has been shown to be among them. For this reason, saliva can be used to determine the pharmacokinetics of paracetamol.

Graham, 1982 reported that the measurement of saliva concentration of a drug is of most value in studies on compounds that are low ionized at physiological P^H values like neutral drugs, phenytoin and weakly basic drugs e.g. carbamazepine and antipyrine where good parallelism is shown between saliva and plasma drug concentration.

Ahmed and Enever, in 1981 showed that correlation between saliva and plasma level of paracetamol allow for the estimation of some simple pharmacokinetic parameters from their salivary concentration. The objective of this single dose study is to observe the profile of interaction without any disturbance by the multiple drug regimens which will mask the pharmacokinetic interaction profile (Garba et al 1998). Also the work of Garba et al 1998 shows that the salivary level of paracetamol

demonstrates a good correlation to their plasma levels. This also goes along way with the findings of Graham, 1982

The most important pharmacokinetic parameters considered in order to evaluate the rate and extent of absorption of paracetamol in this study are: absorption rate coefficient (Kα), time corresponding to maximum absorption (t_{max}), maximum concentration absorbed (C_{max}), lag time and t½α. While those parameters indicative of metabolism and/or excretion was also considered. These include the following: time taken for quantity of a drug available to be reduced by half ($t_{½\beta}$), Coefficient of elimination (K_β), Clearance (Cl) and Volume of distribution (Vd).

It was observed that the mean maximum concentration (C_{max}) of paracetamol achieved after oral administration of 1g of paracetamol was 36.08ug/ml. In comparism, this value was a little above that found by Garba et al (1997) using plasma (30ug/ml) and 29.45ug/ml of Bukhari (1996), and may be due to possibly inter subject variability or influence of food. The absorption half life (t½α) was found to be 0.2546hrs with corresponding absorption constant (kα) of 2.4013hr^{-1}. It was observed that Garba et al (1997) reported a value of 2.1hr^{-1} for Kα (using plasma) indicating a good correlation. The t½α of 0.3096 found by Bukhari (1996) agrees with the figure of 0.34hr for this study.

The time taken to reach the peak absorption (t_{max}) for this phase is 1hr, The value obtained by Ameer and Greenblat (1977), and Payan and Katzung was within 60-82 minutes (1995); while Goodman Gilman (1990) reported a value of 60-90minutes.
The lag time (time taken between the administration of a drug and the time absorption begins) for this phase was found to be 0.1560hrs. This indicates a good correlation when compared with of 0.150hrs reported by Garba et al (1997), However Ameer et al (1983) reported 3-4 minutes this may be related to physiological conditions or other factors.

The AUC_{0-5} was found to be 104.191ug/hr/ml while $AUC_{0-\alpha}$ was 136.246ug/ml/hr. Sambo et al (2002) reported $AUC_{0-\alpha}$ of 140.14ug/hr/ml, while Bukhari (1996) reported value of 129.98. The elimination half life and corresponding elimination constant were found to be 1.698hr and 0.6801 respectively. The $t_{½\beta}$ is within the range of 1-2 hrs reported in some literature (Clissold, 1986; Drug facts and comparisms, 1989a. Goodman and Gilman 1990; Payan Katzug, 1995). It can be seen then the value for $t_{½\beta}$ and that of k_{β} obtained in the study are within the normal rage. Bukhari (1996) and Bello (1990) reported a volume of distribution (Vd) of 43.012 L/Kg and 40.75 L/Kg respectively using saliva. However, the volume of distribution found in this study was 30.2011 which is shorter compared to the previous studies.

There has been an extensive investigation of pharmacokinetic of paracetamol such as that of Prescott, 1974, Rawlings, 1977 and Thomas, 1993; all of which they

established individual variability in the absorption and disposition of paracetamol when subjects are given the same time. This could be seen in the study conduct by Prescott, 1974 who reported that under practical clinical conditions, the variation among the plasma concentrations of paracetamol in 43 fasting patients 1 hour after oral administration of therapeutic close can be as much as 80 folds.

Heading et al, 1973 suggested that the variation in paracetamol pharmacokinetics among individuals might be due to individual difference in the rate of gastric emptying.

Other physical and environmental factors that may course inter-subject variation in paracetamol pharmacokinetics include, age body weight, six, smoking, diet, disease condition etc. the pharmacokinetics of paracetamol after a single oral dose of 1g paracetamol tablets in fowl to be linear. (First order kinetics) all the value for the pharmacokinetic parameters found in this studies are within the normal range established by some literatures and other research work.

The mean pharmacokinetics parameters when paracetamol was administered alone are now compared with those when paracetamol was given with Cimetidine and methyldopa concurrently and at time interval. The variation in figures will now be used to establish a fact whether the second drug (cimetidine or methyldopa) has influence on the absorption of paracetamol or not. In addition to this, the influence of

time variation i.e. whether there is any change in absorption of paracetamol when the two drugs are administered concurrently or at one hour delay. Consequently the change in these parameters stand as a determining factor as to whether cimetidine interacts with paracetamol and inhibit its absorption.

It is obvious from the above data when compared, that there is no significant changes in the pharmacokinetic parameters of the two phases, this mean that the P-value is $P>0.05$, Which indicates that there no influence in the absorption of Paracetamol by cimetidine as well as methyldopa when either was orally administered concurrently with paracetamol. The fndings of Ameer and Greebat, 1977 and Payan and Katzurg 1995 which reported that the time to reach peak plasma and other biologic fluid of paracetamol concentration is between 0-5-1 hr as well as that of Walkentein et al, 1978 who stated that the time to reach the peak concentration of cimetidine after oral administration is 1-2 hours, support the proposal that there is no interaction or absorption inhibition of paracetamol by cimetidine on concurrent administration of the two drugs. Thus, when the two drugs are concomitantly administered paracetamol will appear at a reasonable concentration in the plasma and other biologic fluids earlier enough that cimetidine and the effect of cimetidine on pharmacokinetics of paracetamol will not be manifested (Garba et al, 1997). He further stated that the effects of cimetidine on the absorption paracetamol were only evidence where the H_2 antagonist was given 1 hour before ingestion of paracetamol.

In order to determine the influence of cimetidine in the absorption of paracetamol the pharmacokinetics parameters of phase I (paracetamol alone) and those of phase III (cimetidine paracetamol delayed) are to be compared and analyze the changes in the two figures.

In this phase Paracetamol administration was analyzed by an hour after cimetidine ingestion. There are so many changes observed in pharmacokinetic parameters when paracetamol was administered alone compared to that when paractamol was administered an hour after cimetidine, this is as a result of possible drug interaction squeal to the inhibition of Paracetamol a absorption by cimetidine. It could be seen that there is considerable reduction in the values of C_{max} and Kα by 36.45 % and 47.05% respectively (P< 0.05) which clearly indicates that there is a reduction in the absorption of Paracetamol.

Tmax, $t_{½α}$ and lag time had significantly increased by 52.40% 52.5% and 43.78% respectively (P<0.05).

Looking at the two results, it could be seen that, there is an increase in the value of t½β by 44.61% (p<0.05) volume of distribution by 35.04% (P< 0.05) and AUC by 42.09% (P< 0.10). This has shown that there is a marked change in these parameters.

In general, the study showed that cimetidine inspired the absorption of Paracetamol under the protocol of delayed administration by one hour.

The reduction in the value of elimination (clearance) and subsequent increase in that of AUC indicated that there is inhibition in the metabolism of Paracetamol. However, an elevation of paracetamol concentration was not noticed, this may be as a result of impairment and inhibition of metabolism takes place simultaneously. Welkins et al, 1974 reported that cimetidine a substitution imidazole, compound is known to be a potent inhibitors of oxidation, an effect which can be explained by the interactions with the microsamal cytochrome P- 450system (Pruning and pelkonen 1979).

In summary, the significant decrease in C_{max} and Kα as well as increase in t_{max}, t½α and lag time all lead to a suggestion that absorption of paracetamol was impaired by cimetidine. The therapeutic implications of the impairment of absorption of paracetamol by cimetidine are: decrease rate of absorption which can alter pharmacological effects of paracetamol in that it may be absorbed slowly leading to the failure of reaching the minimal effective concentration (MEC), that result in delaying the onset of analgesic activity.

The exploration for the decrease in C_{max} would go along way with Heading et al (1973) which reported that Paracetamol absorption is related to gastric empting. This is further supported by Nimmon et al (1973) that showed propanthaline reduced gastric emptying for paractamol with associated reduced rate of absorption. The

synonymous activity of Propanthaline with Cimetidine (H2 – receptor blocker) as anticholinergic agents might be the reason for the delay in gastric emptying of Paracetamol.

The parameters obtained when Paracetamol was administered alone and those, when Paracetamol was taken concurrently with methyldopa was compared.

It could be seen that in all the two Phases, there was no significant changes in the pharmacokinetics parameters on Comparism i.e. $p>0.05$. This simply means that there is no/ insignificant interaction between Paracetamol and methyldopa when taken concurrently or delayed after a certain period of time. The little changes observed in some pharmacokinetic parameters may be attributed to individual difference and other physiochemical factors earlier mentioned.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendation

It has been seen from the study that there is no effect on the absorption of Paracetamol by Cimetidine when the two drugs were concomitantly administered together. This may be due to the fact that the maximum concentration of Paracetamol is usually achieved within 0.5-1 hour as explained by Ameer and Greenbat (1977). Whereas for cimetidine it is between 1-2 hours (Walkestein et al 1978). This may lead to a suggestion that the pharmacological benefits of Paracetamol and cimetidine could be maintained when the two tablets are taken together and such administration may be safe and effective.

However, as a result of decrease in the concentration of Paracetamol and change in the pharmacokinetics parameters seen when taken an hour after cimetidine lead to a suggestion that there is interference in its absorption by cimetidine. The findings of Ameer and Greenblat (1977) and that of Walkenstein et al 1978 best explain why the interaction, hence it is not advisable for one to take Paracetamol tablet when Cimetidine tablet has been administered between 1-2 hours.

The other part of the study has shown that there is no effect on Paracetamol absorption observed by methyldopa irrespective of time interval. This may lead to a suggestion that it is effective and safe to administer Paracetamol and methyldopa at the same time or at a time interval.

The study has also shown that it is possible to estimate Paracetamol concentration in human via saliva equally as with plasma; hence it is possible to determine Paracetamol concentration through saliva in to confirm paracetamol toxicity. This may be useful to clinicians in taking a specific line of action to treat such cases.

The methodology and the protocol adopted show that it is possible to determine interaction of some other drugs if such drugs concentration could adequately appear in saliva like plasma.

It is recommended that paracetamol should not be administered an hour after Cimetidine to avoid possible interaction.

Also it could be seen from the findings that it is safe to administer paracetamol and methyldopa concurrently or at time interval without fear of any possible interaction between the two.

It may also be safer to administer paracetamol and Cimetidine concurrently.

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