THE INFLUENCE OF ALCOHOL ON THE PHARMACOKINETICS OF ANTIPYRINE

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DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Prof. Ibrahim Abdu-Aguye, Dr.(Mrs) H.O. Kwanashie and Prof. Abdullahi Mustapha. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referred to accordingly.

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CERTIFICATION

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GLORY BE TO GOD ALMIGHTY.

To the memory of John and Adolphus Obiako.

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ABBREVIATIONS

AP	Antipyrine
AUC	Area Under the Drug Concentration Curve
AUC_{0-15h}	Area Under the Drug Concentration Curve, Zero to 15 Hours
AUC_{0-X}	Area Under the Drug Concentration Curve, Zero to Infinity
BA	Benzoic Acid
CC	Cubic centimeter
Cl.	Plasma (systemic) Clearance
C_{max}	Maximum (Peak) Drug Concentration
Co	Concentration of Drug at Zero Time
CV	Coefficient of Variation %
g	Gram
GIT	Gastro-intestinal Tract
HPLC	High Performance Liquid Chromatography
H	Hour
IU	International Unit
Ka	Absorption Rate Constant
Ke	Elimination Rate Constant
Kg.	Kilogram
MEOS	Mixed (Microsomal) Ethanol Oxidixing System
ML.	Milliliter
MM	Millimeter
N	Newton
NEFA	Non Esterified Fatty Acids

°C	Degree Centigrade (Celsius)
PH.	Peak Height
PHR	Peak Height Ratio
%	Per cent
Rt	Retention Time
S.D.	Standard Deviation
t ½ ∝	Absorption Half - Life
t½ β	Elimination Half - Life
t_{max}	Time to Attain Maximum Drug Concentration
µg	Microgram
µm	Micrometer
UV.	Ultra-violet
Vd.	Volume of Distribution
Yrs.	Years

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ABSTRACT

Alcohol-Drug/Food interaction is a common pharmacokinetic interaction that can cause inter-individual variability in drug kinetics and response by influencing hepatic drug metabolism and related factors. This study was aimed at establishing the influence of alcohol on the pharmacokinetics of antipyrine which has often been used as an index of hepatic metabolising capacity of the individual tested.

Eleven healthy Nigerian adult male alcohol consumers, aged 18 - 32 years, and weighing 60 - 80kg, were each given 500mg antipyrine tablets orally. Blood samples were then collected from each subject at times 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 15.0 hours. Plasma antipyrine was extracted by admixing the plasma with methanol and distilled water, centrifugation and filtration. The antipyrine concentrations were then determined by injecting the filtrates into HPLC using reversed phase Bondesil CI8 (0.5um) column, with benzoic acid as internal standard and acetonitrile: acetic acid (1% in water) 35: 65 as mobile phase.

The pharmacokinetic parameters of antipyrine in these subjects were compared with those in a comparable group of non-alcohol consumers (the control) using the Students' "t" test with the following findings: $P < 0.05$ in the values of the

elimination rate constant, the elimination half-life, the systemic (plasma) clearance, the lag time, the area under the drug concentration-time curve (zero to 15 hours and zero to infinity respectively) and the time to attain peak drug concentration. Other pharmacokinetic parameters such as the volume of distribution, the peak plasma drug concentration, the extrapolated plasma drug concentration at zero time, the absorption rate constant and the absorption half life were not significant different in the two populations (P> 0.05).

The precision of the method used in the study was checked by manual Peak Height (PH) method of quantitation with precision of 1.1 - 5.5%. This study also showed that alcohol enhances the elimination of antipyrine from the body by increasing its systemic clearance and decreasing its elimination half-life. The results also showed that alcohol does not significantly affect the absorption and distribution of antipyrine in the body.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 BASIC CONCEPTS AND PRINCIPLES OF CLINICAL PHARMACOKINETICS

Clinical pharmacokinetics deals with the study of the time course of drugs and metabolite(s) concentrations and amounts in various body fluids, tissues and excreta, and how the knowledge of this can be used in the therapeutic management of patients. To interprete properly pharmacokinetic data, suitable mathematical models are usually constructed to describe the movement of the drug(s) and metabolite(s) in various body fluids and tissues at any given time. Pharmacokinetics, therefore, can be described in various terms: as the mathematical description of concentration changes within the body, and the principles relate specifically to the variation with time of drug concentration particularly in the blood, plasma or serum. By extrapolation, they may be interpreted in terms of drug effects.

Pharmacokinetics can also be viewed as the changes of concentration of drug product and its metabolite(s) in the different body fluids and tissues, in the dynamic system of liberation, absorption, distribution, body storage, binding, metabolism and excretion. In other words, it can be seen as the study of the action of the organism on the administered drug(s), especially as it affects the drug penetration or absorption into the body, its

dilution, distribution, transformation, metabolism and the excretion of its active principles, factors all of which may diminish its availability at the site of action. Applied to therapy, pharmacokinetics is concerned with how to obtain the appropriate amount of drug at the receptor site for the appropriate length of time for the particular drug, thus obtaining maximum therapeutic benefit with minimum adverse effects. This is because for most drugs, the extent and duration of drug action depends on how much of the drug gets to the receptor sites and how long it stays there.

1.2 KINETIC DETERMINANTS OF DRUG ACTIONS AND EFFECTS

For a drug to produce an effect, it must first be absorbed (except intravenously administered drugs) into the body and must enter the organ or tissue where it exerts its effects. For the same effect(s) to be terminated, the drug must be rendered inactive or be excreted from the body. Therefore, drug effects and actions are determined by factors which influence its absorption, distribution, and elimination in the body. These factors are also responsible for individual variability in drug response.

1.2.1 Drug Absorption

All routes of drug administration (oral, sublingual, buccal, inhalational, subcutaneous, intramuscular and rectal) except topical and intravenous, require the drug molecules to cross biological membrane barriers to reach the systemic circulation. Topical drugs rarely reach the bloodstream, and the intravenous drugs are placed directly into the bloodstream. The oral route is the commonest route of drug administration with absorption occurring mainly from the gastrointestinal tract (G.I.T.). The rate and extent of drug absorption from the G.I.T. is determined by the physicochemical properties of the

drug; pH of the G.I.T.; gastric emptying time; intestinal residence time; G.I.T. secretions; nature of G.I.T. mucosal covering; blood flow to the G.I.T. (including enterohepatic circulation) and the presence of food, drugs and other substances in the G.I.T.

Physicochemical properties of a drug which affects its absorption include: its formulation, pka and lipid solubility. Some drug formulations are superior to others in their ability to give up their contained drug product, which will have an effect on the amount that ultimately reaches the systemic circulation. Drug formulation factors which determine its bio-availability are the molecular size, disintegration and dissolution time, solubility, salt form, surface area available for dissolution, nature and volume of the formulation vehicle and other pharmaceutical parameters such as the ability to withstand degradation by gastric acids and enzymes.

Many drugs are either weak acids (pka <1) or weak bases (pka >1), being able to exist in an ionized or un-ionized form, depending on the pH of the medium in which it is dissolved. At low pH, acidic drugs are largely unionized and basic drugs are ionized. In contrast, acidic drugs are ionized and basic drugs are unionized at high pH. Since only unionized drugs can cross biological membrane barriers and be absorbed, the pH of the drug and the pH of the dissolving medium govern the rate and extent of its absorption. Apart from pka and pH, lipid solubility of a drug determines its absorption. The presence of lipophilic groups in a drug molecule enhances its passage through the lipoproteins of the biological membrane mainly by passive diffusion, while absorption is delayed if the drug has predominant hydrophilic groups.

The G.I.T has a wide range of pH values (4.3 – 7.6) which allows the absorption of a wide variety of drugs. However, numerous factors within and/or outside the G.I.T. may enhance or delay the absorption process. Hunger, mild exercise, cold meals, dilute solutions, mild alcohol ingestion, alkaline salts, cholinergics and anti-adrenergics enhance drug absorption by shortening gastric emptying time and prolonging intestinal residence time; while anxiety, vigorous exercise, hot meal, concentrated solutions, anti-cholinergics, and sympathomimetics delay drug absorption by prolonging gastric emptying time and shortening intestinal residence time. The presence of food, drug and diseases in the G.I.T. will reduce drug absorption by decreasing absorptive mucosal surface area while factors which increase intestinal motility and reduce the residence time reduce the contact time of the drug at the absorptive surface. Other factors which reduce drug absorption include reduction in blood flow to G.I.T. including enterohepatic circulation of some drugs, and increased G.I.T. secretions of acids, enzymes and proteolytic substances which enhance drug degradations.

1.2.2 Drug Distribution

This is governed by the pka and lipid solubility of drug, the extent of binding to plasma proteins and tissues, pH of the body fluids and regional blood flow. The pka and lipid solubility of drug and the pH of the body fluid in which the drug is distributed affects drug distribution the same way they affect drug absorption. The pH of the body fluid is normally maintained at a mean pH 7.40 $\pm$ 0.04. Thus only drugs substantially unionised at this pH will be able to diffuse across membrane barriers and become widely distributed. Acidic drugs such as aspirin (pk 3.5) which are ionised at this fluid pH have

low volume of distribution, while neutral to basic drugs eg. Antipyrine (pk 5.8 – 7.0) which are unionised at the fluid pH are widely distributed into body fluids and tissues.

Drugs which are extensively bound to plasma proteins especially albumin, are pharmacologically inert, not diffusable and not available for distribution into body fluids. Such drugs are therefore sparsely distributed eg. warfarin and thiopentone. But poorly plasma protein bound drugs, (e.g. antipyrine) are diffusable and widely distributed and so have a large volume of distribution. Many factors affect the level of plasma proteins in the body and therefore protein drug binding. For example, protein-drug binding is decreased in disease conditions associated with proteinuria such as hepatic failure, nephotic syndrome, cardiac failure, malnutrition etc, and in hyperbilirubinaemia due to reduced protein binding sites. In contrast, acute phase reactant plasma proteins (alpha acids lipoproteins) which bind basic drugs increase in stressful situations such as acute myocardial infarction and shock.

Drug effects are usually correlated to plasma levels which do not take bound drugs into account. But when it is considered that protein binding is an important locus of drug interaction where drugs with affinity for some binding sites may complete, with consequent displacement of weakly bound drugs by strongly bound ones, and therefore increased (unaccounted) plasma levels of displaced drugs, this phenomenon usually results in altered kinetics and possible pharmacokinetic problems. For instance, the displacement of warfarin by phenylbutazone from binding sites have been known to cause fatal haemorrhage.

In contrast to drug protein binding, tissue bound drugs are pharmacologically active, diffusable and available for elimination. They therefore tend to produce an uneven and unpredictable large volume of distribution and local toxicity. Extensively tissue bound drugs include digoxin (cardiac muscles), chloroquine (retinal cells); tetracyclines and heavy metals (bones and teeth).

Regional blood flow also affect drug distribution. In highly perfused organs like the heart, liver, kidney, brain and endocrine glands, equilibrium between plasma and tissue is reached faster than in poorly perfused organs such as adipose and skeletal tissues where distribution is slow and limited.

1.2.3 Drug Metabolism and Biotransformation

Drug metabolism occurs mainly in the liver, but can occur also in the plasma, G.I.T., kidneys and lungs (Brodie *et al.* 1958). The biochemical processes are complex, but they usually lead to deactivation of drug product, decreased lipophilicity of drug and increased hydrophilicity of the metabolites, all of which decrease absorption and enhance renal excretion. The rate of drug biotransformation and the spectrum of metabolic products are related to the relative activity of microsomal enzyme system and the predominance of certain enzymatic mechanisms over others. Hepatic drug metabolism can be influenced by age, sex, hormonal and nutritional status, genetic inheritance, microsomal enzyme inducers /inhibitors and disease states.

The clinical observations of hyper responsiveness to drugs in the extreme age groups are thought to be due to immaturity of the liver in the very young and reduced hepatic

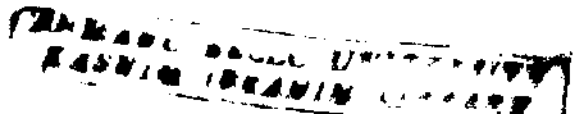
metabolizing capacity in the very old. Gender does not appear to play any major role in man, except in the altered state of pregnancy. Except in hyperthyroidism when all metabolic processes including drug metabolism are increased, hormones do not appear to affect hepatic drug metabolism.

Hepatic drug activity is impaired in disease conditions e.g. liver cirrhosis, malnutrition, hypothyroidism, diabetes mellitus as part of the de-arrangement in enzyme synthesis. Also, heavy metal ions, cyanides and other enzyme inhibitors prolong drug effects by delaying their metabolism by microsomal liver enzymes. This is in contrast to the inductive effect of barbiturates, antipyrine, phenylbutazone, phenytoin, benzpyrene, alcohol (Rubin and Lieber, 1968), caffein and smoking (Vestal *et al*, 1975), lindane and chlorpropamide on the microsomal enzymes. This is not withstanding, the genetic control of the metabolism of drugs such as antipyrine, suxamethonium and succinylcholine (Vessel and Page, 1968, 1969, Vessel *et al*, 1971).

1.2.4 Drug Excretion

Although the urine is the main route of excretion of unchanged drugs and /or metabolite(s), other routes may be important for some drugs e.g. biliary (sulphonamides, chloramphenicol), faecal (neomycin, paraffins), skin (sulphonamides), breast milk (tetracyclines, aspirin, metronidazole), saliva (cimetidine, chloroquine), the lungs (paraldehyde, anaesthetics).

Renal excretion of drugs is determined by pka and water solubility of the drug metabolite, pH of urine, presence of competitive renal tubular transport system inhibitors and



functional state of the renal system. The renal pH range 4.6 – 8.2 enables most drugs to remain ionized. This coupled with the hydrophilicity of the deactivated drug metabolite discourages absorption but encourages renal excretion. Thus the process of urine acidification and alkalinisation can be used to enhance the elimination of basic drugs and acidic drugs respectively.

Compounds of similar structure may competitively inhibit the excretion of each other at the renal tubular transport system. For instance, weak acids (aspirin, probenecid, para-aminohippurate etc) block the excretion of penicillins and methotrexate, while procainamide excretion can be blocked by similar basic drugs such as ethambutol, quinacrine, N-methyl nicotinamide, mecamylamine, tetraethylamine, and tetraethyl ammonium.

Disorders of renal systems such as acute renal failure, Chronic renal failure, nephrotic syndrome and renal hypertension are characterized by impaired renal function including excretion of drugs and metabolites.

1.3 PHARMACOKINETIC PARAMETERS

Important pharmacokinetic parameters which describe the kinetics of drug absorption, distribution and elimination are: absorption (half-life), elimination (half–life), absorption rate constant, elimination rate constant, lag time, concentration at zero time, peak concentration, time to attain peak concentration and systemic (plasma) clearance, area under the drug concentration – time curve, and apparent volume of distribution.

1.3.1 Absorption Half-Life (t½ α)

This is the time taken for half the dose of the administered drug to be absorbed. Its value is affected by all factors influencing drug absorption. It is of no significance in intravenously administered drug. It can be calculated manually using the method of residuals on semi-log graph of plasma drug level-time, or by computer analysis.

1.3.2 Elimination Half-Life (t½ β)

This is the time taken for the post absorption level of the drug in the plasma and tissues, to be reduced by half of its original value. It is related to systemic clearance (Cl) and apparent volume of distribution (Vd) by (t½) = 0.693Vd/Cl.

Elimination half –life (t½β) is a poor index of drug elimination as it increases whenever the Vd increases, even in the absence of a change in systemic clearance (Cl). However, it is useful in the determination of suitable dosage intervals and the time required to attain steady state during intravenous or chronic oral therapy.

1.3.3 Absorption Rate Constant (Ka)

This is a proportionality constant relating to the rate of drug absorption in the body. In first order kinetics, the rate of absorption is directly proportional to the drug concentration at the absorption site per unit time (time-1)

i.e. *rate of absorption* = *Ka .Aa* ..... ***(1)***

where:

Aa = *amount of drug remaining to be absorbed.*

In this case, the driving force for absorption is the difference between the concentration of the diffusing drug at the absorption site *(Ca)*, and the amount unbound in the arterial blood perfusing the absorptive membrane *(Cu)*, thus

rate of absorption = *pA (Ca - Cu)* ..... **(2)**

where

p = *permeability constant,*
A = *effective surface area.*

Since distribution and elimination of absorbed drug often ensure that the value of *Cu* is much less than *Ca*, the rate of absorption is thus,

rate of absorption = *pA. Ca* ..... **(3)**

Assuming that the volume of fluid at the absorptive site *(Va)* remains relatively constant, then substitution yields:

rate of absorption = *(pA/Va).Aa* ..... **(4)**

Thus the value of Ka is determined by the permeability constant of the drug *(p)*, surface area *(A)* and volume of fluid at the absorptive site *(Va)*.

As with other first order processes, absorption rate is characterized by a $t_{½\alpha}$, and is related to $t_{½\ \alpha}$ exponentially via the equation

Ka = $0.693/t_{½}$.

1.3.4 Elimination Rate Constant (Ke)

This, like Ka, observes a first order kinetics too. i.e. the rate at which a drug is removed from the body is directly proportional to the drug concentration in the body per unit time

(time-1) expressed in min –1, h –1. It is important to note that Ke refers to fractional rate of drug elimination and not to the actual amount of drug eliminated per unit time. It is therefore represented by the equation:

$$Ke = F.D/Vd.\ Auc \qquad \textit{or} \qquad Ke = Cl/Vd,$$

where F = fraction of administered drug observed (bio-available)

D = dose of administered drug

Vd = Volume of distribution of drug

Cl = Plasma clearance of drug.

Auc = quantity of drug observed per unit time

Ke is also exponentially related to $t_{½}\beta$ via the equation

$$Ke = 0.693/t_{½}.$$

The relationship between Ka and Ke determines the rate of change of drug concentration in the body, thus:

rate of change of drug concentration = rate of absorption – rate of elimination

$$\textit{i.e} \quad \frac{(d.Ab)}{dt} = (Ka.\ Aa) - (Ke.Ab)$$

where: *Aa* = amount of drug remaining to be absorbed

Ab = amount of drug absorbed in the body

d.Ab = total quantity of drug in the body

d.t = total time

This means that when Ka > Ke, blood drug level will rise and will continue to do so until at equilibrium (Ka = Ke) when the rise will stop at the peak level. This peak level will remain constant as long as the equilibrium is maintained, but drug level will start to decline once Ke > Ka until the whole drug is eliminated from the body.

1.3.5 Lag Time

This is the delay between drug administration and the beginning of absorption; and can be from a few seconds to many hours. The value is largely affected by factors which influence drug absorption, but is insignificant in intravenously administered drugs. For instance long lag times are commonly observed when enteric – coated capsules or tablets are ingested, which may be due to the delay in release of drug product from the protective coating into the intestinal fluids. The same effect is observed when drugs are taken on a full stomach. Lag time can be calculated manually using the method of residuals on a semi – logarithm plasma drug concentration – time graph or by computer analysis.

1.3.6 Concentration at Zero Time (Co)

This is the drug concentration at zero time. It is an extrapolated value estimated by dividing the dose of administered drug with the volume of distribution.

Co = Dose of drug/Vd.

In practice, Co is estimated by extrapolating the straight line of elimination phase of plasma drug concentration – time graph to zero time.

1.3.7 Maximum (Peak) Drug Concentration (C_{max})

This is the peak blood level of drug per unit time (time –1). It is reached when the rate of absorption equals the rate of elimination in non-intravenously administered drugs. Its value is instantaneous for intravenous drugs. C_{max} is important in determining drug dosages during therapy.

1.3.8 Time to Attain Maximum Drug Concentration (t_{max})

This is the time it takes to reach C_{max}, so like C_{max} its value is instantaneous in intravenous drug administration. t_{max} is useful in the determination of dosages and dosage intervals.

1.3.9 Area Under Drug Concentration –Time Curve (AUC)

This is the integral of blood drug level over time, from zero to infinity ($AUC_0 - \alpha$). It is a measure of the quantity of drug absorbed (bio-available) in the body, and is important for calculating relative bioavailability, plasma clearance and apparent volume of distribution. AUC can be calculated using various methods such as planimetry, cut and weigh, trapezoid rule and computer application. It can be represented by the equation :

$$AUC = \int Co.dt$$

where Co = concentration of drug at zero time
dt = total time.

1.3.10 Apparent Volume of Distribution (Vd)

This is that volume of fluid in which the amount of drug in the body would need to be uniformly distributed to produce the observed plasma concentration. It is inversely proportional to blood drug level i.e. the larger the Vd, the more extensively the drug

distributes from the blood stream into the various tissues, organs and binding sites of the body with consequent low plasma drug level. Vd does not relate to a real physiological or anatomic space but it provides an estimate of the extent of drug distribution through the body fluid compartments and its uptake by tissues. A large value of Vd implies wide distribution or extensive tissue uptake or both. Vd is used to relate the plasma concentration of a drug to the administered dose, as the body is obviously not homogeneous, even if it can be treated as such in a mathematical model. Therefore, drug concentrations in the liver, kidneys, muscles, fat and other tissues will differ from one another as well as from the plasma concentration. Vd is also useful in calculating the amount of drug in the body when the plasma concentration is known or predicting the plasma concentration following a given dose. After oral drug administration, Vd can be determined from the equation:

$$Vd = F.D/Ke\ AUC \qquad \textbf{or} \qquad \textbf{Vd} = Cl/Ke$$

1.3.11 Systemic (Plasma) Clearance, (Cl)

This is the volume of plasma from which a drug is totally and irreversibly removed per unit time. Total plasma clearance of a drug is the sum total of individual clearance of the drug by the various organs of drug elimination viz lungs (expired air), liver, renal (urine) and GIT (faeces). Cl is therefore a direct index of drug elimination, and indicates the efficiency of drug eliminating organs. It is thus subject to the influence of drug distribution, metabolism and excretion, and is expressed in volumes per unit time as:

$$\textbf{Cl} = \frac{F.D}{AUC}$$

(absorbed dose of drug)
(total bioavailable drug eliminated.)

Cl is also related to Ke and Vd by the equation,

$$\textbf{Cl} = Ke.Vd.$$

1.4 PHARMACOKINETIC MODELS

Attempts are made in pharmacokinetics to describe biological events in mathematical terms, by a system of compartments with no apparent physiological or anatomic reality. A compartment is an entity which can be described by a definite volume and a concentration of drug contained in the volume. Compartment models are used to describe the behaviour of drugs in biological terms, and can be classified into one, two or multi-compartment models. However, drugs which are primarily eliminated by biotransformation and/or by zero-order processes cannot be analyzed using these methods.

1.4.1 One – Compartment Model

Here the whole model is considered mathematically as a single homogeneous unit, in which the drug entering the body distributes instantly between the blood and body fluids or tissues, i.e. the exchange of drug between the plasma and tissue proceeds rapidly compared with the rate of elimination. This, therefore, assumes that any changes that occur in the plasma quantitatively reflects changes occurring in tissue drug levels, but does not mean that the drug concentration in all body fluids and tissues at any given time are the same. This model is useful for the pharmacokinetic analysis of blood, plasma or serum concentrations. It is also useful for analysis of salivary and urinary excretion data for drugs which are rapidly distributed between plasma and other body fluid and tissues upon entry into the systemic circulation.

1.4.2 Two–Compartment Model

This model contains both a central and a peripheral compartment. The central compartment correspond to the plasma or blood volume, together with the extracellular fluids of highly perfused organs: heart, liver, kidney, brain and endocrine glands. Drugs distribute within a few minutes through this compartment and equilibrium between plasma and tissues is rapidly established. The peripheral compartment is then formed by less perfused tissues of the skin, muscle and fat in which drugs enter more slowly. This model is useful for drugs in which distribution is so slow that it cannot be disregarded. The combined effect of the two compartments gives rise to biphasic curve on intravenous injection with two distinct linear portions when drawn on a semi-logarithm scale. Although drug distribution is slow, it is usually much faster than elimination. Thus the initial rapid fall in concentration (known as the alpha or distribution phase) mainly represents the relatively rapid process of drug distribution from central to peripheral compartment. Once distribution is complete, the curve enters the relatively slow beta or elimination phase during which drug disappearance is determined mainly by irreversible elimination from the central compartment.

1.4.3 Three–Compartment Model

This is a modified model for oral dosing in which an additional compartment is incorporated to represent the volume from which absorption occurs at a first order rate. In this model, it is assumed that the entire dose is rapidly introduced into the site of absorption, from which it is absorbed into the central compartment. A typical concentration time plot after a single oral dose (and other routes which involve a

preliminary absorptive phase) will show an uphill absorption and distribution phase, and a downhill elimination phase.

1.5 SIGNIFICANCE OF PHARMACOKINETIC STUDIES

Knowledge of the pharmacokinetics of a given drug and the various factors modifying them is very useful in therapy and in development of a rational dosage regime that will achieve the maximum therapeutic benefit with minimum toxic effects. It also leads to a better understanding of inter-individual variations in drug response and the factors responsible for these variations. It is especially of value in many clinical situations such as: when a drug with a narrow therapeutic margin is used in therapy; when therapy appears to be going wrong; where there is drug toxicity; therapeutic management of the very young or the very old or even of individuals with diseased organs of drug elimination.

1.6 INTER-INDIVIDUAL VARIABILITY IN DRUG KINETICS AND RESPONSE

Marked variations in responsiveness to drugs occur in man. For the most part, this is due to large inter-individual variations in drug disposition, resulting from differences in age, gender, diet, genetic inheritance, health status and disease states, exposure to environmental pollutants and others drugs.

1.6.1 Age

Volume of body fluids and systemic clearance change with age and growth. In the very young, the volume of body fluids is about 40% of total body weight compared to 20% in

adults. Plasma proteins, lean body mass, liver mass, total body water, cardiac output, liver and renal perfusion and glomerular filtrate rates are lower in the extreme age groups than in adults. These factors contribute to reduced clearance of most drugs in them, so that drugs tend to act longer in their body than in adults.

1.6.2 Sex

The effect of gender per se in drug kinetics is minimal, but sex linked differences in hormonal balance, body composition, and activity of certain enzymes manifest themselves in both pharmacokinetics and responsiveness. For instance, intra-muscular absorption of some drugs is slower in females than in males because of differences in muscles mass, fat content and blood flow. Physiological changes in pregnancy such as increases in plasma volume, plasma proteins, progesterone production, non esterified fatty acids (NEFA), blood flow to the gut, intestinal residence time and improved liver/renal perfusion and clearance are known to cause altered drug kinetics. Progesterone induces microsomal enzyme activity leading to enhanced drug metabolism. Non esterified fatty acids may displace some drugs from albumin binding sites leading to increase free drug fractions in plasma.

1.6.3 Diet

Food is a complex mixture of chemicals, each potentially capable of interacting with drugs, and altering their kinetics and response. Milk and fatty foods reduce the absorption of alcohol and other water soluble drugs by delaying gastric emptying time, but this produces improvement in the absorption of sparingly soluble drugs e.g. griseofulvin (Lin *et al*, 1976). Calcium ion (in milk), kaolin, magnesium salts etc form an

insoluble complex with tetracyclines, thereby reducing their absorption. Protein deficient diet impairs microsomal enzymes synthesis and thus metabolizing ability.

1.6.4 Genetic Inheritance

This accounts for over 60% of the inter-individual variability in drug kinetics and response. It is also responsible for differences in height, physical stature, color of skin, hair and eyes between individuals. Genetic polymorphism is either monogenic or polygenic, but monogenic control of metabolic pathways account for most of the inherited variations in drug kinetics and response (Vessel and Page, 1968). It produces a polymodal (or discontinuous) distribution curve in a population as opposed to the unimodal or continuous distribution curve produced by polygenic factors. Drug absorption, distribution and excretion are not under genetic control. Thus, the renal clearance value for any drug is essentially the same in age and weight matched healthy subjects, suggesting that much of the inter-individual variability in pharmacokinetics could be avoided if drugs were entirely excreted unchanged. Drugs with genetically controlled metabolic pathways include isoniazid, dapsone, hydrallazine, debrisoquine, succinylcholine, suxamethonium, alcohol (Vessel *et al.* 1971), antipyrine and phenobarbital (Vessel and Page, 1968, 1969).

1.6.5 Disease States

These alter drug kinetics and response by producing abnormal physiological states. Gastrointestinal disorders (eg. Lactose intolerance, gastroenteritis, irritable bowel syndrome) reduce drug absorption by reducing intestinal residence time. Others such as

Coeliac Disease, Tropical Sprue, Giardiasis, and Crohn's Disease produce atrophic mucosal covering which lead to widespread disturbances of nutrients and drug absorption.

Circulatory disorders produce widespread haemodynamic disturbances that significantly alter drug kinetics and response in the individual. Heart failure, myocardial infarction, malignant hypertension, shock etc, individually and collectively cause diminished vascular perfusion of the gut, liver, kidneys and other parts of the body leading to reduced GIT drug absorption, poor clearance of highly hepatic extracted drugs (e.g., lignocaine, propranolol, quinidine) and drugs primarily excreted in the urine. Secondary haemodynamic changes may alter physiological pH of body fluids, reduce plasma protein/ tissue binding sites, or increase circulating NEFAs and acute phase reactant proteins, all of which can influence the binding, distribution and excretion of some drugs.

Pulmonary insufficiency from any cause, leads to increased pulmonary vascular resistance, body gases disturbances and secondary haemodynamic changes similar to those seen in cardiac disorders with attendant altered drug kinetics and response.

Liver diseases do not always produce consistent or predictable effect on drug disposition, even though the liver is the main site of drug metabolism. This is because each liver disease whether local or diffuse affects the various levels of hepatic organizational structure to a different extent. Due to its unique position between the systemic circulation and the vasculature draining the GIT, liver cirrhosis or viral hepatitis will produce a low first pass effect for highly hepatic extracted drugs, but not for poorly hepatic extracted drugs (eg. diazepam, antipyrine, isoniazid). The massive hepatic cell damage, increased

total body water, decreased plasma proteins and impaired microsomal enzyme activity, associated with acute or chronic liver failure, help to alter drug kinetics and response.

Renal impairment causes the accumulation of all end-products of metabolism, both toxic and non-toxic, including the unchanged drug, thus leading to prolonged pharmacological effects and perhaps toxic manifestations. Added to these are the complications of renal diseases such as uraemia, ascites, hypoproteinaemia, electrolyte imbalance and accumulation of endogenous protein binding substances which may displace drugs from binding sites. While NEFA, may displace some acidic drugs from binding sites thereby increasing their plasma free fraction levels, the alpha acid glycoproteins bind basic drugs thereby reducing their plasma free fraction.

1.7 PHARMACOKINETIC EFFECTS OF DRUG INTERACTIONS

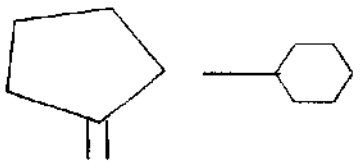
Drug interactions is next to genetic inheritance as a cause in inter-individual variations in drug kinetics and response (Azarnoff *et al,* 1969). Repeated exposure to tobacco and cigarette smoke, dichlorodiphenyltrichloroethane (DDT), lindane, 3,4-benzpyrene and other polycyclic aromatic hydrocarbons, alcohol, barbiturates and phenytoin result in induction of liver microsomal enzyme activity and therefore enhance drug metabolism. In contrast, drugs such as allopurinol, disulfiram and phenelzine inhibit xanthine oxidase, alcohol dehydrogenase and monoamine oxidase respectively, thereby blocking the inactivation of drug metabolism by these enzymes and prolonging their action in the body (Brodie *et al,* 1958).

Some drugs alter the kinetics of others by reducing their absorption in the GIT (calcium and tetracylines); displacing them from binding sites (sulphonamides and phenylbutazone, warfarin and tolbutamide), or blocking their renal excretion (probenecid and penicillins, procainamide and ethambutol).

These inter-individual differences in drug kinetics and response and the factors causing them must be considered when developing a dosage regime for an individual, because giving a fixed dose or a weight adjusted dose may not compensate for these large variations, as it can lead to ineffective therapy in some patients and toxicity in others.

1.8 PHARMACOLOGY OF ANTIPYRINE

1.8.1 Physicochemical Properties (Including Structure)



Antipyrine (Phenazone) is a derivative of pyrazolone, with molecular formula $C_{11}H_{12}N_2O$ and molecular weight of 188.2. It is a white crystalline powder, odourless, with a bitter taste, melting point of 110-113°C, soluble in water (1:1), alcohol (1:1), chloroform (1:1), ether (50:1). A 5% solution in water has a pH of 5.8 –7.0 while a 6.8% solution is iso-osmotic with serum. Solutions are sterilized by autoclaving. The powder is stored in air-tight containers and protected from light. It is incompatible with nitrites (in acid solutions), cinchona alkaloids, iodides and tannic acid. It forms liquid mixtures with acetanilide, betanaphthol, camphor, chloral hydrate, sodium salicylate and many other substances.

1.8.2 Pharmacokinetics

Antipyrine is rapidly and completely absorbed from the GIT with peak plasma levels attained in 1-2 hours. It is distributed throughout body fluids and tissues and so can be used to measure total body water. It is not appreciably bound to plasma proteins, and is slowly but extensively metabolized by the hepatic microsomal enzyme system, so that less than 5% is excreted unchanged in the urine. It disappears from the plasma at a rate of about 6% per hour, with a half-life ($t^{1}/_{2}$) of about 12 hours. Metabolism by the mixed enzyme oxidizing system (MEOS) is genetically controlled and leads to the production of metabolites (4-hydroxyantipyrine, 30-40%; 3-hydroxyantipyrine, 13-17%; norantipyrine, 6%; 4,4 dihydroxyantipyrine, 3-6%) which are rapidly and completely excreted in the urine (Bottcher *et al.* 1984; Moncrieff, 1986; Vessel and Page, 1968). Antipyrine enhances its own metabolism by microsomal enzyme induction. Its half-life is reduced by other MEOS inducers like phenytoin, barbiturates, tobacco and cigarette smoke, nicotine, caffeine, halogenated hydrocarbons (lindane, DDT), polycyclic aromatic hydrocarbons, anabolic steroids, rifampicin, and alcohol (1ml/kg/day for 21 days) (Rubin and Lieber, 1968; Vessel and Page, 1968; 1969; Vessel *et al.* 1971). In contrast, antipyrine half-life is increased by MEOS inhibitors such as isoniazid, androgens, testosterone, cortisol and oral contraceptives and in conditions of low hepatic enzyme activity in disease states (liver diseases, myxoedema etc), in the newborn and in the elderly and in acute ethanol intoxication (Rubin *et al.* 1970).

1.8.3 Toxic Effects

Antipyrine was introduced in 1884 as antipyretic/analgesic, but was withdrawn in many countries because of its severe toxic effects which included haematuria, allergy, multiform

skin eruptions, kidney failure and leucopenic reactions among others (Baer and Harris 1967; Baran and Rowles, 1973; Kadar and Kalow, 1980; Payne *et al,* 1966). Oral adult dose was 0.3-0.6g given as tablet or capsule every four hours.

1.8.4 Importance in Pharmacokietic Studies

Antipyrine has been widely used as a drug for various pharmacokinetic studies. It has been used to study the influence of diseases (Eichelbaum *et al,* 1974, Eichelbaum and Spannbruker, 1977; Elfstrom and Lindgren, 1974; Lichter *et al,* 1973), drugs, (Awazu *et al,* 1987; O'Malley *et al,* 1972; Rubin and Lieber, 1968; Vestal *et al,* 1975), diet (Kappas *et al,* 1976) and genetic factors (Vessel and Page 1968, 1969) and environment (Kolmodin *et al, 1969)* on drug metabolism in man. Antipyrine is well suited for this purpose because it is almost completely absorbed in the GIT; is widely distributed in body fluids; not appreciably bound to plasma proteins; and is extensively metabolized by hepatic microsomal enzymes. Also all the metabolites are excreted completely in the urine. Therefore the rate of its decline in the plasma or the appearance of its major metabolites in the urine can be taken as a measure of drug metabolizing capacity of the individual tested.

1.9 THE ALCOHOLIC AND ALCOHOLISM

The Oxford Advanced Learners Dictionary. New Student's edition defined an 'alcoholic' as a person who regularly drinks too much alcohol or suffers from a physical disorder caused by this. It also defines "alcoholism" as a physical disorder caused by regular drinking of too much alcohol. The Concise Medical Dictionary Oxford Edition, defines alcoholism as a syndrome due to physical dependence on alcohol, such that sudden deprivation may cause withdrawal symptoms of tremors, anxiety, hallucinations and delusions. Alcoholism impairs

intellectual function, physical skills, memory and judgement. Social skills such as conversations are usually preserved until a late stage. Organic lesions such as liver cirrhosis, peripheral neuritis, cardiomyopathy, enteritis and altered drug metabolism are common (Shearman and Finlayson, 1982; Sotaniem *et al,* 1977; Ugarte *et al,* 1977).

Various authors have attempted to characterize the alcoholic personality and to establish a commonly accepted or defined aetiology of alcoholism (Criteria committee of the National Council on Alcoholism, N.Y., 1972). As a result, the alcoholic has been described in various terms: criminally minded, weak character, self-degrading, compulsive-neurotic, hysterical; suffers deprivational stress, mal-adjustments and psycho-biological allergy and prone to abuse of drugs (Texon, 1949; East, 1936). The lack of a universally accepted definition of alcoholism and a biochemical marker of alcoholism has greatly hampered research and treatment efforts directed at the alcoholic. Many biochemical tests proposed for alcoholism have been generally unsatisfactory (Israel and Mardones, 1971). This is because during casual sampling, ethanol was found in the blood of only a minority of both "heavy drinkers" (subjects consuming more than 80g/ethanol/day) and patients with alcohol liver disease (Homilyn *et al,* 1975). Furthermore, alcohol may be absent from the blood after relatively brief abstinence, although the presence of ethanol in the blood does not distinguish acute from chronic alcohol consumption. (Ugarte *et al,* 1972). Similarly, serum glutamic dehydrogenase and gamma glutamyl transpeptidase enzymes are found elevated in only a minority of "heavy drinkers" and in-patients with non-alcoholic liver disease (Rollason *et al,* 1972). Of all the physiological, biochemical, clinical, behavioral and attitudinal criteria of alcoholism, the most reliable is the biochemical observation of elevated plasma alpha aminobutyric acid/leucine ratio in 65-100% of alcoholics (Criteria committee of the National

Council on Alcoholism, N.Y. 1972; Shaw *et al*, 1976). Because of the enumerated problems associated with alcoholism and chronic alcohol use, the volunteers used in this study were carefully selected; based on satisfactory history of non-alcoholism and or ill/health; normal liver function and serum protein values and a positive history of alcohol consumption as a primary vehicle for socialization.

1.10. THE ALCOHOLIC BEVERAGES

1.10.1 Types:

Alcoholic beverages can be classified according to the percentage ethanol content as beer (2-6%), wines (10-14%) distilled spirit (30-40%), palm wine (5-10%), crudely brewed spirits (40-70%). Clinical and biochemical studies have shown that most of the organic lesions of alcoholism are due to the ethanol content of the alcoholic beverages, and not the congeners, and that this effect is proportional to the percentage ethanol content. (Lelback, 1974), although specific lesions have been identified with contaminants in local brews e.g. siderosis from iron metal containers (Buchanan, 1970), arsenic, lead and cobalt poisoning.

1.10.2 Alcohol–Drug/Food Interactions

In Nigeria and other societies where alcohol use is not legally restricted , alcohol consumption is common in social gatherings. On such occasions, alcohol beverages are commonly consumed together with food, kolanut, spice, tobacco and even drugs. Significant interactions therefore occur between the alcohol and these substances at the various pharmacokinetic loci of absorption, distribution, metabolism and excretion.

Alcohol (ethanol) is rapidly and completely absorbed from the GIT, the rate and extent of absorption being influenced by the percentage ethanol content of the beverage, and the presence or absence of food, drugs and other substances in the gut. For example, distilled spirits are better absorbed than beers etc. Also hunger, alkaline salts, carbonated drinks (e.g. coca-cola drink), cholinergics and anti-adrenergics enhance alcohol absorption by shortening gastric emptying time and prolonging intestinal residence time (Lin *et al*, 1976). The opposite effect of reduced absorption is produced by food, anticholinergics and sympathomimetics (Lin *et al*, 1976).

Once absorbed, ethanol is uniformly distributed to body fluids and tissues, as it is poorly bound to plasma proteins. Peak plasma levels are reached in 0.5-1 hour. Its distribution is therefore not appreciably affected by kinetic interactions.

Ethanol is metabolised in the liver by a mitochondrial enzyme (alcohol dehydrogenase) and microsomal ethanol oxidising system (MEOS) to carbon dioxide and water (Lieber, 1966-1973; Mezey and Tobon, 1971). About 10% are excreted unchanged in breath, sweat and urine (Payne *et al*, 1966). Most of the kinetic interactions between alcohol and other drugs occur in the liver MEOS; although a few occur in the alcohol dehydrogenase metabolic pathway (e.g. alcohol-disulfiram). The MEOS activity can be induced or inhibited by many drugs. MEOS inducers accelerate their own metabolism as well as others metabolised by the enzyme system (Azamoff and Hurwitz, 1969; Carulli *et al*, 1971; Mezey, 1976). Chronic intake of alcohol induces its own metabolism as well as those of antipyrine, barbiturates, phenytoin, meprobamate, benzpyrene, bilirubin, tolbutamide, chloral hydrate, chloroform, acetaminophen, halogenated hydrocarbons etc.

This effect may in part account for the increased tolerance of alcohol drinkers to the effect of alcohol and their resistance to many of the microsomal metabolised drugs. Since many of these drugs enhance the metabolism of alcohol, persons on any of them may tolerate the action of alcohol better than normal persons. Drugs such as trichloroethanol, trichloroacetaldehyde, chloral hydrate and disulfiram decrease ethanol clearance by competitive inhibition of alcohol dehydrogenase enzymes, an effect which is accentuated by diminished drug metabolising capacity of the liver in alcoholic liver damage (Ugarte *et al*, 1977).

1.11 QUANTITATIVE HPLC ANALYSIS

Quantitative analysis is carried out by measuring the areas or heights of the peaks in the elution chromatogram. This can be done either manually or electronically. The measurement of peak areas is an inherently more accurate method of quantitation than peak height measurement, there are occasions when the latter method is preferred. For example, in the trace analysis problems peak height measurement are used, since peak height are less affected by the presence of interfering peaks than are peak areas. Furthermore, peak area measurement is much more affected by flow rate variations than is peak height measurement, therefore, in quantitative analysis in general and particularly when using peak areas, the pumping system must be capable of delivering a constant flow rate or the signal must be recorded against volume rather than time. Peak height reproducibility on the other hand, requires a reproducible injection distribution and this is often difficult to achieve with an on-column syringe injection. Provided a calibration plot of peak height or peak area against amount injected is linear over the required range, either method is satisfactory.

Measurement of peak heights is straight forward. Peak areas are best measured by multiplying the peak height by the peak width at half-height. This gives a precision of around 3% (precision values are quoted in terms of the coefficient of variation which is the standard deviation divided by the mean expressed as a percentage). Measurement of peak area by computing ½ base width x peak height gives a precision of around 4%. In both cases a sufficiently fast chart speed should be used to ensure that peak widths can be measure accurately. Other less common procedures involve counting the small squares under a peak on the chart paper. This method is tedious but this destroys the chromatogram. (A photocopy of the chromatogram can also be cut out and weighed). A planimeter can also be used to measure the area.

There are several points to remember when performing a quantitative HPLC analysis. These are:

i. Since the common HPLC detectors respond differently to different solutes, a response factor must be determined for each component in a mixture before the peak area (or height) can be converted into the amount of each particular solute. Response factors must be calculated individually for each component by injecting standard amounts of the pure component and measuring the peak area.

ii. Internal standards can be used to calibrate the system for quantitative analysis. An internal standard is a compound which is added in known amounts to the mixture to be analysed as well as to the standard solutions use to calibrate the system. Quantitation is carried out by comparing the solute peak area or height with that of the standard peak area or height. The column capacity ratio (or k' value) of the

internal standard must be chosen such that it does not interfere with any of other peaks. The method has the advantage that any loss of sample solution (e.g. on syringe injection against relatively high pressures) does not affect the analysis since both solutes of interest plus internal standard are lost proportionately. Internal standards may also be added to body fluids before the extraction steps are carried out. If the standard has a similar chemical constitution to the compound of interest to be extracted, losses of both the standard and the component of interest should be the same throughout the procedure.

iii. External standards can also be employed. This method involves alternate analysis of standard solutions and of the samples containing the component of interest. From the injection of standard solutions a plot of peak area or height against amount injected may be constructed and used to quantitate the peak areas or heights from the sample solutions. If the analyses are carried out in duplicate or triplicate, much of the error involved in injecting sample and standard consecutively can be eliminated.

iv. Accuracy and Precision: The accuracy of an analysis is the ability of the method to measure the quantity being determined. Accuracy can be checked by weighing the components of a mixture and comparing the HPLC result for the amount present with the expected result. Precision on the other hand is the ability of the method to give the same result in a series of replicate determinations. The accuracy of the method is governed by the ability to calibrate the system using standards as described above. Precision depends on the method used for quantitation. The

manual methods as described above have precision in the region of 3 – 4% while electronic integration can give a precision of 0.5 - 1.0%

v. Reproducibility: The reproducibility of a method is governed by the ability of independent analysts in different laboratories to give the same result using the same method, this not withstanding the effect of temperature, solvent composition and solute mass on peak retention times and column efficiencies.

1.12 THE AIMS OF THIS STUDY

Alcohol is a social drug commonly abused, and also consumed with food, fruits, drinks, tobacco and drugs. This study was aimed at establishing the effect of alcohol on the pharmacokinetics of antipyrine in the Nigerian environment. Antipyrine was used because of its excellent pharmacokinetic properties *(see 1.8.4)* so that alteration of any of these properties by alcohol may explain the kinetic variables responsible for altered drug response in alcohol consumers.

CHAPTER TWO

MATERIALS AND METHODS

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals

1	Antipyrine powder	Sigma U.S.A.
2	Methanol Analar P10158	BDH U.K.
3	Acetonitrile Analar P29220	BDH U.K.
4	Acetic acid Analar	BDH U.K.
5	Benzoic acid powder Analar	BDH U.K.
6	Heparin Sodium 500 IU. USP per ml	B.Braun Germany
7	Distilled water	NAFDAC Kaduna
8	Antipyrine tablet 250mg	A.B.U. Zaria

2.1.2 Human Plasma (heparinised with 160 i.u/ml USP heparin sodium)

2.1.3 Equipments

1. High performance liquid chomatography (HPLC) consisting of Philips PU 4100 pumping device: Philips Pye Unicam PU 4020 UV detector; Philips PM 8251 A one line chart recorder; 25cm X 4.6mm column packed with 5um Bondesil C18 and 5-20ul microsyringe with hypodermic needle.
2. Mettler AE 240 digital weighing machine (10^{-4}-10^{3}g).
3. Vortex mixer
4. Centrifuge machine with test tubes (Gallen Kamp)

5. Blood collection apparatus (sample bottles, syringes and needles, tourniquet, cannulae, methylated spirit, cotton wool, hand gloves).
6. 0.6um paper filter.
7. Blood storage system (refrigerator and cold-chain flask).
8. Miscellaneous: Volumetric flasks; microsyringes (0.1-10cc) with hypodermic needles, detergents, sponges, tap water, wash bins, stop watch.

2.2 METHODS

2.2.1 Formulation of Antipyrine Tablets

(a) Preparation of the antipyrine powder mass for compression.

Crystals of antipyrine were size - reduced using a mortar and pestle. It was subsequently mixed with micro-crystalline cellulose (Avicel PH 101) as diluent, such that the powder mass contains 200mg of antipyrine in 270mg of the tritrate.

(b) Tablet Compression.

Production of the tablets was carried out on an Eureka Single punch tablet press (Type EKO) employing the direct compression procedure. The powder mass obtained from the mixture of antipyrine crystals and the micro-crystalline cellulose were forced-fed into the die- cavity of the tablet press. Punch and dies of 10mm diameter size was used. Compression force of 2kgN was employed. Powder was force-fed into the die cavity, which had been adjusted to contain approximately 270mg of the powder mass. The produced tablets were then subjected to quality control tests.

(c) Quality Control of Produced Tablets

The produced tablets were subjected to various quality control tests as prescribed in the British Pharmacopoiea 1988 namely – weight variation, thickness, hardness, friability and disintegration tests as described below.

Using a Mettler balance, ten tablets were weighed and the mean weight calculated. Individual tablets were then weighed and their percentage deviation from the mean weight computed.

Friability was determined using an Eureka Friability Apparatus (Type TA 3R) for 10 tablets. The friabilator was adjusted to rotate 25 times per minute and was timed for 4 minutes. The weight of the tablets before subjection to the test and after the test were recorded. The loss in weight calculated in percentage (friability value) was determined. The hardness of the tablets was also determined using a Monsanto Hardness Tester.

Disintegration test was carried out in an Eureka Disintegration Apparatus (Type ZT 3) as described by the British Pharmacopoiea 1988. Distilled water thermostated at 37°C was used as the disintegration medium. Time taken for the last particles of the tablets to pass though the mesh was noted using a stop watch.

2.2.2 Research Population

Healthy adult male volunteers, aged 18-32 years, weighing 50-80kg and domiciled in Ikara, Northern Nigeria were randomly selected. The inclusion criteria were:

(i) known history of alcohol consumption as a primary vehicle of socialisation, for at least one year.

i) absence of physical or mental features of alcoholism and/or ill health.

ii) normal liver function and serum protein values.

iii) proximity to Ikara General Hospital where the blood samples were collected.

iv) voluntary participation.

2.2.3 Calibration Curve

Preparation of stock solution antipyrine standard was done by dissolving 0.5g of antipyrine (AP) powder in 100ml of distilled water to get 500µg/ml solution which was stored in the refrigerator. Sequential dilutions with distilled water to 5µg/ml, 10µg/ml, 20ug/ml, 40ug/ml, 60ug/ml, 80ug/ml and 100ug/ml were made from the stock solution.

A 500µg/ml stock solution of benzoic acid (BA), as internal standard, was made by dissolving 0.5g of the powder in 100ml of distilled water. Sequential dilutions to 100µg/ml were made from the stock solution.

Standard samples were prepared by adding 0.1ml of AP of serial concentrations 5µg/ml to 100µg/ml to seven consecutive test tubes. To each tube was also added 0.2ml plain plasma. 0.1ml BA and 1.6ml methanol to give a final volume of 2.0ml.

The content of each test tube was mixed on a vortex mixer for 15 seconds, then centrifuged at 3,000g for 10 minutes to remove plasma proteins. The supernatant was filtered through a 0.6um paper filter and the filterate put in a labelled container. 20µl of the filtrate was then injected into the H.P.L.C. machine.

The HPLC was operated under the underlisted conditions for optimum results:

i) mobile phase comprising acetonitrile: acetic acid (1% in water) at a ratio of 35:65, flow rate of 1ml/minute, pressure of 68 bars and room temperature of 35°C.

ii) mobile phase was degassed by ultra sonic stirring at a maximum temperature of 40°C for at least one hour, and then cooled to room temperature before use.

iii) the machine was primed with methanol for at least 30 minutes to attain a steady baseline on the chart recorder before use.

iv) the stainless steel column, 25cmx4.6mm ID, packed with 5µm Bondesil C18 was maintained at room temperature with output filter of 0.5 and back off coarse of 4.

v) UV detector sensitivity was at 254nm and 0.08, 10mv X 1 range

vi) loading of 20µl of the filtrate into an external loop in the micro-volume sample valve and its introduction into the mobile phase by an appropriate rotation of the valve.

Peak height (PH) measurement of the HPLC tracing (Chromatogram) for the serial concentrations of AP and the specific concentration of BA were determined manually, the ratios of PH (PHR) of AP and BA were calculated through the equation

$$\textbf{PHR} = \frac{\text{PH of AP}}{\text{PH of BA}}$$

The PHR was then plotted against the serial (standard) AP concentrations, with PHR on y-axis and AP concentration on x-axis. (Fig 2). The coefficient of linear regression and the slope were determined. The retention times (Rt) of AP and BA were noted.

2.2.4 Definitive Study Using Eleven Alcohol Consuming Volunteers

Two tablets of AP (250mg each) was administered orally with 200ml of water to each of the eleven volunteers on an empty stomach at zero time. A pre-dose blood sample was

collected from each subject and used to produce a calibration curve. Blood samples were then taken at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 15h post-drug ingestion. Subjects were allowed to eat only food and drink water 2 hours after dosing. Alcohol ingestion or cigarette smoking was not allowed. Each blood sample was put in a heparinised labelled container and refrigerated pending analysis. This definitive study was preceded by an earlier one (a pilot study involving one volunteer) done in order to optimize the methodology.

2.2.5 Analysis of Plasma Antipyrine

0.2ml of each of the timed post-dose plasma samples was added to the corresponding labelled test tube containing 0.1ml of benzoic acid (100µg/ml), 1.6 ml of methanol and 0.1ml of distilled water to make up a mixture of 2.0ml volume.

The content of each tube was mixed on a Vortex mixer for 15 seconds, then centrifuged at 3,000g for 10 minutes to remove plasma proteins. The supernatant was filtered though a 0.6µm paper filter and the filtrate was put in a labeled container.

20µl of the filtrate was injected into the H.P.L.C. machine. The retention times of antipyrine (AP) and Benzoic acid (BA) were noted while the peak heights of AP and BA were manually measured on the chromatograms. A typical chromatogram is shown in Fig 1.

The peak height ratios of the AP and BA in the post-dose chromatograms were also measured manually and used to derive the plasma AP concentration, thus:

$$\text{Plasma AP concentration} = \frac{\text{PHR}}{\text{Slope of Calibration Curve}}$$

The plasma AP concentration in the eleven volunteers are shown in *Table 3. 2.*

A semi-logarithmic graph of mean plasma AP concentration (on y-axis) and time (on x-axis) was plotted. This graph *(see appendix II)* was used to get a manual estimate of lag time, Co, Ke, t½β, Ka, t½ α.

2.3 DATA ANALYSIS

The calculated values of lag time, Co, Ke, and Ka, derived from the semi-log plot of the mean plasma AP concentration versus time were fed into the computer together with the plasma AP concentration of individual volunteers *(Table 3.2).* These were subjected to interactive weighted non-linear best least-square regression analysis using a modified version of SIMP which computed the following pharmacokinetic parameters: Vd, Cl, Co. Ke, Ka. Lag time, t½β, t½α, $AUC_{0\text{-}15h}$, $AUC_{0\text{-}\infty}$, $C_{max,\ and}$ t_{max}.

The kinetic parameters obtained were compared with those of a collaborating colleague (Dr. Nwanze) who was at the same time doing a similar work on normal volunteers drawn from the same study population.

There was a working collaboration throughout this study and Dr. Nwanze's . The results from the two subsets were subjected to statistical analysis using Students' 't'-test for paired samples. P values less than 0.05 were accepted as being significant.

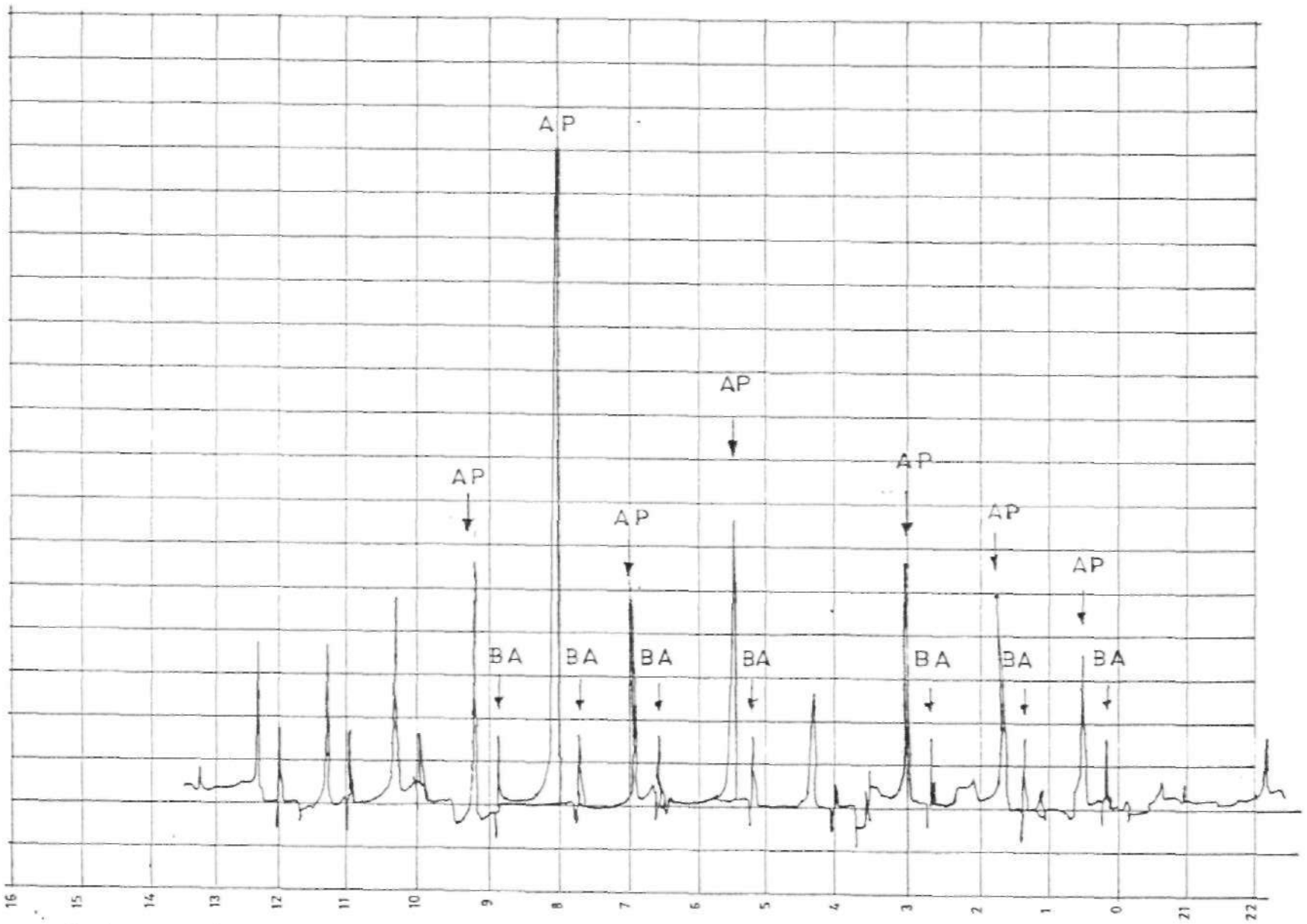


Fig. 1 Typical Elution Chromatogram Showing Peaks For Antipyrine (AP) And Benzoic Acid (BA)

CHAPTER THREE

RESULTS

CHAPTER THREE

3. 0 RESULTS

3. 1 QUALITY CONTROL OF PRODUCED ANTIPYRINE TABLETS

The mean weight of ten antipyrine tablets using a Mettler balance was 270 ± 10 mg. No tablets was found to deviate more than 10% from the mean weight. Similarly no significant difference was found to exist among the produced tablets when the tablet thickness was determined using a Vernier caliper. The friability value or loss in weight of tablet calculated in percentage was 0.3%. much below the maximum limit of 0.8%. No tablet capped during the friability test. Tablets were generally of average tensile strength when tested using Monsanto Hardness Tester. Average disintegration time of the tablets was 2 minutes.

3. 2 *VOLUNTEERS*

There were eleven (11) volunteers in the study. They were all males between the ages of twenty (20) years and thirty-two (32) years (mean ±S.D of 26±5years). Their weights varied from 50kg to 80 kg (mean ±S.D of 62.5 ±10.4kg). The mean alcohol consumption was 15.0 ± 3.59g/ ethanol per week. The mean ethanol use per kilogram body weight was 0.24 ± 0.06g. Table 3.1 below shows the details of the volunteers characteristics.

TABLE 3. 1:

The Age, Sex, Weight, and Amount of Alcohol use of the Volunteers.

S/No.	Volunteer	Sex	Age (yrs)	Weight (kg)	Alcohol use (g/ethanol) per week	Alcohol use per week (g/ethanol/kg)
1	MD	M	20	52	15	0.20
2	NA	M	22	54	15	0.28
3	DK	M	25	54	15	0.28
4	AI	M	30	70	10	0.14
5	TG	M	32	78	15	0.19
6	MT	M	32	74	10	0.14
7	AD	M	28	60	20	0.33
8	MM	M	18	58	15	0.26
9	KD	M	20	58	20	0.34
10	SI	M	31	80	20	0.25
11	SY	M	25	50	10	0.20
Mean±S.D			**25.73±4.95**	**62.54±10.4**	**15.0 ± 3.59**	**0.24 ± 0.06**

3.3 Calibration Curve

AP concentrations used in the calibration curve ranged from 5µg/ml to 100µg/ml. The BA concentration was constant at 100µg/ml throughout the study (*See Appendix 1)*. The graph of PHR (y-axis) against AP concentration (x- axis) was linear with a slope of 0.10 ± 0.006 and Pearson's coefficient of correlation of 0.99 (Fig. 2).

3.4 Plasma antipyrine (AP) Concentrations (µg/ml) in eleven alcohol Consumers

The plasma AP concentrations (µg/ml) in all eleven volunteers from 0.25h to 15h. are shown in table 3.2. The plasma level rose rapidly from a mean value of 3.80 ± 0.09µg/ml at 0.25h to a peak mean level of 17.33 ± 0.30µg/ml at 1.0h. It then slowly declined from this peak level to 3.32 ± 1.10µg/ml in 15.0h. Fig 3 illustrates this trend graphically.

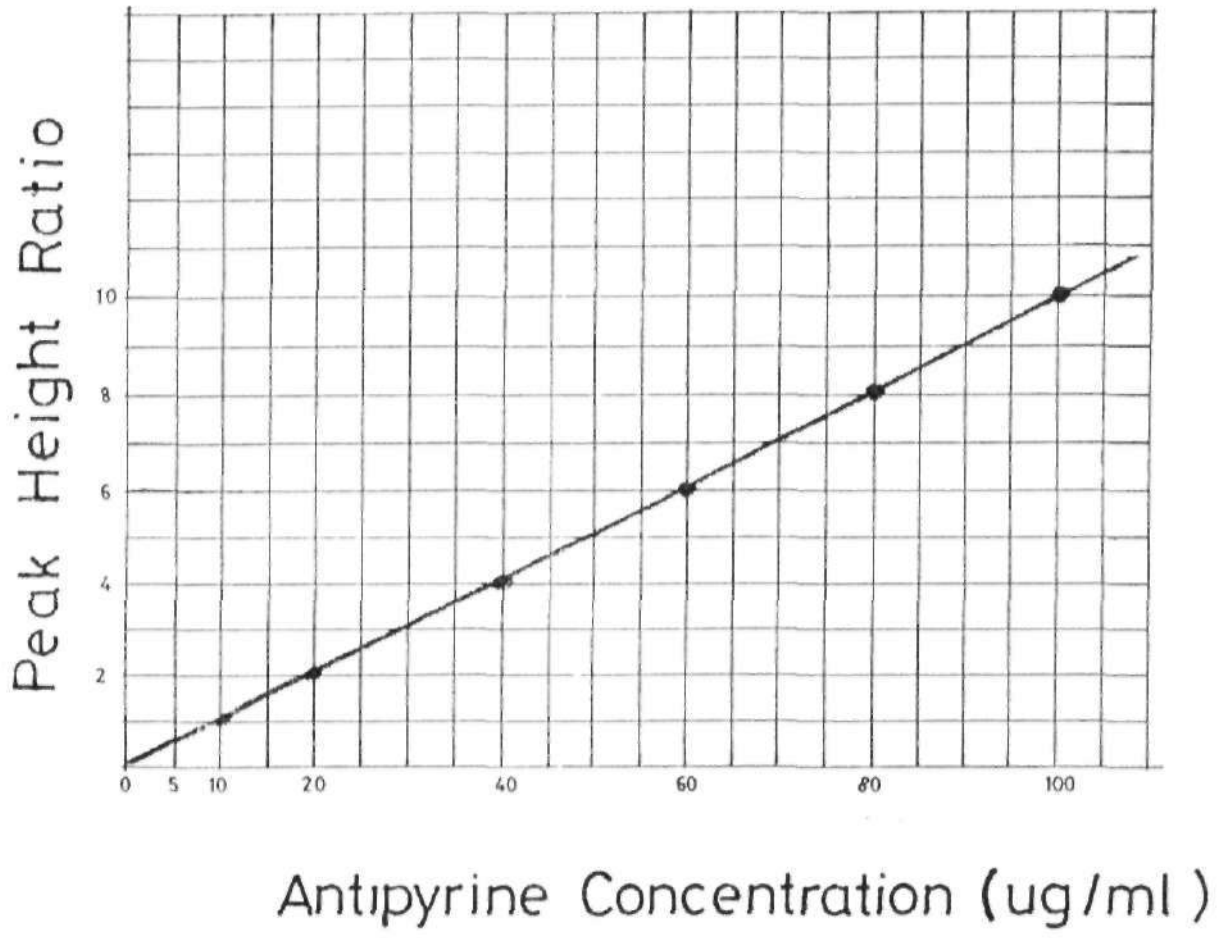


Fig .2. Antipyrine Concentration Curve

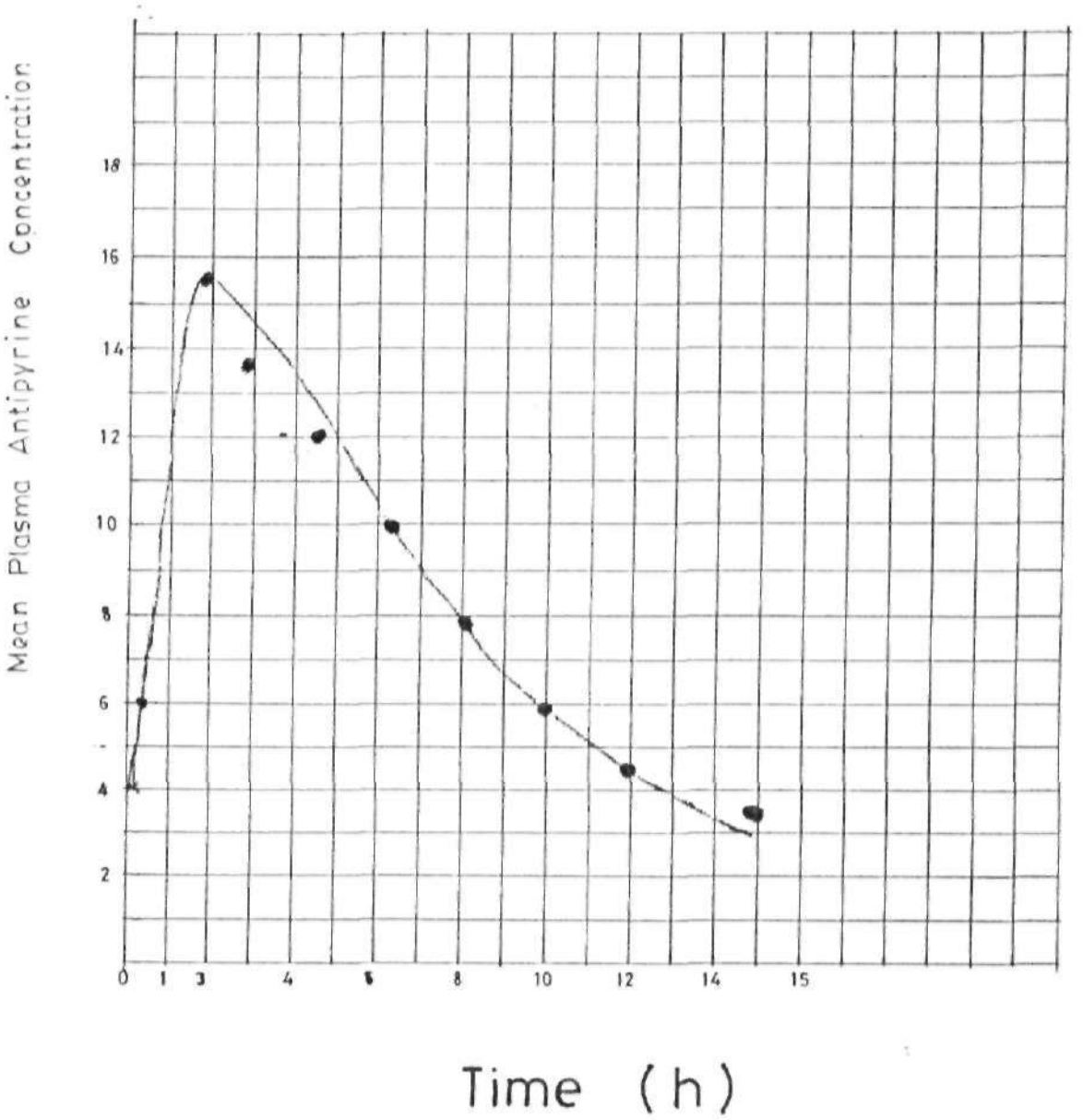


Fig. 3 Mean Plasma Antipyrine Concentration (ug/ml) Versus time

TABLE 3.2:

Plasma Antipyrine Concentrations (μg/ml) in Eleven Alcohol Consuming Volunteers

Time (Hours)	1	2	3	4	5	6	7	8	9	10	11	Mean	± SD
0.25	3.58	3.78	3.39	3.75	3.61	3.63	3.73	3.40	4.55	4.66	3.68	**3.80**	**0.088**
0.5	5.54	5.74	5.05	6.03	5.51	5.57	5.79	5.49	5.84	6.42	5.93	**5.72**	**0.33**
1.0	17.29	17.32	16.80	17.75	17.26	17.37	17.49	17.21	17.25	16.95	17.88	**17.33**	**0.30**
2.0	15.33	15.82	15.44	15.60	15.22	15.33	15.42	15.44	15.55	15.39	15.31	**15.44**	**0.04**
3.0	13.38	13.87	14.36	13.43	13.33	13.48	13.57	13.28	14.36	13.43	13.35	**13.62**	**0.38**
4.0	11.42	11.81	11.82	11.02	11.60	11.25	11.61	12.22	10.62	11.47	11.37	**11.47**	**0.093**
6.0	8.97	7.87	9.46	9.00	8.48	9.43	9.26	9.40	9.52	9.95	9.65	**9.18**	**0.45**
8.0	7.30	6.32	7.70	7.01	7.11	7.43	7.96	7.78	7.70	7.61	7.50	**7.40**	**0.10**
10.0	5.34	5.25	5.54	5.50	5.58	5.56	5.52	6.03	5.57	6.00	5.93	**5.62**	**0.25**
12.0	4.07	4.17	4.17	4.56	4.56	4.65	4.66	4.37	3.78	4.46	4.14	**4.33**	**0.10**
15.0	3.09	3.58	3.31	3.58	2.87	3.71	4.23	3.03	3.15	2.88	3.12	**3.32**	**0.10**

3.5 Pharmacokinetic Parameters

Tables 3.3 and 3.4, show both the individual and mean pharmacokinetics parameters of antipyrine (AP) in the alcohol consuming volunteers. The extrapolated drug plasma concentration at zero time (Co) ranged from 19.95µg/ml to 22.76µg/ml with a mean of 20.83 ± 0.75µg/ml. The absorption rate constant (Ka) was between 0.97 and 2.07/h (mean 1.87±0.32/h), while the absorption half-life (t½ α) ranged from 0.30h to 0.40h (Mean 0.35 ± 0.01h). The lag time varied from 0.14h to 0.20h (mean 0.18 ± 0.002).

The volume of distribution (Vd) of the drug ranged from 22,867.78ml to 26,458.25ml with a mean value of 25,154.40 ± 918.86ml. The area under the drug concentration versus time curve, $AUC_{0\text{-}15h}$ and $AUC_{0\text{-}\infty}$ were 116.74µg-h/ml, to 123.91µg-h/ml, (mean 122.10±2.56µg-h/ml) and 139.29µg-h/ml to 159.75µg-h/ml (mean 146.88±6.63µg-h/ml,) respectively

The range of the peak plasma drug concentration recorded in the study were 16.80µg/ml and 17.78µg/ml respectively, with a mean of 17.33 ± 0.3µg/ml. The time to achieve this peak plasma drug level (t_{max}) was at 1.0h post drug ingestion in all the volunteers. The elimination rate constant (Ke) of the drug ranged from 0.12/h to 0.14/h with a mean of 0.14 ± 0.003/h, while elimination half life (t½ β) ranged from 4.42h to 5.55h (mean 5.11 ± 0.30h) The plasma clearance values varied from 3,192.88 ml/h to 3,641.87ml/h with a mean value of 3,415.62 ± 157.93 ml/h.

TABLE 3.3

INDIVIDUAL PHARMACOKINETIC PARAMETERS OBTAINED FROM PLASMA OF ALCOHOL CONSUMING VOLUNTEERS AFTER INGESTING 500mg ANTIPYRINE

	Pharmaco-kinetic parameters	1	2	3	4	5	6	7	8	9	10	11
1	Vd (ml)	25,173.92	22,867.78	25,023.36	24,490.71	25,772.74	25,691.45	24,665.40	26,035.00	26,458.25	26,458.25	24,949.72
2	Cl (ml/h)	3,537.23	3,581.83	3,312.28	3,378.93	3,641.87	3,293.64	3,129.88	3,258.56	3,522.6	3,519.35	3,395.59
3	Co (µg/ml) (intercept on y-axis)	20.79	22.76	20.83	20.86	20.78	20.18	20.22	19.95	21.34	20.56	20.81
4	Ke (h-1)	0.14	0.16	0.13	0.14	0.15	0.13	0.15	0.12	0.14	0.12	0.13
5	Ka (h-1)	1.98	1.83	1.83	2.07	0.97	2.01	2.04	2.06	1.78	1.94	2.06
6	Lag Time (h)	0.19	0.18	0.20	0.17	0.18	0.17	0.18	0.19	0.20	0.15	0.14
7	$t_{1/2}\ \beta$ (h)	4.93	4.42	5.33	5.03	4.90	5.24	5.21	5.55	5.10	5.31	5.24
8	$t_{1/2}\ \alpha$ (h)	0.35	0.38	0.38	0.33	0.35	0.34	0.32	0.30	0.39	0.40	0.36
9	$AUC_{0\text{-}15}$ (µg-h/ml,)	119.36	116.74	123.91	122.20	118.89	123.88	125.31	124.84	123.07	122.53	122.34
10	$AUC_{0-\infty}$ (µg-h/ml,)	141.35	139.59	150.95	148.19	139.29	151.81	159.75	153.44	141.94	142.07	147.25
11	Cmax (µg/ml)	17.29	17.32	16.80	17.78	17.26	17.37	17.49	17.21	17.25	16.95	17.88
12	t_{max} (h)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

TABLE 3.4:

Pharmacokinetics of Antipyrine in Eleven Alcohol Consuming Volunteers (Mean Values)

No	Pharmacokinetic Parameters	Mean	± SD
1	Vd (ml)	25,154.4	918.86
2	Cl (ml/h)	3,415.62	157.93
3	Co (µg/ml) (intercept on y-axis)	20.83	0.75
4	Ke (h^{-1})	0.14	0.003
5	Ka (h^{-1})	1.87	0.32
6	Lag Time (h)	0.18	0.002
7	T½ β (h)	5.11	0.30
8	T½ α (h)	0.35	0.03
9	AUC_{0-15} (µg-h/ml)	122.10	2.56
10	$AUC_{0-\infty}$ (µg-h/ml)	146.88	6.63
11	C_{max} (µg/ml)	17.33	0.31
12	t_{max} (h)	1.00	-

3.5

3.6 Comparison of Pharmacokinetics of Antipyrine in Normal Volunteers and those who Consume Alcohol

A comparison of the pharmcokinetic values of antipyrine in non-alcohol consuming volunteers and their alcohol consuming counterparts in table 3.5 showed significant diffeences in the values of the elimination rate constants, the elimination half-life, the systemic clearance, the lag time, the area under the drug concentration-time curve (0 – 15 hours and 0 infinity respectively), and the time to attain peak plasma drug concentration. Other pharmacokinetic parameters such as the volume of distribution, the peak plasma drug concentration, the extrapolated plasma drug concentration at zero time, absorption rate constant and absorption half-life, were not significantly different in the two study populations.

Table 3.5

Comparison of Pharmacokinetics of Antipyrine in Normal

(non-alcohol comsuming) * Volunteers and those Consuming Alcohol

S/No.	Pharmacokinetic Parameters	Non-alcohol Consuming Volunteers		Alcohol Consuming Volunteers		Probability (P) Value	Remark
		Mean	±SD	Mean	±SD		
1	Vd (ml)	25848.12	867.82	25,154.4	918.86	0.138	NS
2	Cl (ml/h)	2169.35	109.40	3,415.62	157.93	0.0	S
3	Co (µg/ml) (intercept on y-axis)	20.08	1.29	20.83	0.75	0.109	NS
4	Ke (h^{-1})	0.083	0.004	0.14	0.003	0.0	S
5	Ka (h^{-1})	1.89	0.17	1.87	0.32	0.84	NS
6	Lag Time (h)	0.10	0.003	0.18	0.002	0.0	S
7	$t_{½}$ β (h)	8.27	0.43	5.11	0.30	0.0	S
8	$t_{½}$ α (h)	0.37	0.001	0.35	0.03	0.187	NS
9	AUC_{0-15} (µg-h/ml)	161.52	1.15	122.10	2.56	0.0	S
10	$AUC_{0-\infty}$ (µg-h/ml)	231.14	11.72	146.88	6.63	0.0	S
11	C_{max} (µg/ml)	17.47	0.57	17.33	0.31	0.47	NS
12	t_{max} (h)	1.98	0.005	1.00	-	0.0	S

P < 0.05 is Significant (S)

P> 0.05 is not Significant (NS)

* **Data from Dr Nwanze's work (See Appendix IV)**

CHAPTER FOUR

DISCUSSION

CHAPTER FOUR

DISCUSSION

4.1 EVALUATION OF METHODS

4.1.1 Reproducibility:

The retention times (Rt) of benzoic acid and antipyrine were 2.94 minutes and 6.68 minutes respectively throughout the work. The peak height ratios of the standard antypyrine were also reproduced in all cases. The relationship between the standard antipyrine concentrations and antipyrine-to-benzoic acid peak height ratios were linear, up to 100µg/ml. Pearson's correlation coefficient was 0.99 and the slope of the regression line was 0.1±0.006. The linearity of the calibration curve in the range 5-100 µg/ml demonstrated that this drug can be assayed within this range with the methods being used. There were no interference or ghost peaks in the chromatograms. The reproducibility of the analytical method as demonstrated by the retention times and constant peak height ratios confirmed its accuracy.

4.1.2 Precision:

The precision of the methods was checked by the manual peak height method of quantitation and found to be within the range of 1.1 to 5.5%.

4.1.3 Sensitivity

The sensitivity limit of the method ranged from 5µg/ml to 100µg/ml for the standard antipyrine. The plasma antipyrine concentrations from the volunteers were within these limits.

4.2 EVALUATION OF RESULTS

The semi-logarithmic graph of plasma antipyrine concentration – time *(Appendix II)* showed that the data of this study fitted into a single compartment model. This is compatible with the known pharmacokinetic behaviour of antipyrine i.e. rapid absorption, large volume of distribution and slow elimination from the body. The short lag time of 0.12 ± 0.004h confirmed the rapid absorption of antipyrine tablet into the intestinal fluids.

Comparison of the pharmacokinetics of antipyrine in the non-alcoholic consuming volunteers as obtained by Nwanze, (1998) and that obtained in this study from alcohol consuming volunteers showed significant differences in the values of the elimination rate constant, the elimination half-life, the systemic clearance, the lag time, the area under the plasma drug concentration-time curve (zero to 15 hours and zero to infinity respectively) and the time to attain peak plasma drug concentration. On the other hand, the mean values of Co (20.83 ± 0.75µg/ml), C_{max} (17.33 ± 0.30µg/ml), absorption rate constant (1.87 ± 0.32/h), absorption half-life (0.35 ± 0.03h) and volume of distribution of drug ($25,154.40 \pm 918.86$ml) obtained in this study were not significantly different from the mean values obtained by Nwanze

for the same parameters in his work using a similar group of non–alcoholic healthy Nigerian volunteers and conducted concurrently with the present study. His values were: absorption rate constant (1.89±0.17/h), absorption half-life (0.37±0.001h) and volume of distribution of drug (25,848.12±867.82ml), Co (20.08±1.29µg/ml), C_{max} (17.47±0.57µg/ml).

The increased elimination rate constant and drug clearance values coupled with the decreased elimination half-lives obtained in this study are also in agreement with the results of earlier studies by Rubin and Lieber, (1968); Seixas, (1975) and Vestal *et al,* (1975). This result and those of other workers thus confirm that alcohol does not affect the absorption and distribution of antipyrine significantly, but definitely increases its elimination from the body.

Alcohol has also been shown to increase the elimination of tolbutamide (Carulli *et al,;* 1971), meprobamate (Ugarte *et al.* 1972) barbiturates, phenytoin, acetaminophen, bilirubin and benzpyrene (Mezey, 1976, Seixas, 1975). Also drugs which are known to induce liver microsomal enzymes e.g. antiypyrine, (Vestal, *et al,* 1975), alcohol (Mezey, 1971 and 1976), barbiturates and chlorinated hydrocarbon insecticides (Kolmodin *et al.* 1969), caffein and tobacco smoke (Vestal, *et al,* 1975) have been shown to enhance their own metabolism as well as those of one another. This thus may suggest that the effect of alcohol on the pharmacokinetics of antipyrine could be due to its ability to induce liver microsomal enzymes.

The present data for Nigerian subjects which were comparable to other reports for non-Nigerians may suggest that racial and ethnic factors may not be important determinants of the influence of alcohol consumption on the pharmacokinetics of antipyrine. The time to attain plasma peak concentration (t_{max}) obtained at 1.0 hour in all the volunteers was compatible with the normal pharmacokinetics of antipyrine and was not affected by alcohol consumption.

Sultatos *et al,* 1980 suggested that systemic clearance, elimination rate constant and elimination half-life are the only parameters of drug elimination kinetics. They evaluated the relationship between the systemic clearance, volume of distribution and elimination half-life in man and found that the relationship between the increase of antipyrine elimination half-life and clearance was approximately linear; while no significant correlation was found to exist between elimination half-life and volume of distribution. They then concluded that clearance is a more appropriate parameter for estimating drug elimination, even though, the elimination half-life is also a valid parameter because it is a hybrid constant dependent upon both volume of distribution and clearance (Gibaldi and Koup, 1981) and also because clearance is its primary source of variability. Also changes in elimination half-life may reflect changes in clearance, volume of distribution or both.

There are circumstances in which elimination half-life is more easily measured than systemic clearance. For example, in paediatric or other patients where limited blood samples are available, adequate data may not exist to calculate the area under the drug concentration (AUC) – time curve accurately. AUC is necessary to calculate

clearance accurately and independently. In addition, it is sometimes desirable or even necessary to estimate drug elimination through non-invasive methods such as estimation of antipyrine urinary excretion rate and breath tests; but such methods would not provide an accurate reflection of drug metabolizing capacity.

The value of the intercept on y-axis or concentration of drug at zero time when multiplied by the volume of distribution produced approximately the dose of antipyrine administered to each volunteer. For a drug such as antipyrine, which has a low hepatic extraction ratio, and a large volume of distribution, its concentration at zero time can be used to determine the validity of results of pharmacokinetic studies.

4.3 CONCLUSION

The results of this study has shown that alcohol consumption, even without features of alcoholism or overt liver disease influences the elimination of antipyrine from the body; the effect which is thought to be due to alcohol-induced microsomal enzyme activity in the liver. It should be assumed that this effect can be produced by other hepatic microsomal enzyme inducers such as cigarette and tobacco smoke, halogenated hydrocarbon insecticides, phenobarbitone etc, many of which assail Nigerians regularly. The method used in the study was rapid, sensitive, specific, precise, reproducible and accurate; it involved the use of small aliquots of plasma (0.2ml) which did not require extraction into organic solvents. The method may also be suitable for studies in laboratory animals and paediatric patients with limited blood samples. It also eliminates the requirement for quantitative extraction into organic

solvents, and may prove useful for simultaneous determination of antipyrine and other compounds.

4.4 SUGGESTIONS FOR FURTHER WORK

In view of the abuse potential of alcohol, a possible extension of this work would be to determine the influence of chronic alcoholism or even addiction on the pharmacokinetics of antipyrine. Determination of blood alcohol levels and types of alcoholic beverages consumed may provide additional useful information. The concurrent presence of endemic conditions like malnutrition, malaria, or other parasitic diseases may also constitute interesting studies.

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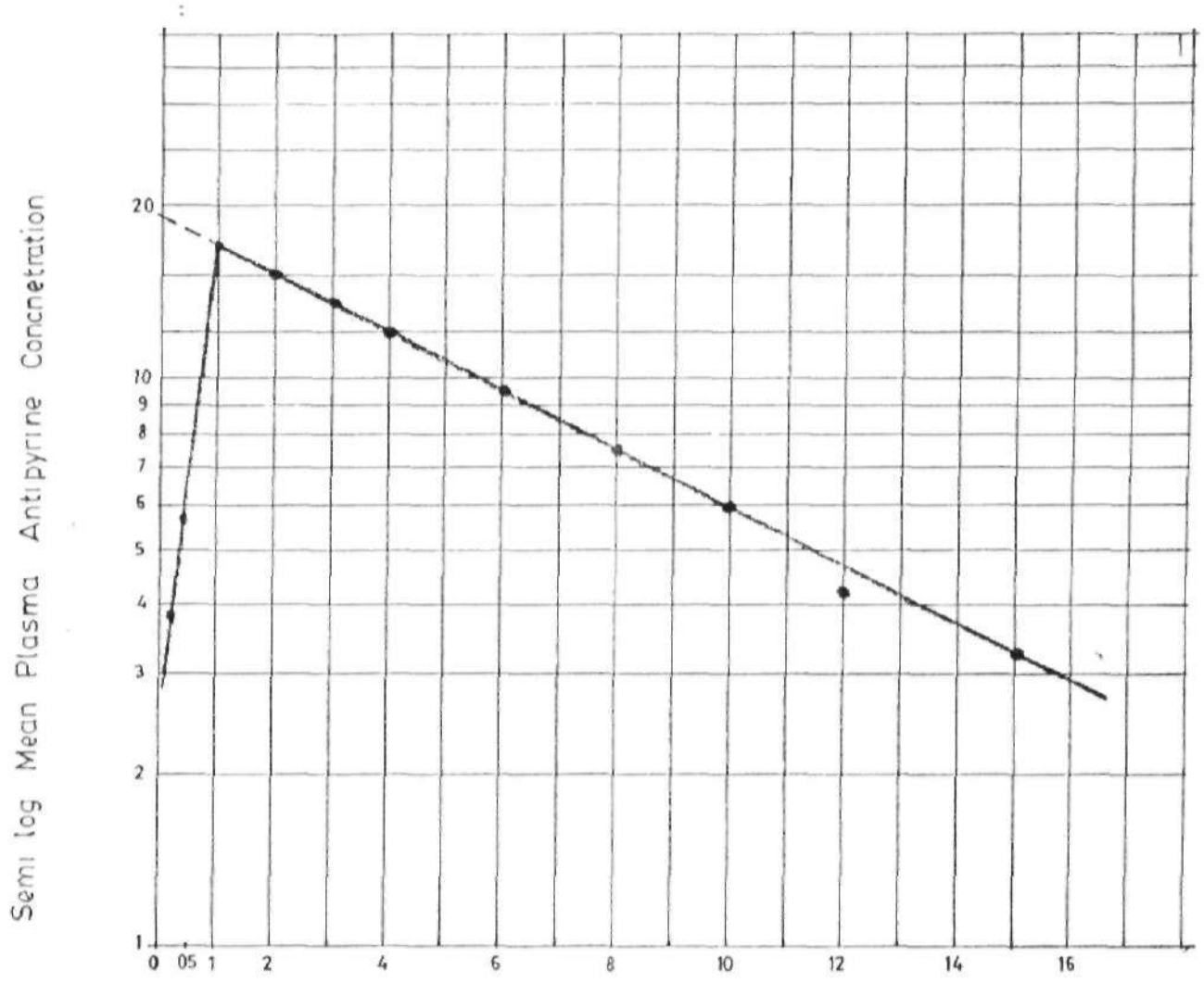
APPENDICES

APPENDIX I

Data Used in Standard Calibration Curve

COMPOUND (μg/ml)		PEAK HEIGHT (mm)		PHR
AP	BA	AP	BA	
5	100	3.1	5	0.62
10	100	5.5	5	1.1
20	100	10.0	5	2.0
40	100	21.0	5	4.2
60	100	31.0	5	6.2
80	100	41.0	5	8.2
100	100	51.0	5	10.2

Appendix II



Semi Log Mean Plasma Antipyrine Concentration Versus Time (**h**)

APPENDIX III

PHARMACOKINETIC PARAMETERS OBTAINED FROM INDIVIDUAL PLASMA CONCENTRATIONS AFTER INGESTING 500mg ANTIPYRINE

PHARMACOKINETIC PARAMETERS.	VOLUNTEERS												Mean	Std. Devt.	Coeff. Corr %
	A	B	C	D	E	F	G	H	I	J	K	L			
Vol. Of Distribution (Vd) (ml)	24574.45	24952.01	25387.93	26260.85	27427.42	26421.25	25433.28	26149.20	26380.02	25318.78	24984.01	26888.19	25848.12	±867.83	3.35
Clearance (Cl) (ml/h)	1952.66	2207.80	2089.70	2106.26	2224.59	2237.37	2277.82	2261.11	2134.07	2350.18	2099.24	2091.31	2169.35	±109.40	5.04
Intercept on Y-axis (Co)(µg/ml)	20.99	21.54	20.77	19.91	19.30	19.90	21.22	19.98	19.65	20.26	20.94	19.48	20.08	±1.29	6.42
Elimination rate constant (Ke) (h^{-1})	0.079	0.088	0.082	0.080	0.084	0.084	0.089	0.086	0.081	0.092	0.083	0.077	0.083	±0.004	4.80
Absorption rate Constant (Ka) (h^{-1})	2.01	1.47	1.74	2.03	1.83	2.11	1.81	1.96	2.00	1.87	1.88	1.98	1.89	±0.17	8.90
Lag time (h)	0.10	0.05	0.04	0.11	0.12	0.11	0.09	0.11	0.11	0.14	0.09	0.12	0.10	±0.006	6.00
Elimination half-life $t_{½}\beta$ (h)	8.72	7.83	8.45	8.66	8.54	8.18	7.74	8.01	8.57	7.47	8.25	8.91	8.27	±0.43	5.20
Absorption half-life $t_{½}\alpha$ (h)	0.34	0.47	0.40	0.34	0.38	0.35	0.38	0.36	0.35	0.37	0.37	0.35	0.35	±0.001	2.70
A.U.C (0 – 15h) µg.h/ml	171.91	163.75	165.89	162.08	156.33	157.93	163.01	156.94	159.63	152.96	166.38	161.43	161.52	±1.15	0.71
A.U.C (0 - α) µg.h/ml	256.13	226.47	239.27	237.39	224.76	223.48	219.51	221.13	234.29	212.75	238.30	239.08	231.14	±11.72	4.90
T_{max} (h)	1.96	2.00	1.80	1.98	2.01	2.01	2.00	2.00	1.98	2.00	1.99	2.01	1.98	±0.005	2.50
C_{max} (µg/ ml)	18.53	17.79	17.79	17.30	18.04	17.80	17.54	16.80	17.48	16.85	17.61	16.77	17.47	±0.57	3.25