THE EFFECT OF RANITIDINE ON THE ABSORPTION AND PHARMACOKINETICS OF PRAZIQUANTEL IN *SCHISTOSOMA HAEMATOBIUM* -INFECTED SUBJECTS

BY

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DECLARATION

I hereby declare that this thesis has been written by me and it is a record of my research work carried out under the joint supervision of Dr. Abdullahi Mustapha and Dr. Ibrahim Abdu-Aguye. 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

This thesis entitled "THE EFFECT OF RANITIDINE ON THE ABSORPTION KINETICS OF PRAZIQUANTEL IN SCHISTOSOMA HAEMATOBIUM INFECTED SUBJECTS" by MUSA UMAR meets the regulations governing the award of the degree of MASTER OF SCIENCE (PHARMACEUTICAL ANALYSIS) OF AHMADU BELLO UNIVERSITY, and is approved for its contribution to Science, and literary presentation.

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DEDICATION

This work is dedicated to my wife Khadija, and my Children, Kellu Shuwa and Abubakar Sadiq Girei.

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ABSTRACT

Praziquantel tablets were assayed for content of active ingredient. Tests for disintegration and dissolution were carried out. The results complied with the official specifications for praziquantel tablets. The dissolution rates were however lower at pH 6.8.

The effect of ranitidine, ISOmg on the mean absorption and pharmacokinetics of praziquantel following single dose of 40mg/kg was studied in six *Schistosoma haematobiutn-'mi'ecied* subjects. The study was conducted in a crossover experiemental design in which ranitidine was given one hour prior to praziquantel administration. Pharmacokinetic parameters were derived from the praziquantel plasma concentration - time profiles of subjects using PCNONLIN non linear estimation program. The pharmacokinetic data obtained were compared using the student t-test: paired - two sample for means and values of $p<0.05$ were considered statistically significant and values of $p>0.05$ were considered statistically not significant. Ranitidine coadministration caused statistically significant changes in Cmax, Kabs and Tlag ($P<0.001$, $P<0.05$ and PO.05 respectively). The change in Tmax was however not significant ($P<0.5$).

The effect of ranitidine on Tl/2, Kel, and the AUC was also significant (PO.01, $P<0.01$ and P- 0.001 respectively). The total systemic clearance increased significantly ($p<0.01$). This may suggest that the liver may not be the only site of praziquantel presystemic elimination. Considerable information can therefore be obtained from measuring both drug and metabolites alter drug administration, although using mean pharmacokinetic values may be misleading and should be cautiously interpreted especially where quantitative aspects of drug metabolism are concerned.

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ABBREVIATIONS

Abbreviation	Full Meaning
AUFS	Absorbance units full scale
^{o}C	Degrees Centigrade
Conc.	Concentration
cm	Centimeter
et al	and others
h	hour
HPLC	High Performance Liquid Chromatography
i.e.	That is to say
IV	Intravenous
kg	kilogram
L_n	Natural logarithm
L	liter
M	molar
mcg, ug	microgram
mg	milligram
ml	milliliter
min.	minutes
NaOH	Sodium hydroxide
nm	nanometer
pH	hydrogen ion concentration
PSI	pound per square inch
SEM	Standard error of the mean
µl	microliter
µg	microgram
α	alpha

β	beta
$<$	less than
$>$	greater than
$+$	plus
$-$	minus
%	percent

CHAPTER ONE

1.1 INTRODUCTION

The concomitant administration of two or more drugs may elicit responses different from the simple sum of the observed effects when the same doses of the drugs are given separately. These changes may be brought about either by one drug influencing the pharmacokinetics of the second drug, or by one drug modifying the pharmacodynamic actions of the second drug. The result of a drug-drug interaction may be for one drug to increase or decrease the pharmacological effects of another drug (quantitative changes) or the observed results may bear no obvious relation to the pharmacological properties of the drugs when used separately (qualitative changes) There may also be the importance of the sequence and time interval between taking the two drugs.

1.2. SIGNIFICANCE OF DRUG INTERACTIONS.

Drug interactions are usually reported as been statistically significant or clinically significant, but there is no consensus in the medical literature as to the clinical significance of the many statistically significant drug interactions. Drug interactions may vary from person to person and from species to species. They may be of major clinical significance or of no clinical significance at all (Riegalman *et al.*, 1971)

1.3. PHARMACOKINETIC DRUG INTERACTIONS.

Pharmacokinetics is the study of the kinetics of absorption, distribution, metabolism and excretion of drugs and their pharmacological, therapeutic or toxic response in animals and man (Riegalman *et al.*, 1973).

The goal of rational drug therapy envisages the attainment of desired plasma drug concentration and successful control or eradication of a disease state through the appropriate selection, proper administration and efficient distribution of drugs.

1.4. PHARMACOKINETIC MODEL SYSTEMS.

The most common method used to describe the pharmacokinetic behaviour of a drug in the body is by the use of models depicting the body as a single compartment or series of compartments. These compartments do not usually have any literal anatomic or physiological reality but visualisation of the body as such helps explain the observed disposition characteristics of various drugs and allows prediction of drug concentrations in the body as a function of time, dose and route of administration.

The single compartment with intravenous bolus administration, assumes that the drug is instantaneously available for distribution throughout the vascular system. Thus following an intravascular dose, one is only concerned with distribution, drug transfer to and from the various tissues and drug elimination. The rate of drug elimination from the body is assumed to follow first-order or linear pharmacokinetics (Figure 1.4.1)

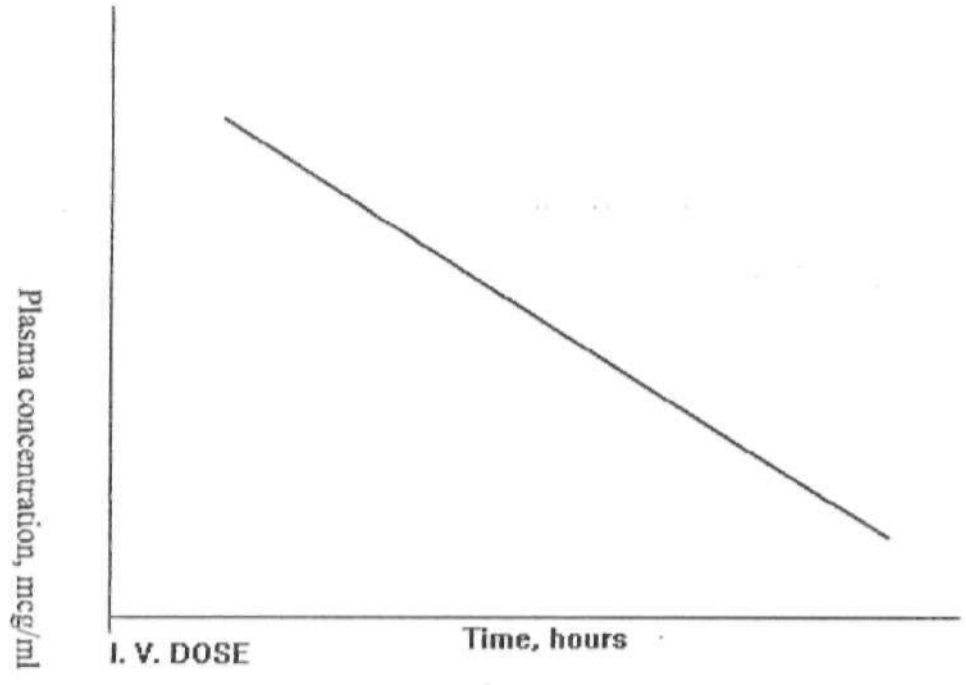


Figure 1.4.1 Plasma concentration - time profile after intravenous administration for a drug characterised by a one - compartment model.

The two-compartment model separates the body into a central compartment (usually with a small volume) and a peripheral compartment with larger volume. Initial distribution of a drug occurs within the central compartment, assumed to consist of plasma/blood, other body fluids and highly perfused tissues which are in rapid equilibrium with the blood. Slower and reversible drug distribution then

occurs between the central and peripheral compartments. The peripheral compartments are those body fluids that slowly equilibrate with the drug in plasma/blood. Drug elimination from the body is assumed only to occur from the central compartment. Both distribution between the compartments and elimination from the central compartment are again assumed to obey first - order pharmacokinetics. (Figure 1.4.2)

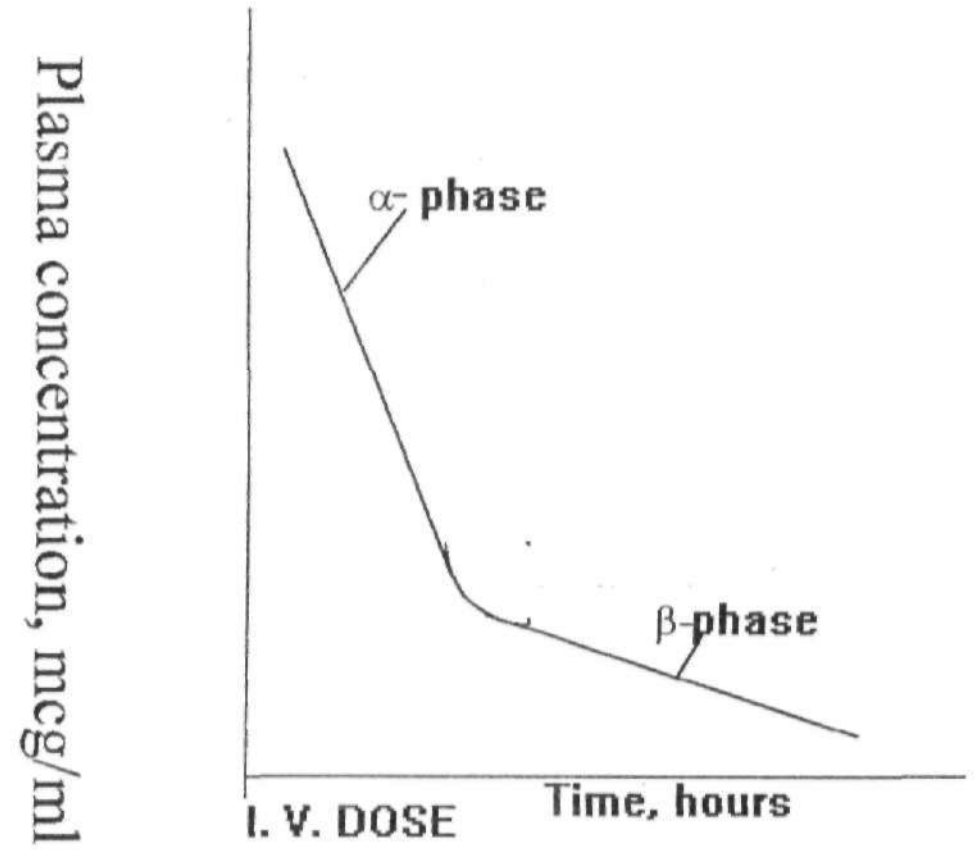


Figure 1.4.2. Plasma concentration - time profile after intravenous administration for a drug characterised by a two - compartment model.

The combined effect of two compartments gives rise to a biphasic decline curve on intravascular injection, designated as α-phase for the relatively rapid distribution between the central and peripheral compartments, and a β-phase which is determined mainly by the irreversible elimination from the central compartment.

Multicompartment models may be necessary to fully explain observed pharmacokinetic behaviour of a drug but the increased complexity of these models may reduce their clinical value. The disposition of gentamicin is best described by a two - or three - compartment (Schentag *et al*; 1977) but the use of a one - compartment model to predict plasma concentrations and individualise dosing regimens for gentamicin has proven successful clinically (Sawchuk *et al*., 1976)

1.5. DRUG ABSORPTION AND BIOAVAILABILITY.

Drug absorption encompasses those processes through which a drug may go after administration, up to the point at which the drug molecules reach the systemic circulation (Rowland, 1981). The commonest route of administration of drugs is the oral route where the majority of the dose is absorbed from the small intestine into the portal vein, through the liver and into the systemic circulation. Smaller amounts of drug are absorbed through the stomach walls and through the walls of the large intestine.

In some cases, high plasma concentrations achieved during rapid absorption of a drug may be undesirable. For this reason and to reduce the frequency of dosing for drugs with short half-lives, formulations which release their drug dose more slowly into the gastrointestinal tract have been designed. Such slow - release or controlled - release products (Dressman *et al*, 1986) have found increasing acceptance by physicians and patients.

The process of drug absorption is dependent on many factors, including the surface area available for absorption, existence of drug concentration gradient, adequate blood flow to and from the absorption site and the physico-chemical properties of the drug particularly the lipid solubility and degree of ionisation. The retention index of a compound on a C-18 reversed-phase HPLC has been used as a measure of lipophilicity (Carlson *et al*., 1975). Many lipid-soluble drugs are absorbed more readily than some small polar molecules (e.g. urea) and it was thought that the main route of absorption is through the lipid membrane and not through the aqueous pores (Schanker *et al*., 1958).

However at high octanol/water partition coefficient (Log P>3) the absorption rate reaches a plateau level (Higuchi *et al*., 1979, Taylor *et al*., 1985). It has been suggested that this is due to a change

in the rate limiting factor from permeability control to aqueous diffusion control across the unstirred layer (Higuchi *et al.*, 1979).

For drugs that are weak acids or bases an increase in the proportion of unionised drug usually increases their ability to cross lipid membranes. Thus weakly acidic drugs should be more rapidly absorbed in the stomach due to the low pH of gastric fluids. However, the absorption of these compounds is much faster and more complete in the more basic environment of the small intestines because of the greater blood flow to the intestines and larger surface area available for absorption compared to the stomach (Crouthamel *et al.*, 1971) Another factor which has to be taken into account when considering the absorption of solid dosage forms is the solubility of the drug substance in the gastrointestinal fluids. Acidic compounds will tend to dissolve more slowly in the acidic contents of the stomach but basic compounds will dissolve readily. Some acidic compounds of low aqueous solubility may even be precipitated from solution on entry into the stomach.

The main disadvantage of the oral route is that most of the absorbed dose passes directly through the liver before entering the systemic circulation. The liver is the major metabolising organ of the body and can cause extensive deactivation of some drugs during this initial exposure to the liver immediately after absorption. This presystemic elimination ('first pass effect') may limit the effectiveness of the oral route of administration for these drugs by considerably reducing the amount of unchanged drug reaching the site of action, even if actual absorption of the drug is complete (Riegalman *et al.*, 1973). The systemically available (bioavailable) dose is a major determinant of the therapeutic efficacy of a drug. Bioavailability is the rate and extent to which the active drug or therapeutic moiety is absorbed from the drug product and becomes available at the site of action. Some authors have suggested methods for determining bioavailability using pharmacological data (Smolen, 1976, Holford *et al.*, 1981). In practice bioavailability studies are almost always conducted using blood, plasma or urine drug concentrations following intravenous administration. Urinary data may be less reliable if the PKa of the drug is similar to the urinary pH , due to changes in the fraction reabsorbed.

Relative bioavailability refers to the percentage of a test drug product absorbed relative to a standard preparation (Wagner, 1971), and is usually determined when the intravenous route is not used for comparison either by design or because an intravenous dosage form is not available. Bioequivalence of

two drug products is attained if the products do not differ significantly in the bioavailable dose or in the rate of supply (Riegalman *et al.*, 1973). The rate of drug absorption is dependent on many factors, including the rate of gastric emptying. The presence of food in the stomach may delay gastric emptying thereby decreasing the rate of absorption for most drugs (Toothaker *et al.*, 1980). Drugs such as propantheline, atropine and narcotic analgesics delay gastric emptying, whereas metoclopramide increases the rate of gastric emptying (Nimmo, 1981).

The bacteria of the large intestines are able to change many drug substances chemically and thus to reduce their absorption.

The process of dissolution is important when dealing with compressed oral dosage forms. It is difficult for a drug with low water solubility to dissolve and remain in solution long enough to have significant absorption.

Drug absorption from the gastrointestinal tract is therefore not a simple process and co-administration of other drugs may either slow down or speed up absorption.

1.6. DRUG DISTRIBUTION

Distribution governs the access of drugs to the various physiological areas of the body (nonspecific affinity) and particularly to the effector biophase (specific affinity). Tissues and organs with similar distribution characteristics can be grouped together in a single compartment. A distribution compartment is a space in which the drug distributes with the same dynamics to achieve a homogenous steady state concentration. This space is characterised by the apparent volume of distribution (Vd).

The concept of distribution is used to relate the plasma concentration of a drug to the dose administered if no elimination has occurred. This relationship can reasonably predict the dose of a drug required to achieve a desired plasma concentration. The magnitude of Vd for any drug is determined by the extent of tissue and plasma protein binding and the lipid versus water solubility of the drug.

Rowland and Tozer (1980) have discussed the pharmacokinetic basis of drug - drug interactions based on displacement from plasma proteins. The free unbound drug is capable of diffusing into tissues and it is this concentration which relates to the degree of pharmacological action exerted by the drug. It is possible for one drug to displace another drug from a common binding site, thus

increasing the free concentration of the displaced drug. Displacement becomes significant as a basis for drug - drug interaction when the displaced drug is extensively bound and hence the free concentration is low and secondly that the displacing drug has a high affinity for the binding sites and is present in sufficient amount to occupy a large proportion of binding sites. An important practical result of drug - drug interaction involving plasma protein displacement is that the total plasma concentration of the displaced drug will fall and so the use of plasma concentrations to monitor dosage will be compromised. Serum digoxin levels are significantly increased when quinidine is given. This is caused by digoxin clearance being reduced (Fenster *et al.*, 1984), and also by digoxin being displaced from tissue binding sites. During combined therapy, serum digoxin levels rise while the cardiac tissue concentration and tissue to serum ratio of digoxin decline. Under such conditions the serum digoxin levels do not reflect the same relationship to cardiac performance as they normally do. Hence decreased cardiac effects despite elevated serum digoxin levels can be the result of this interaction (Das *et al.*, 1984).

1.7. DRUG METABOLISM.

Metabolites are of interest because many of them exhibit pharmacologic and toxic effects. Sometimes an administered compound produces no biological effects. It must be converted into active metabolites to exhibit its effects. When the administered compound has no effect, it is called a prodrug, a term especially applied when the prodrug was developed to improve the delivery, increase the stability or diminish the undesirable properties of the active principle (Higuchi, 1987).

First-pass metabolism has a number of consequences that depend on whether most of the pharmacologic activity resides with the administered compound or an active metabolite. Drugs with low oral bioavailability because of first - pass metabolism can show large route - dependent differences in the doses required to achieve a given level of response. Phenylephrine requires 250mg oral dose to achieve same pressor response after 0.8mg of intravenous dose (Goodman *et al.*, 1980).

When most of the activity resides with the metabolite, the therapeutic implications may be quite different. Propranolol and 4-hydroxy-propranolol (active metabolite) both produce ß-blockade because they are both pharmacologically active. The concentration of the metabolite early in time after

oral administration of the drug can greatly exceed that of the expected level after intravenous bolus of the same dose size. At the same plasma propranolol concentration, ß-blockade is observed at early times to be greater after oral rather than after intravenous administration (Cleaveland *et al.*, 1972).

Saturable first-pass metabolism may produce nonlinear metabolism during the absorption phase and consequently the bioavailability of a drug that is highly extracted in the liver increases with oral dose or absorption rate. Nonlinear behavior becomes more common as a rule in drug overdose rather than the exception that applies to the therapeutic range. Hydrallazine (Talseth, 1976) and Metoprolol (Regardh *et al.*, 1980) are drugs that exhibit nonlinear behaviour during absorption due to saturable first pass metabolism.

The metabolism of exogenous lipophilic compounds by higher animals occurs mainly in the liver via a two-phase process. In phase I, a nucleophilic substituent group (-OH, NH_2) is inserted or revealed through oxidation, reduction or hydrolytic enzyme action. In phase II, this substituent group is conjugated, typically with a carbohydrate, amino acid or organic acid. The net effect is to convert lipophilic products which tend to pass readily into cells and bind to cellular components into more hydrophilic products which can be excreted. The enzymes carrying out these two phases of metabolism are collectively referred to as drug metabolising enzymes. The true function of these enzymes may be to facilitate the clearance from the body of exogenous lipophilic substances, or control the synthesis and degradation of endogenous bioactive compounds such as steroids and prostaglandins. The cytochrome p-450 - containing microsomal mixed-function oxidases, coupled with other metabolically linked enzymes provide an important pathway whereby the cell can metabolise and eliminate drugs. These drug metabolising enzyme systems are generally inducible (Testa *et al*; 1978). Enzyme induction is an important factor modifying the pharmacokinetics of many drugs. This enzyme induction is only demonstrable in vivo and there is a delay before increased enzyme activity can be observed.

Enzyme-inducing agents of clinical importance can be substances used in therapeutics or substances found in the environment but to which man is exposed, and both may influence the efficacy of co-administered drugs or cause toxicity such as cancer, tissue necrosis or thrombotic lesions (Breckenridge, 1987). The important inducing agents in clinical use are anticonvulsants (phenobarbitone, phenytoin, carbamazepine, etc) and the antibiotic, rifampicin. They affect mainly the

pharmacodynamics of co-administered drugs with low therapeutic index (e.g. oral anticoagulants, antiarrhythmics). The oral contraceptives may be less effective when given with rifampicin, phenobarbitone or phenytoin. There may be increased risk of break through bleeding or pregnancy (D'Arcy, 1986).

Environmental agents which affect the efficacy of co administered drugs include cigarette smoke which contains inducing agents, principally polycyclic aromatic hydrocarbons. Vestal and Wood (1980) suggest that the effect of cigarette smoking on drug metabolism occurs mainly in the young rather than in the elderly. However it is known that only the reactivities of certain forms of cytochrome p-450 are increased by smoking (Gonzalez, 1989), and it is difficult to predict those drugs whose metabolism will be increased.

On the other hand, chemical agents and drugs can equally inhibit the enzymes involved in drug metabolism. The works of Cook *et al* (1954) and Cooper *et al* (1954) remained an important tool in the understanding of inhibition of drug metabolism. It led to the identification of drug hydroxylation as an essential reaction in the manifestation of biological effects in vivo. Inhibitors can act in several ways, but the most frequent basis of inhibition found in man is competition for active sites on the enzyme by alternative substrates which can also be metabolised. This form of inhibition is reversible. But covalent binding ('suicide inhibition') causes irreversible destruction of the enzyme (Netter, 1987).Imidazole compounds have been recognised as inhibitors of the hepatic enzyme mixed - function oxidase system.

Back *et al* (1985) have shown a clear structure - activity relationship with respect to position of substitution on the imidazole ring and the ability to inhibit drug oxidation. Imidazole derivatives are thought to be potent inhibitors of cytochrome p-450 enzymes because they can bind to both the oxygen and substrate binding sites of these enzymes. Cimetidine, a substituted imidazole and an H_2-antagonist, is an important inhibitor of drug oxidation (Serlin *et al*, 1979). Important and interesting clinical examples of inhibitions of drug metabolism include interactions with oral hypoglycaemic agents, anticoagulants and antihypertensive agents. The significance of the inhibition is partly because of the steep dose-response relationship of these agents.

A kinetic expression for the effect of inhibition on the ratio (R) of the new t½ of a drug in the presence of the inhibitor to the normal t½ has been developed (Rowland, 1975).

$$R = \frac{t½\ (inhibited)}{t½(normal)} = \frac{1}{fm/\{[\ 1+(I/k_i) + (1-fm)\}}$$

Where fm = fraction of dose eliminated by the pathway of interest.

I = amount of Inhibitor.

k_i = the Inhibitor constant.

When all the drug is eliminated by the inhibited route (fm = 1) the ratio R changes drastically with increasing inhibitor concentration. Below fm = 0.5, the maximum increase in the ratio is twofold and is inconsequential from clinical point of view; in such cases, unless the therapeutic index of the drug is small, alteration in dosing is not necessary.

1.8 DRUG ELIMINATION AND PLASMA HALF - LIFE

The two most important eliminating organs for most drugs are the liver and the kidneys. The concept of clearance which was first introduced to describe renal excretion of endogenous compounds (creatinine, urea) as a measure of kidney function has been applied to other eliminating organs (such as the liver) and elimination pathways (biotransformation) for both endogenous and exogenous compounds, and has become central to the development of pharmacokinetics (Wilkinson, 1987). Hepatic clearance is influenced by blood flow to the liver, drug plasma protein binding and the intrinsic metabolic capacity of the liver enzymes (Wilkinson *et al*, 1975). Clearance values most often cited in the literature refer to the total body clearance (Cl_t) of a drug. This is the sum of all clearance processes in the body. Thus the entire body is considered to be a single drug elimination system. Since the kidneys and liver are the major eliminating organs for most drugs, then:

$$Cl_t = Cl_r + Cl_m$$

where, Cl_r = renal clearance

Cl_m = metabolic (hepatic) clearance.

Classifying drugs by hepatic extraction ratio (the fraction of drug removed from the blood in a single pass through the liver) helps predict which of these factors will be the primary determinant of Cl_m for a given drug. For drugs with high extraction ratio (>70%), the Cl_m is considered flow-limited and is directly related to changes in hepatic blood flow. The Cl_m for drugs with low extraction ratio (<20%) is capacity-limited and is more dependent on the intrinsic metabolic capacity of the liver enzymes than on hepatic blood flow. Alterations in protein binding or metabolic capacity of the liver enzymes due to enzyme induction or liver disease may more greatly affect the Cl_m for this latter group of drugs (Blaschke, 1977). The intrinsic metabolic capacity of the liver enzymes is considered to be the actual clearance of a drug if the Cl_m were not limited by protein binding or hepatic blood flow (Wilkinson *et al*, 1975).

For most drugs, this clearance is an apparent first-order (linear) process, and any change in dose results in a proportional change in plasma drug concentration. However, if the drug metabolising capacity of the hepatic enzymes is limited, the clearance may follow non-linear pharmacokinetics and Cl_m would proceed at a constant or fixed rate regardless of the dose administered. Therefore larger doses of a drug would have lower time-averaged clearances and longer half-lives when compared to smaller doses (Wagner, 1975).

The liver is irrigated by both the hepatic artery and the portal system. Therefore, orally administered drugs are subject to hepatic elimination prior to reaching the systemic circulation. A first-pass presystemic elimination, generally referred to as the 'first pass effect' (Pond *et al*, 1984) may result. The kidney, like the liver, is a major route of excretion for most drugs whether they are excreted unchanged and/or as metabolites. Renal clearance, Cl_r, is the resultant of three basic physiological processes involved in urine production: glomerular filtration, tubular secretion and reabsorption (Garett, 1978).

Many drugs are either weak organic bases or organic acids. Hence they ionise in aqueous solution so that there is present a neutral, usually lipid soluble species, and a charged conjugate acid or base. *The rate of renal excretion of such a drug, whether acid or base, will be influenced by the degree*

of its ionisation in the tubular fluid, which is in turn controlled by the PKa of the substance and the pH of the tubular fluid. The effect of making the urine alkaline has been shown to increase the proportion of doxycycline excreted, the rate of excretion, and to reduce the serum levels of doxycycine during both single and multiple dosing (Jaffe *et al*, 1974). A weak acid, chlorpropamide (Pka 4.8) is cleared primarily by renal excretion. Sodium bicarbonate taken concomitantly raised the urinary pH (range 7.1 to 8.2) and reduced the plasma half-life from a mean of 50h to a mean of 13 h. Ammonium chloride had the opposite effect, lowering the urinary pH (range 4.7 to 5.5) and prolonging the plasma half-life to a mean of 68.5h (Neuvonen *et al*, 1983).

The elimination half-life (t½) is the time required for the plasma drug concentration to decrease by one half. The apparent first-order elimination rate constant (ß) is a proportionality constant that relates the elimination rate to plasma drug concentration. The relationship between ß and t½ is:

$$ß = \frac{\ln 2}{t½} \cong \frac{0.693}{t½}$$

The t½ and ß are after often inappropriate expressions of the elimination characteristics of a drug because they only describe the rate or removal of drug from the plasma and not necessarily from the body.

The clearance, Cl, is a fundamental concept in pharmacokinetics and is a proportionality constant that relates the rate of elimination to the plasma (or blood) concentration, C, that is:

$$\text{Rate of elimination} = Cl.C$$

on integrating this relationship from time zero to time infinity,

$$\text{Dose} = Cl.\ AUC$$

Where dose, the amount absorbed, is equal to the amount eliminated, CL. AUC, and AUC is the area under the plasma concentration-time curve.

The AUC is the most useful and direct measure of the body's exposure to the drug. Its value depends on both the dose given and the systemic clearance. When AUC is normalised to the dose;

$$\frac{1}{Cl} = \frac{AUC}{Dose}$$

Therefore, 1/CL is the normalised index of exposure; when multiplied by the dose, gives AUC, the more direct index of the body's exposure to the drug.

Another useful measure of exposure, particularly for reversibly acting drugs for which drug at the site of action is in rapid equilibrium with that in plasma, is the initial plasma concentration, C_o. The actual value of C_o depends on the initial apparent dilution of the drug as measured by the initial apparent volume of distribution, Vd.

The value of vd can be estimated from:

$$V_d = \frac{Dose}{C_o}$$

Co is the initial plasma concentration and is obtained from extrapolation to time zero. This volume parameter, like clearance is proportional to the dose administered. Clearance can then be described in a simple but clinically useful form for those drugs adequately described by a one-compartment model:

$$Cl = ß.Vd$$

But it is inappropriate to consider that Cl is dependent on ß and Vd simply because of the above relationship. Rather values of ß and t½ are dependent on Cl and Vd. The Vd and Cl may change independently of one another, resulting in a change in ß and t½. A drug may have a long t½, because either it has a large Vd or it is slowly removed from the body by the eliminating organs or both.

Although t½ is a poor index of drug elimination, it is a useful parameter in the determination of suitable dosage intervals and the time required to attain the steady state during constant intravenous or chronic oral therapy.

Most drugs exhibit linear pharmacokinetics in the therapeutic dose range and total body clearance is a parameter of primary importance. It is a physiologically more appropriate term than half - life to describe drug elimination from the body. Non linearities in the pharmacokinetic behaviour must

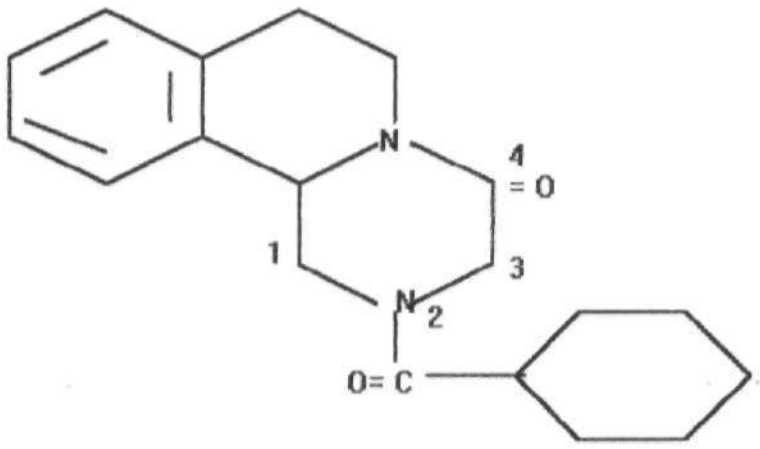
be recognised early during drug research and development since they affect the outcome of pharmacological and toxicological studies and the safety of clinical dose escalation studies (Clark *et al*, 1984).

CHAPTER TWO

LITERATURE REVIEW

2.1. PRAZIQUANTEL:

Praziquantel is a heterocyclic pyrazino-Isoquinoline derivative. Chemically it is 2-cyclohexylcarbonyl [1, 2, 3, 6, 7, 11b]- hexahydro-4H- Pyrazino [2,1-a] isoquinolin-4-one. The molecular formula is C_{19} H_{24} N_2 O_2, the molecular weight 312.4.



Structural formula of Praziquantel.

The drug is internationally available as Biltricide from E. merck (Darmstadt) and Bayer AG (Leverkusen) of Germany. It is a colorless crystalline powder with a bitter taste. It is hygroscopic in nature and melts at 136°- 140°C with decomposition.

Praziquantel is freely soluble in chloroform and dimethylsulfoxide, soluble in ethanol but only slightly soluble in water. It has a log *p* value of 3.43 (octanol/water).

2.1.1. CHEMISTRY:

The major antihelmintic activity of this asymmetric molecule is related to the pyrazino - [2,1-a] isoquinoline, while its broad spectrum appears to be dependent on an oxo group at position 4 and one

acyl or thioacyl group in position 2 (Andrews, 1985). It is used in therapy as its racemate although the S-(+) enantiomer is ineffective (Andrews, 1985; Standt *et al*, 1992)

2.1.2. MECHANISM OF ACTION

The exact mechanism of action of praziquantel is unknown. In vitro studies have shown that the earliest detectable effect of the drug on schistosomes is spastic paralysis of the parasite musculature (Pax *et al*, 1978), The other observable phenomenon is vacuolation and degeneration of the worm (Xiao *et al*, 1984). Both effects are associated in the influx of Ca^{++} ions and may be blocked by high concentrations of Mg^{++} ions. In the schistosomes, drug effects are not affected by calcium channel blockers or neurotransmitter antagonists (Andrews, 1985). Experimental evidence suggests that parasite destruction in vivo may require additional host-dependent immunologic events. Drug treatment of T-cell-deficient mice infected with Schistosoma mansoni has been shown to be less effective than therapy of immunologically intact mice (Sabah *et al*, 1985). Additionally vacuolization at several sites in the tegument of adult schistosomes is followed by attachment of phagocytes to the parasite and ultimately by death (Mehlhorn *et al*, 1981).

2.1.3. PHARMACOKINETICS

Praziquantel is a broad spectrum schistosomicidal agent with high hepatic clearance. The drug is rapidly and efficiently absorbed after oral administration with bioavailability of about 80%. However extensive first-pass hepatic hydroxylation occurs and probably accounts for inter individual variation in pharmacokinetics (Ofori-Adjei *et al*, 1988).

As single oral dose of 50mg/kg body weight in healthy adults results in peak serum concentrations of 1mcg/ml of unchanged drug in 1 to 3 hours. The unchanged drug has a serum half-life of 0.8 to 1.5 hr. This may be extended in some individuals.

A higher plasma concentration that is associated with a higher incidence of side effects occurs in those with hepatic disease (Watt *et al*, 1988). But thioacetamide-induced hepatic cirrhosis does not affect the pharmacokinetics of orally administered praziquantel in the rat (Kokwaro *et al*, 1990).

Small concentrations of the drug have been detected in bile, faeces, cerebrospinal fluid and breast milk. The concentration in the cerebrospinal fluid is approximately 14% to 20% the total amount of praziquantel in the plasma (Thomas *et al*; 1982). The drug appears unchanged in human breast milk in concentrations about 25% those in maternal serum (Putter *et al*, 1979).

Although the drug binds reversibly to plasma proteins, it undergoes pronounced first-pass metabolism in the liver yielding preferentially monohydroxylated and polyhydroxylated metabolites (Bühring *et al*, 1978) that are excreted through the kidneys. No unchanged drug is excreted in the bile or urine. The antihelmintic activity of metabolites is less than that of the parent drug. About 30mcg/ml concentration is required to induce significant tegumental damage in Schistosoma japonicum worms in vitro (Xiao *et al*, 1991). The metabolites have a half - life of 4 to 5hrs. The cumulative renal excretion of praziquantel and its metabolites is 80% by the fourth day with more than 90% of that in the first 24 hours.

2.1.4. TOXICITY

Praziquantel is well tolerated by humans and appears to have no long-term toxicity. The drug has been associated with mild gastrointestinal disturbances, transient headaches, and, at high doses (25mg/kg three times a day for one day) malaise and dizziness (Davies *et al*; 1979; Frohberg *et al*; 1981). These symptoms have not been associated with objective clinical findings. No unusual effects have been seen in patients with glucose-6- phosphate hydrogenase deficiency or the haemoglobinapathics that are found in areas where helmintic infections are common (Pearson *et al*; 1983). A wide range of investigations have failed to show mutagenic activity in tissue-, and urine-mediated assays with S*almonella typhimurium* (Obermeir *et al*; 1978). There has been little experience with the drug in children under 4 years and safety in pregnancy has not been established.

2.1.5. THERAPEUTIC USES

The development of praziquantel is considered a major breakthrough in pharmaceutical research on antischistosomal agents. The drug is highly effective against a broad range of helminths,

some of which respond poorly, or not at all to previously available drugs (Pearson *et al*; 1983). It can be administered orally and is effective after single dose or several doses on one day.

Praziquantel is the drug of choice for treating all pathogenic schistosoma infections in man i.e. those caused by *Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum, Schistosoma mekongi and Schistosoma intercalatum* (Pearson and Guerrant, 1983, King and Mahmoud., 1989).

Alternative chemotherapy for *Schistosoma mansoni* (oxamniquine) and *Schistosoma haematobium* (metrifonate) infections are available which are less expensive than praziquantel but equally effective and may remain the drugs of choice for the treatment of Schistosomiasis where one or the other of these schistosoma species is endemic. However both metrifonate and oxamniquine have shown mutagenic activity in *Salmonella typhimurium* (Batzinger *et al*., 1977). Neither drug is active against *Schistosoma japonicum* or *Schistosoma mekongi*.

Praziquantel is effective in both acute and chronic Schistosomiasis. End - stage hepatic fibrosis is not reversed by drug treatment; however, the pathogenic process usually can be halted and faecal egg counts markedly reduced or eliminated. Standard single day therapy is safe and very effective in cerebral schistosomiasis (Watt *et al*., 1986). A single oral dose of praziquantel is considered the treatment of choice in infections caused by *Clonorchis* and *Opisthorcis* (Chinese and oriental liver flukes) (Yangco *et al*., 1987). A second course of therapy occasionally is necessary to effect a cure. It has been suggested that praziquantel replace bithionol as the drug of choice in the treatment of Paragoniasis (Pachuki *et al*; 1984).

Praziquantel is as effective as niclosamide and appears to be quite safe in treating intestinal infections caused by *Taenia saginata, Taenia solium, Diphyllobothrium latum* and *Dipylidium canimum*. In dwarf tapeworm infections, a single dose praziquantel seems to be as effective as a seven - day regimen of niclosamide.

Cysticercosis caused by *Taenia solium* is an important medical problem and a frequent cause of seizures in some areas of south and central America. Praziquantel has been shown to be effective in the treatment of cysticercosis caused by *Taenia solium* when it is given daily for two weeks (Sotelo *et al*., 1990).

Albendazole is preferred for therapy of parenchymal cysticercosis because it has equivalent activity and can be given in a shorter course than praziquantel (Sotelo *et al.*, 1990). Adjunctive therapy with antiepileptic drugs, corticosteroids and/or surgery may be required. Praziquantel is only partially effective or ineffective in patients with a malignant form of the disease (cysticercotic encephalitis). When cysts are located in the ventricular fluid, surgery or albendazole is a more definitive treatment (Vasconcelos *et al.*, 1987).

In *Hymenolepis nana* infections the efficacy of praziquantel is similar to that of niclosamide, but praziquantel may emerge as the drug of choice for *Hymenolepis nana* infections because of its high cure rate, and simple single dose regimen (Schenone, 1980).

Combination chemotherapy is more effective in post spillage prophylaxis for hydatid disease than either albendazole or praziquantel alone (Taylor and Morris, 1989).

2.1.6. RECOMMENDED DOSING.

King and Mahmoud (1989) have summarized the recommended dosings for humans infected with trematodes and cestodes (Table 2.1.6.1). Praziquantel is supplied in 600mg tablets which are easily quartered. The drug has a bitter taste and if chewed before swallowing may produce gagging and emesis.

The interval between doses should not be less than 4 hours and not greater than 6 hours. The drug is dosed per kilogram of body weight

.2.1.7. SIDE EFFECTS:

Praziquantel is related chemically to sedative and antianxiety agents and drowsiness develops relatively frequently. The drug has fewer side effects than the antimonial schistosomicidal drugs it has replaced.

Most studies report an incidence of 10% to 15% The symptoms include abdominal pain or discomfort, nausea, vomiting, anorexia and diarrhoea.

Some patients with heavy *Schistosoma mansoni* or *Schistosoma japonicum* infections may have bloody diarrhoea. Neurologic symptoms include headache, vertigo, dysphoria and dizziness. Systemic symptoms include fever urticarial rash and lassitude.

In summary, although praziquantel does cause some side effects, because the duration of adverse effects is short, compliance with repeat therapy, if needed, has been very good in longitudinal treatment programmes (King and Mahmoud., 1989).

Table-2.1.6.1. Praziquantel therapy for Humans infected with trematodes and cestodes.

PARASITES	DOSE
Blood flukes	
Schistosoma mansoni +	20mg/kg 2 to 3 times daily for 1 day
Schistosoma haematobium +	40mg/kg in a single dose
Schistosoma japonicum +	20mg/kg 3 times daily for 1 day.
Schistosoma mekongi +	20mg/kg 3 times daily for 1 day.
Schistosoma intercalatum	40mg/kg 3 times daily for 1 day.
Tissue flukes	
Clonorchis senensis	25mg/kg 3 times daily for 2 days
Opisthorcis viverrini	25mg/kg 3 times daily for 1 day
Opisthorcis felineus	25mg/kg 3 times daily for 1 day
Paragonimus wstermani	25mg/kg 3 times daily for 2 days
Other paragonimus species	25mg/kg 3 times daily for 1-2 days
Fasciola hepatica	25mg/kg 3 times daily for 2 days (Investigational).
Intestinal flukes	
Heterophytes heterophytes	25mg/kg 3 times daily for 1 day
fasciolopsis buski	25mg/kg 3 times daily for 1 day
Tissue cestodes	
Taenia solium (cysticercosis)	50mg/kg/ daily in three divided doses for 14-30 days.
Echinococcus granulosus	Not recommended - therapeutic use presently under investigation.
Intestinal cestodes	
Diphyllobothrium latum	10-20mg/kg in a single dose
Taenia saginata	10-20mg/kg in a single dose
Taenia solium	10-20mg/kg in a single dose
Dipylidium caninum	10-20mg/kg in a single dose
Hymenolepis nana	25mg/kg in a single dose

+ Approved use by the United States Food and Drug administration (FDA).

2.1.8. DRUG INTERACTIONS.

The initial reports of praziquantel therapy for neurocysticercosis described occasional exacerbation of neurologic symptoms after the onset of therapy, presumably due to inflammatory response to the dying cysts (de Ghetaldi *et al.*, 1983, Sotelo *et al.*, 1984). This finding prompted some physicians to treat all neurocysticercosis patients with high dose of corticosteroids before and during praziquantel therapy. Recent studies indicate however, that concurrent administration of dexamethasone and praziquantel may lower plasma praziquantel levels by 50% (Vazquez *et al.*, 1987). Subsequent clinical experience indicates that not all patients require concurrent steroid therapy, and suppression of antiparasitic inflammatory responses must be assessed individually for every patient (Vasconcelos *et al.*, 1987). Combination of praziquantel and oxaminiquine has been shown to be synergistic in mice infected with schistosoma mansoni (Brammer *et al.*, 1981, Shaw *et al.*, 1983 and Botros *et al.*, 1989). A 2.1 $^{w}/_{w}$ combination was shown to be equi-active with 4- to 5- fold higher doses of oxamniquine alone or 8- to 10- fold higher doses of praziquantel alone. It may therefore be significant using low doses to reduce side effects. Prankerd and Ahmed (1992) have shown that a combination solid dosage form is feasible, because the two drugs did not interact on fusion. Mixture of the two drugs appeared only to interact with stearic acid of all the nine common pharmaceutical excipients. No adverse interactions with alcohol, tobacco or dietary constituents have been reported (Pearson *et al.*, 1983). It is also interesting to note that since the introduction of praziquantel in Europe (1978) and in the United States (1982), no serious pharmacokinetic drug interactions have been reported (Drug Evaluations Annual, 1993).

2.1.9. LIMITATIONS FOR USE

Praziquantel represents an important advance in the chemotherapy of several helmintic infections. It can be given in simple dosage regimens which are well tolerated. However the relatively high cost of praziquantel therapy, even with the support of international agencies, has put widespread use of the drug beyond the reach of many countries health programmes.

The potential for drug resistance is a theoretical limitation for population - based control programmes based on praziquantel alone. Studies in Brazil and Kenya have already reported rapid emergence of resistance for oxamniquine (king and Mahmoud., 1989).

2.1.10. *THERAPEUTIC MONITORING*

There are no published reports adequately relating serum praziquantel concentrations to clinical response. Andrews (1985) hypothesised that the important factor in praziquantel therapy is exposure of schistosomes to at least a minimal level of the drug for as yet unknown period of time that is sufficient to damage the tegument of the parasite. And absolute plasma levels of praziquantel do not appear to be important (Xiao *et al*., 1992). Absorption of the drug from the gastrointestinal tract is variable and the peak plasma concentration may occur anywhere between 1 and 3 hours (Leopold *et al*, 1978). As a consequence, a single blood concentration determination may be of limited value in therapeutic monitoring.

There are a number of methods available for the quantitative determination of praziquantel in the blood. The fluorometric (Putter, 1979) and gas-liquid chromatographic methods (Diekmann, 1979) involve complex sample preparation methods or derivatization. A simple method for the quantitation of praziquantel by high-performance liquid chromatography was developed by Xiao and co-workers (1983). This HPLC method is sufficiently sensitive to permit pharmacokinetic studies of praziquantel in humans. However, quantitative analytic procedures based on UV spectro photometry are of low precision and selectivity. Infra-red spectroscopy is the only method of praziquantel assay that meets the requirements for drug substance measurement (Lopatin *et al*, 1989). IR spectroscopy is the recommended drug assay for praziquantel in the current 1988 international pharmacopoea.

2.2 RANITIDINE

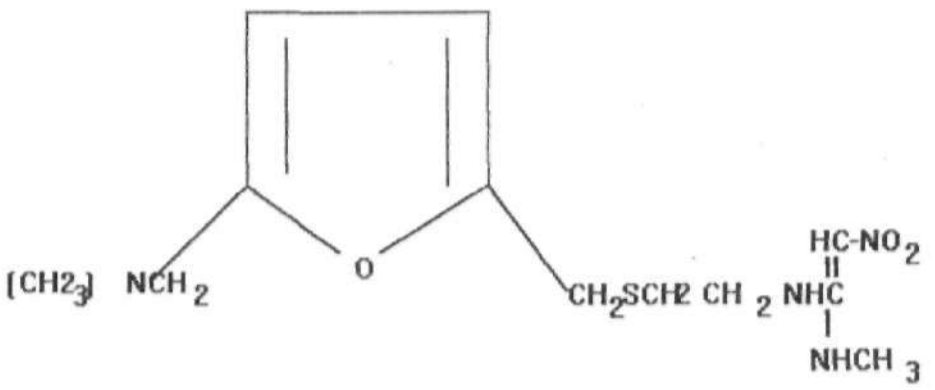
2.2.1 INTRODUCTION

The early antihistamines were unable to antagonise the release of gastric acid caused by the action of histamine. Cimetidine did, and was introduced into clinical practice in 1976 (Brimblecombe *et al*, 1975). Ten years later, ranitidine was introduced and the two became the best selling drugs in the world. Ranitidine is about 5 times more potent than cimetidine as H_2 - antagonist and is almost without inhibitory effect on the cytochrome p-450- mediated oxidative drug metabolism. It has therefore

become the preferred antipeptic agent when other drugs are being administered simultaneously (Hande *et al*, 1986).

2.2.2 CHEMISTRY

Ranitidine is chemically N-[2-[[[5-[dimethyl amino) methyl]-2- furanyl] methyl]ethyl]-N-methyl - 2- nitro - 1,1-ethenediamine. The molecular formula is $C_{13}H_{22}N_4O_3$ 5.



Structural formula of ranitidine.

The furan ring is non-basic and it is able to lower the basic strength of the trimethylamine side chain. It is a white to pale yellow granular substance that is soluble in water, slightly bitter in taste, with sulfur-like odour.

2.2.3 PHARMACOLOGICAL ACTION AND MECHANISM

Ranitidine is a potent competitive reversible inhibitor of the binding of histamine to H_2- receptors. The drug inhibits both daytime and nocturnal basal gastric acid secretions as well as gastric acid secretions stimulated by food, betazole and pentagastrin. Unlike cimetidine, ranitidine binds minimally, if at all, to such other sites as androgen receptors, hepatic mixed-function oxidase system and peripheral lymphocytes. This binding specificity of ranitidine probably accounts for the fact that it appears to lack some of the serious side effects seen clinically with cimetidine (Zeldis *et al*, 1983).

2.2.4 PHARMACOKINETICS

The absorption of ranitidine is fairly rapid. Peak plasma concentrations are usually achieved between 1 to 3 hours after oral dose. Also a second peak after oral administration has been observed (Lin, 1991). Onset of antisecretory effect per oral is within 1 hr. Plasma levels of 36-94 ng/ml of ranitidine inhibit 50% of pentagastrin stimulated acid secretions in healthy subjects (Konturek *et al*; 1981).

The oral bioavailability is about 50-60% and is influenced by hepatic function since the drug is taken up and metabolised by 'first-pass' kinetics. As expected therefore, the bioavailability of ranitidine in liver disease is increased, and the serum t½ slightly prolonged because of decreased hepatic metabolism and a slightly reduced glomerular filtration rate (Young *et al*; 1982). Roberts (1984) has reported the following t½ values for ranitidine; 2.5 - 3 hrs in normal, 4-10hr in renal failure and 2.8hr in liver disease.

About 30% of administered dose is metabolised by the liver to the N-oxide, S-oxide and desmethyl derivatives (Martin *et al* 1982). 70% of an intravenous dose is excreted unchanged in the urine in 24 hours. The remainder is eliminated in the faeces (Roberts, 1984).

Ranitidine is about 15% plasma protein bound. The Vd_{ss} is 1.2 - 1.9L/kg. The distribution into the CNS is minimal. There is a simple, direct correlation between plasma concentration and the inhibition of gastric acid secretion, implying a rapid equilibration between drug concentration in the plasma and at the site of action and a reversible drug receptor interaction (Lin, 1991).

2.2.5 THERAPEUTIC USES

Ranitidine is as effective as cimetidine with the added advantages of a more prolonged period of action and fewer side effects. The drug is indicated in conditions where inhibition of gastric acid secretions may be beneficial, such as duodenal and gastric ulcers, gastroesophageal reflux disease and hypersecretory conditions, such as Zollinger-Ellison Syndrome where up to 6g/day has been administered.

For the prevention of bleeding from stress-related mucosal lesions in acutely ill patients, it is necessary to adjust dose or frequency of intermittent therapy or the rate of continuous infusion to maintain the intragastric pH above 4 (Morris *et al*, 1985).

Drug interactions that suggest ranitidine may affect the bioavailability of certain drugs, e.g. through pH-dependent effect on absorption or change in vd have been encountered but the clinical significance of most of these interactions has not been established.

Symptomatic response to therapy does not preclude the possibility to gastric malignancy. Popular proprietory name is Zantac (Glaxo).

2.2.6 ADVERSE EFFECTS

Adverse reactions are usually minor and occur infrequently. Ranitidine does not elevate basal levels of testosterone, presumably because it does not bind to androgen receptors. Human lymphocytes have receptors for cimetidine but not for ranitidine (Peden *et al*, 1982) and cimetidine has been demonstrated to have effects on cell- mediated immunity (Jorizzo *et al* 1980).

On the whole, ranitidine is well tolerated. General side effects include headache, tiredness, dizziness and mild gastrointestinal disturbance. Clinical studies have not shown a significant pharmacological effect of ranitidine on the cardiovascular system via H_2-receptors, even though individual sensitivities cannot be ruled out. There have been occasional reports of reversible hepatitis especially in the critically ill and multiple therapy patients, or patients with chronic renal or hepatic failure, so that the direct causal link with ranitidine treatment was often difficult to ascertain.

Although few, and poorly documented, white cell injury (i.e. agranulocytosis) appears to be the most frequent haematological complication (Vial *et al*; 1991). Small increases in serum creatinine may occur, but are thought to be as a result of competitive inhibition of creatinine secretion by ranitidine.

2.2.7 DRUG INTERACTIONS

Cimetidine, the predecessor drug of ranitidine interacts with a variety of other agents and has stimulated the interest of clinicians and pharmacologists. The interaction potential of ranitidine has been subject of extensive investigations. Pharmacokinetic interactions of ranitidine with other drugs may occur at the site of absorption, metabolism or renal excretion (Klotz *et al*; 1991), but numerous controlled studies have proven that ranitidine can be safely co administered with other drugs.

The gastrointestinal absorption of Ketoconazole is variable and dependent on the presence of gastric acid (Vander Meer *et al*, 1980).

A similar study with ranitidine has shown that the mean area under the concentration time curve from 0 to 12hr for Ketoconazole was significantly different from that of ketoconazole alone (Piscitelli *et al*, 1991)

The decrease in Ketoconazole bioavailability observed with ranitidine has been correlated to the increase in gastric pH.

The metabolism of theophylline is saturable and about 90% of its elimination is via metabolism. Cimetidine may reduce the elimination of theophylline and precipitate theophylline toxicity (Jackson *et al*, 1980) probably by interaction with one or more of the variants of the cytochrome p-450 drug metabolising system. Ranitidine does not appear to interfere significantly with drugs metabolised via the cytochrome -450 mixed function oxidase system (Kirch *et al*, 1984). Ranitidine is thought to spare phase one hepatic metabolism mediated by cytochrome p-450. A controlled study on the effects of cimetidine and ranitidine on plasma theophylline concentration in patients with chronic obstructive airways disease treated with theophylline and corticosteroids shows that the average increase in the theophylline concentration due to cimetidine was 32% but there was no significant change in plasma theophylline concentration during ranitidine administration (Boehning, 1990). However, contrary to results obtained from controlled studies, two cases of clinically significant ranitidine - theophylline drug interaction have been reported (Hegman and Gilbert, 1991). The report suggests that such drug interaction may occur in select patients.

However ranitidine interferes with the renal tubular secretion of the organic cations procainamide and N-acetyl procainamide in humans through competition for transport via the organic cation transport system. The potassium - sparing diuretic triamterene undergoes renal tubular secretion, hepatic hydroxylation and subsequent sulphate conjugation to a pharmacologically active metabolite. Ranitidine significantly reduced the renal clearances of triamterene (51%) and P-hydroxy triamterene sulphate conjugate (47%), the clearance by hydroxylation of triamterene (30%) and the apparent absorption of triamterene (52%). In turn, triamterene reduced the renal clearance of ranitidine (14%) (Muirhead *et al*, 1988). This interaction resulted in a small attenuation of the pharmacodynamic

response to triamterene. Competition for translocation across membranes is postulated as a common mechanism for the observed renal and hepatic interactions.

The pharmacokinetic interactions of the alkylating agent cyclophospamide with cimetidine and ranitidine in mice provides a good basis for product differentiation. Cimetidine, given 5 or 30 min. before cyclophosphamide, significantly increased the survival of leukemia bearing mice, as well as the t½ and plasma area under the curve of total alkylating metabolites of cyclophosphamide. Cimetidine also reduced the survival of normal (bone marrow stem cell) colony forming units in a dose dependent fashion. Ranitidine did not significantly alter cyclophosphamide anti leukemic activity, pharmacokinetics, or toxicity to normal bone marrow stem cells. These results suggest caution in the use of cimetidine in patients being treated with cyclophsphamide in order to avoid the possibility of enhanced toxicity. Ranitidine may be a safer alternative (Dorr *et al*; 1986).

In the light of the foregoing examples, it may seem reasonable to speculate on the possibility of limited interaction potential of ranitidine in special patient population under certain clinical conditions (Klotz *et al*; 1991). Nonetheless it is generally considered that the potential for such interactions is far less in comparison to cimetidine.

2.3 AIM OF THE PRESENT STUDY:

It is estimated that over 200 million people in Africa, South America and the far-east are infected by Schistosomiasis (Conlon and Ellis, 1985). A further 600 million are constantly at a risk of infection. It is understandable therefore that only large scale chemotherapeutic projects will help control this epidemic. Previous therapy of schistosomiasis has been unsatisfactory. Niridazole was supplanted by metrifonate for the treatment of infection with *Schistosoma haematobium* and by oxamniquine for *Schistosoma mansoni*. These newer agents have no activity against *Schistosoma japonicum* and all the three drugs are more toxic than praziqunatel.

The introduction of praziquantel was a significant advance in the treatment of schistosomiasis and other fluke (trematode) and tapeworm (cestode) infections (conlon and Ellis; 1985). Praziquantel has been well tolerated by patients including those with severe liver disease due to parasitic infections.

Also ranitidine has become established in the medical literature as the preferred anti ulcer drug when other drugs are being coadministered. It is thought to be devoid of significant drug interaction potential, because it lacks inhibitory effect on the cytochrome p-450-mediated oxidative drug metabolism. At the moment there is little need for routine plasma praziquantel monitoring, but it may be necessary to evaluate the absorption of praziquantel in patients whose conditions may affect drug absorption. In the event of clinical failure, the concentrations provide evidence that the drug was absorbed. The present study is therefore aimed at evaluating the effect of ranitidine on the absorption kinetics of praziquantel. The objectives of the study are thus:-

1. To determine the pharmacokinetic profile of praziquantel in *Schistosoma haematobium*-infected subjects.

2. To determine the effect of ranitidine on the pharmacokinetic profile of praziquantel in *Schistosoma haematobium*-infected subjects; and,

3. To evaluate the effect of ranitidine on the bioavailabiliy of praziquantel.

2.3.1 METHODOLOGY

The study was conducted as a cross over experimental design. It was therefore carried out in 2 phases thus:-

PHASE I.

The determination of the pharmacokinetic profile of praziquantel in the volunteers using plasma samples.

PHASE II

After a wash-out period of one week, praziquantel was administered preceded by ranitidine per oral, one hour earlier. The effects of ranitidine on the pharmacokinetic profile of praziquantel was then determined.

CHAPTER THREE

3.0 MATERIALS AND METHODS.

3.1 MATERIALS

The following materials were used in carrying out the project.

3.1.1 GLASS WARES

Glass centrifuge tubes, 15ml.

Measuring cylinders: 500, 250, 100 and 50ml sizes

Conical flasks: 100 and 250ml sizes

Volumetric flasks: 1000, 500, 250, 100 and 50ml sizes

Beakers: 500 and 250ml sizes

Screw - capped sample bottles, 20ml.

Bijoux bottles, 20ml.

Heparinised sample bottles, 5ml.

Plastic syringes: 10 and 5ml sizes

Needles, 21G sizes

Glass pasteur pipettes (with teats).

Micropipettes: 1 and 0.1ml sizes.

3.1.2 EQUIPMENT

Gallemkamp centrifuge, junior.

Gallemkamp oven, BS size one.

Stuart autovortex mixer.

Solvent filtration kits, with 0.5µ and 0.45µ Waters^{R} solvent filters.

Effondorf pipette, 1ml.

Gallenkamp water bath, compenstat.

Dissolution - rate study assembly, Erweka, England.

Disintegration - rate study assembly, Erweka, England.

Thermocool refrigerator, T150, Nigeria.

Kent pH meter, model 7060.

Hamilton Injection microsyringe, 100µl

Hamilton priming syringe, 20ml

Kerry ultrasonicator

Vacuum pump EB 3A, Edwards, England.

Electronic balance, mettler AE 240.

Nitrogen gas, IGL

3.2. DRUGS

i. Praziquantel: Biltricide^{R}

Manufacturer: Bayer Nig. PLC.

Batch Number: 604912

Date of manufacture: November, 1991

Expiry date: November, 1995

Strength: 600mg

ii. Ranitidine, USP: Zinetac^{R}

Manufacturer: Glaxo pharm. Division, India.

Lot No.: NA 090.

Date of manufacture: February, 1994.

Expiry date: January, 1996

Strength: 150mg.

3.3 REAGENTS AND STANDARD SAMPLES

- Absolute Ethanol, Reanal Budapest, Hungary.
- Toluol Zur Analyse, Merck, Germany.
- Boric acid, BDH Chemicals, England.
- Sodium hydroxide, BDH Chemicals, England.
- Distilled and filtered water.
- Praziquantel powder (Pe 319223) - obtained as gift sample from Bayer AG, Germany.

  Sodium lauryl sulfate BDH Chemicals, England.

3.4 SOLVENT SYSTEM

A mobile phase consisting of Ethanol: water in a ratio of 70:30 (v/v) was used to analyse praziquantel in plasma. The solvent system was filtered through waters filtration kit with 0.45µm filter and further degassed by ultrasonication for 15 minutes. The pH of the mobile phase (unadjusted) was about 7.3.

3.5 *IN - VITRO* STUDIES

3.5.1 ASSAY.

The assay procedure used was based on the assay procedure outlined in the USPXXI (Supplement 8).

The quantity of praziquantel, in mg, in the portion of tablets taken was calculated using the formula provided:

Quantity of praziquantel (in mg) in portion of tablets taken = $2500\ (^{C}/_{3})\ (^{ru}/_{rs})$.

Where C = Concentration (mg/ml) of praziquantel standard preparation.

ru = Peak height obtained from assay preparation.

rs = Peak height obtained from standard preparation.

3.5.2 DISINTEGRATION TESTS.

Neither USP XXI nor B. P. 1988 contains disintegration time specifications for praziquantel tablets. Therefore the current official disintegration time specification for film-coated tablets was used.

Procedure:

One tablet each was placed in the tubes of the basket and a disc was added to each tube. The assembly was suspended in the beaker containing distilled water maintained at $37.0^{C} \pm 0.5^{oC}$. The apparatus was then operated.

3.5.3 DISSOLUTION TESTS.

The Rotary - Basket method as described in the B. P. 1988 was used.

Procedure:

One tablet of praziquantel was placed in the basket and placed in the round-bottom flask containing 900ml of the dissolution medium (USP XXI). The stirrer was set at 50 rpm. 10ml samples of the dissolution medium were withdrawn at 30 min.. Sample were withdrawn from midway between the basket wall and the side of the vessel so that the flow pattern of the medium was not disturbed.

The samples were filtered through whatman filter paper (No. 4). The first 1ml was rejected. The procedure was repeated for the simulated intestinal fluid (pH 6.8) containing 2mg sodium lauryl sulphate per ml of fluid volume.

The samples were assayed by reference to a standard calibration curve obtained for known concentrations of praziquantel standard preparation. The ultraviolet absorbances were measured at 263nm wavelength.

3.5.4 PREPARATION OF STANDARD SAMPLES.

3.5.4.1 PREPARATION OF PRAZIQUANTEL SOLUTION.

A stock solution of 100µg/ml of praziquantel was made in ethanol. Working concentrations of 10µg/ml were obtained by serial dilution with ethanol.

3.5.4.2 PREPARATION OF BORATE BUFFER.

A stock borate - sodium hydroxide buffer was prepared in a 1000 - ml volumetric flask by dissolving 12.404g of boric acid in 500ml of distilled water, mixed with 100ml of 1M NaOH and diluted to volume.

A working borate buffer solution was obtained by mixing 50ml of stock solution with 50ml of 0.1M NaOH in a 100ml volumetric flask. The pH of the resultant solution was 11± 0.1

3.6 *IN-VIVO* STUDIES

3.6.1 PROTOCOLS OF STUDY.

Six subjects aged between 12 and 18 years (weight: 32 - 52.5 kg) were involved in the study. The six subjects presented with terminal haematuria and tested positive for *Schistosoma haematobium* through laboratory investigations. Informed consent was given by the volunteers and tutors acting in Loco parentis. The study was conducted as a crossover experiemental design, with a washout period of one week. Volunteers were free from hepatic, renal or gastrointestinal tract disorders.

The cross over study was divided into two phases. In the first phase, single oral doses of praziquantel tablets 40mg/kg were administered after an overnight fast with a glass of water. Two hours later, all the subjects ingested identical standard breakfast of tea and shortbread.

Phase two involved coadministration of praziquantel with ranitidine. Ranitidine, 150mg was given one hour earlier with a glass of water.

Blood sampling was done via a cubitally-placed indwelling cannula. Blood samples (5ml) were withdrawn prior to each dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 hours after the dose. The patency of the cannula was maintained by flushing with 1 ml heparinised saline after each withdrawal of blood.

The blood samples were centrifuged at 2000g for 10 minutes and the plasma was harvested in Bijoux bottles and frozen until subsequent analysis within one week.

3.6.2 ANALYSIS

3.6.2.1 EXTRACTION

The extraction method of Westhoff and Blaschke (1992) was adopted with modifications. Borate buffer was used to neutralise acidic plasma components and improve system selectivity. Single extraction with 3ml of ethanol: toluene (40:60, V/v) instead of double extraction was used.

To 0.5ml of plasma in extraction tube, 1.0ml of borate buffer were added. The mixture was vortex-mixed and extracted with 3.0ml of ethanol; toluene ((40:60, V/v). The organic phase was separated by centrifugation at 2000g for 20 minutes, removed and evaporated under a stream of nitrogen. The residue was dissolved in 100µl of ethanol: water (70:30, V/v), of which 10µl were injected into the reversed-phase column.

3.6.2.2 CALIBRATION CURVE

External calibration with spiked plasma was used instead of the internal standard method. Calibration curves were obtained for five concentrations - 0.2, 0.4, 0.8, 1.0 and 1.5µg. The peak responses were measured and plotted against concentration. The calibration curve was used to determine the concentrations of praziquantel in the plasma samples of individual volunteers.

3.6.2.3 INSTRUMENT

The Chromatographic system was a waters^{R} model 204 liquid chromatograph equipped with model 441 fixed-wavelength ultraviolet detector fitted with 214nm filter and U6k universal septumless injector. The solvent system was pumped through a model 510 reciprocating pump. Analysis was carried out on a radial-pak^{R} reverse-phase column (8x100mm) packed with 10µm µ Bondapak C-18 (Waters No. 85721) placed in a compressed Z-module compartment. The output of the detector was recorded on an SE 120 BBC metrawatt recorder.

Chromatographic conditions:

Solvent system: Ethanol: water (70:30 $^{v}/_{v}$)

Flowrate: 0.8 ml/min.

Pressure: 2000 PSI

Sensitivity: 0.02 Aufs

Sample size: 10µl

Chart speed: 0.25cm/min.

Filter: 214nm

3.6.2.4 DATA ANALYSIS

The PCNONLIN nonlinear estimation program (one compartment model, first order input and output) was used to generate the pharmacokinetic parameters, Kabs, Kel, t½ß, tlag and AUC 0–∞. The AUC (0-6) was calculated using the GRAPHPAD program. The Vd and $Cl_{(t)}$ were calculated using the following mathematical equations:

(i) $Vd_{(area)} = \frac{F.D}{ß.AUC}$

F.D = Dose absorbed.

$Vd_{(area)}$ = Volume of distribution after oral dosing.

(ii) $Cl_{(t)} = \frac{F.D}{AUC}$

$Cl_{(t)}$ =systemic clearance after oral dosing

All pharmacological data obtained were compared, and the statistical significance of the differences determined using the student t- test: paired two - sample for means. Values of $p<0.05$ were considered as significant and values of $p>0.05$ were considered statistically not significant.

CHAPTER FOUR

RESULTS

4.1 *IN-VITRO* STUDIES

4.1.1 ASSAY

The content of praziquantel in tablets was determined using the procedure outline in USP XXI (supplement 8). The peak responses obtained for the assay preparation and standard preparation were used to compute contents of praziquantel tablets used in the in-vivo study. Praziquantel tablets contain not less than 90% and not more than 110% of the labeled amount of praziquantel.

The results were within limits (Table 4.1.1).

Table 4.1.1. Assay of praziquantel tablets using HPLC.

Sample No.	Active ingredient (% of labeled amount)
1	103.57
2	104.57
3	102.48
Mean ± SEM	103.51 ± 0.603

SEM = Standard Error of the mean

n = 3

4.1.2 DISINTEGRATION TEST

Neither USP XXI nor B.P. 1988 contains disintegration time specification for praziquantel tablets. Therefore the current official disintegration time specification for film-coated tablets was used. The results obtained from the disintegration tests are shown in table 4.1.2.

Table 4.1.2. Disintegration tests for praziquantel tablets.

Tablet No.	Disintegration time (Min.)
1	8.90
2	9.50
3	9.20
Mean ± SEM	9.2 ± 0.173

SEM = Standard Error of the mean

n = 3

4.1.3 DISSOLUTION TEST.

The dissolution test was carried out according to the procedure outlined in USP XXI (supplement 8) using the Rotating Basket method. The procedure was also repeated using simulated intestinal fluid, pH 6.8 containing 2.0mg of sodium lauryl sulphate per ml. of simulated fluid volume.

Table 4.13 shows the dissolution rates (percent dissolved after 30min.)

Table 4.1.3 In-vitro dissolution of praziquantel tablets using the Rotating Basket method.

Tablet No	Dissolution rate (% dissolved)	
	Dissolution medium	Simulated intestinal fluid, pH 6.8
1	98.40	89.30
2	99.20	90.50
3	97.60	88.70
Mean ± SEM	98.8 ± 0.326	89.5 ± 0.529

SEM = Standard Error of the mean

n = 3.

4.2. *IN-VIVO* STUDIES

4.2.1 SOLVENT SYSTEM

The High performance liquid chromatogram of blank plasma and plasma of a volunteer 2 hours after dosing with praziquantel 40mg/kg are shown in figures 4.2.1 and 4.2.2 respectively.

Praziquantel has been separated on a μ Bondapak C-18 column using ethanol: water (70:30, V/v) as mobile phase at a flow rate of 0.8ml/min. and detected at 214nm wavelength. The mobile phase was chosen on the basis of the solubility behaviour of the drug. Praziquantel had a retention time of 8.0 min. under the chromatographic conditions selected and capacity factor, K' of 2.1. The flowrate of 0.8ml/min. gave optimum retention time. The effect of ethanol content of the mobile phase on the capacity factor, K' of praziquantel and the effect of ethanol content on the peak height of praziquantel are shown in figures 4.2.3, 4.2.4 and table 4.2.3 respectively. PH had no effect on the capacity factor, K' or the peak height. The chromatogram of blank plasma did not show any interfering extraneous peaks or plasma matrix effects.

Table 4.2.1. Effects of mobile phase composition on the capacity factor, K' and peak height of praziquantel.

Composition (Ethanol: water, v/v)	Capacity factor, K'	Peak height, (cm)
50:50	1.6	1.5
60:40	2.5	1.3
65:35	1.3	1.8
70:30	2.1	2.4

Figure. 4.2.1 High performance liquid chromatogram of blank plasma.

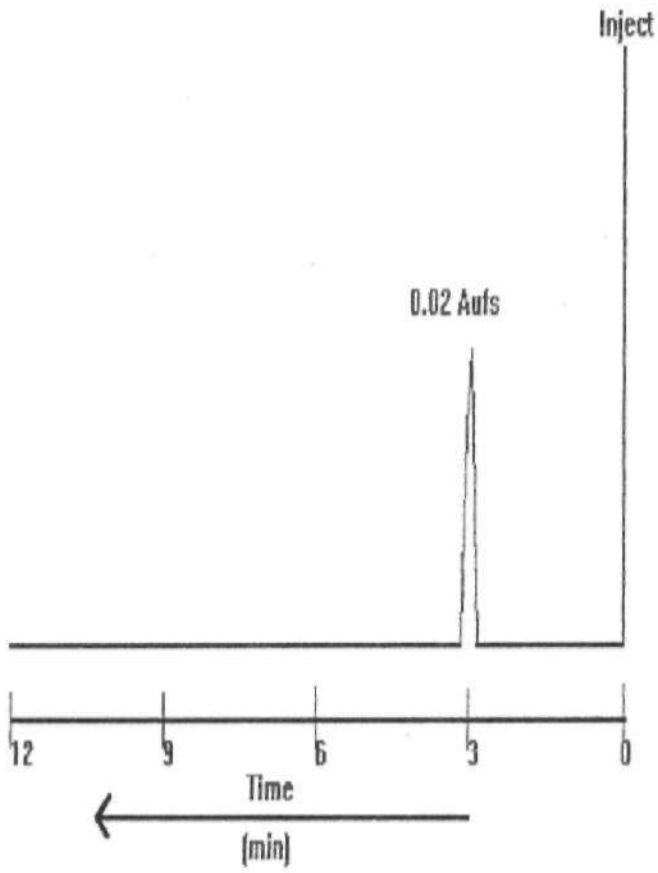


Figure 4.2.2. High performance liquid chromatogram of a volunteer 2 hours after dosing with praziquantel.

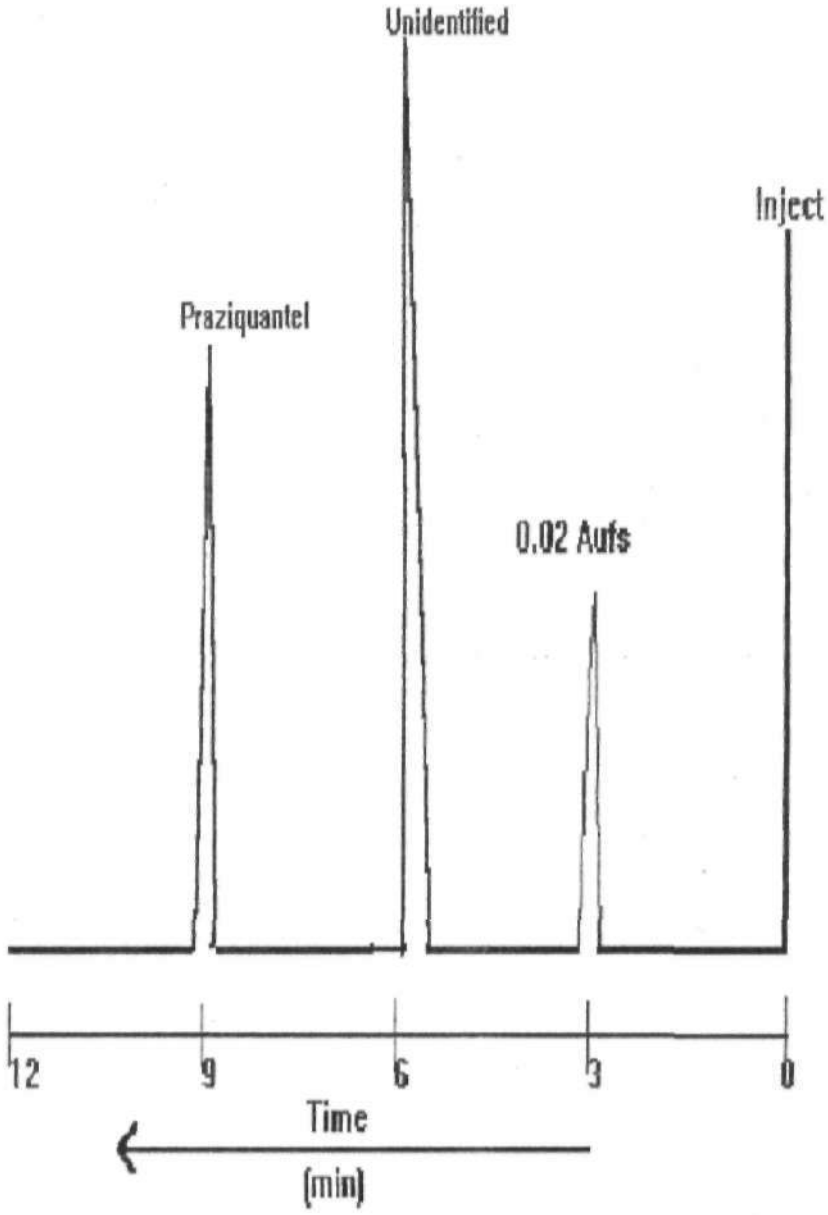


Figure 4.2.3 Effect of ethanol content of the mobile phase on the capacity factor, K' of Praziquantel

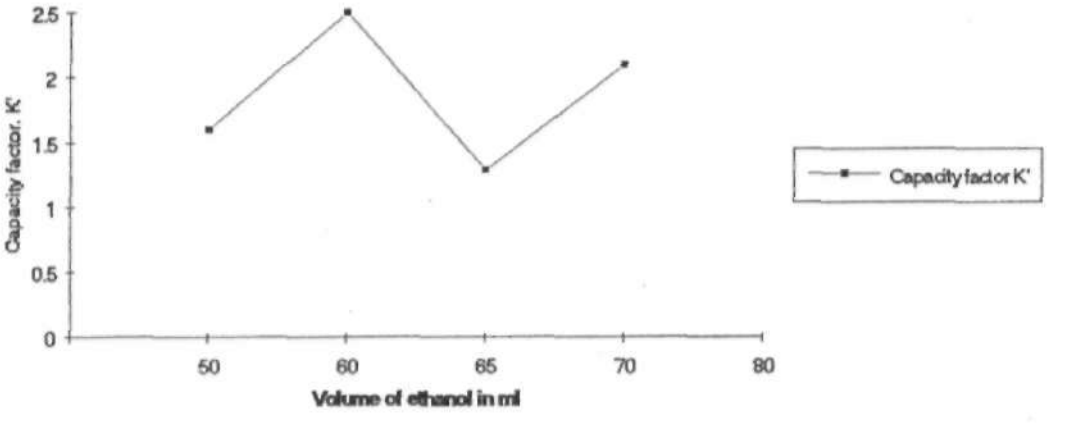
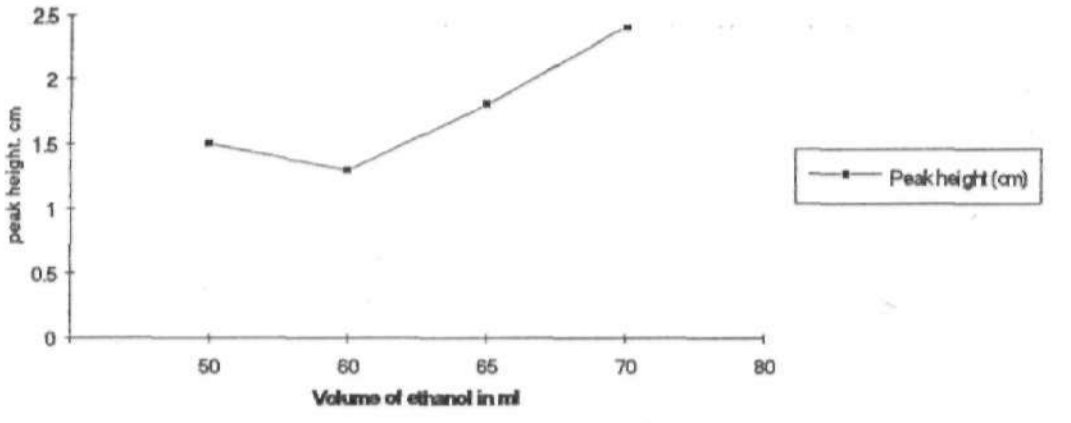
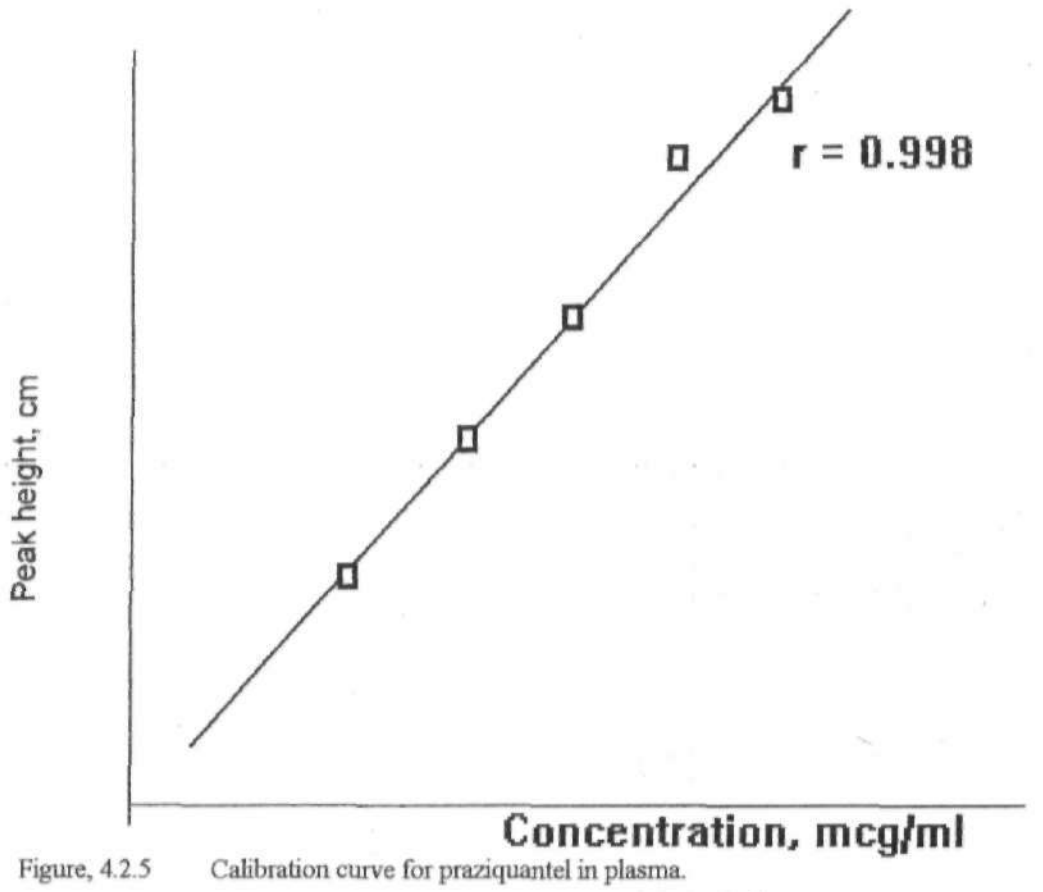


Figure 4.2.4 Effect of ethanol content of the mobile phase on the peak height of Praziquantel



4.2.2 CALIBRATION CURVE

The calibration curve for praziquantel in plasma is shown in figure 4.2.5. The correlation coefficient, r = 0.998 which shows a good correlation of response of the detector at the concentrations used.



Figure, 4.2.5 Calibration curve for praziquantel in plasma.

4.2.3. PRECISION.

The results of the precision of the analytical method are given in Table 4.2.3.

Table 4.2.3 Precision of Analytical method.

Sample: Praziquantel.	Concentration (µg/ml)	C.V. (%)	n
Within run:	0.1	7.0	5
	1.0	4.9	5
	1.5	2.5	5
Between run:	0.1	7.8	5
	1.0	5.0	5
	1.5	2.8	5

The limit of detection was found to be 5ng/ml for a 10.µl injection.

4.2.4 RECOVERY

The percentage recovery of the extraction method are given in table 4.2.2.

Table 4.2.4 Percentage recovery of praziquantel.

Sample	Nominal conc.(µg/ml)	Assayed conc.(µg/ml) Mean ± SEM	Recovery (%)
Praziquantel	1.0	0.896 ± 0.01	89.6
	0.4	0.922 ± 0.08	92.2

SEM = Standard Error of the mean

n = 5.

4.3 *PHARMACOKINETIC STUDIES.*

4.3.1 PRAZIQUANTEL BLOOD LEVELS

The concentrations of praziquantel obtained from plasma of subjects following the protocol outlined in chapter three are presented in Table 4.3.1 and 4.3.2, While figures 4.3.1 - 4.3.3 show the plasma concentration time profiles respectively.

Table 4.3.1 Plasma concentrations of praziquantel following single oral dose of 40mg/kg.

Time (h)	Concentration, µg/ml.						Mean ± SEM
	I	II	III	IV	V	VI	
0	0	0	0	0	0	0	0
0.5	0.38	0.22	0.22	0.3	0.3	0.3	0.2867 ± 0,0246
1.	0.54	0.46	0.54	0.46	0.62	0.54	0.5267 ± 0.0246
1.5	1.46	0.62	1.3	1.08	1.38	1.3	1.19 ± 0.1252
2.0	1.3	0.76	1.08	1.38	1.08	1.08	1.1133 ± 0.0883
3.0	1.14	1.3	0.92	1.0	0.92	0.92	1.0333 ± 0.0638
4.0	0.54	1.0	0.46	0.38	0.3	0.3	0.4967 ± 0.1076
6.0	0.22	0.62	0.22	0.22	0.16	0.16	0.2667 ± 0.0717

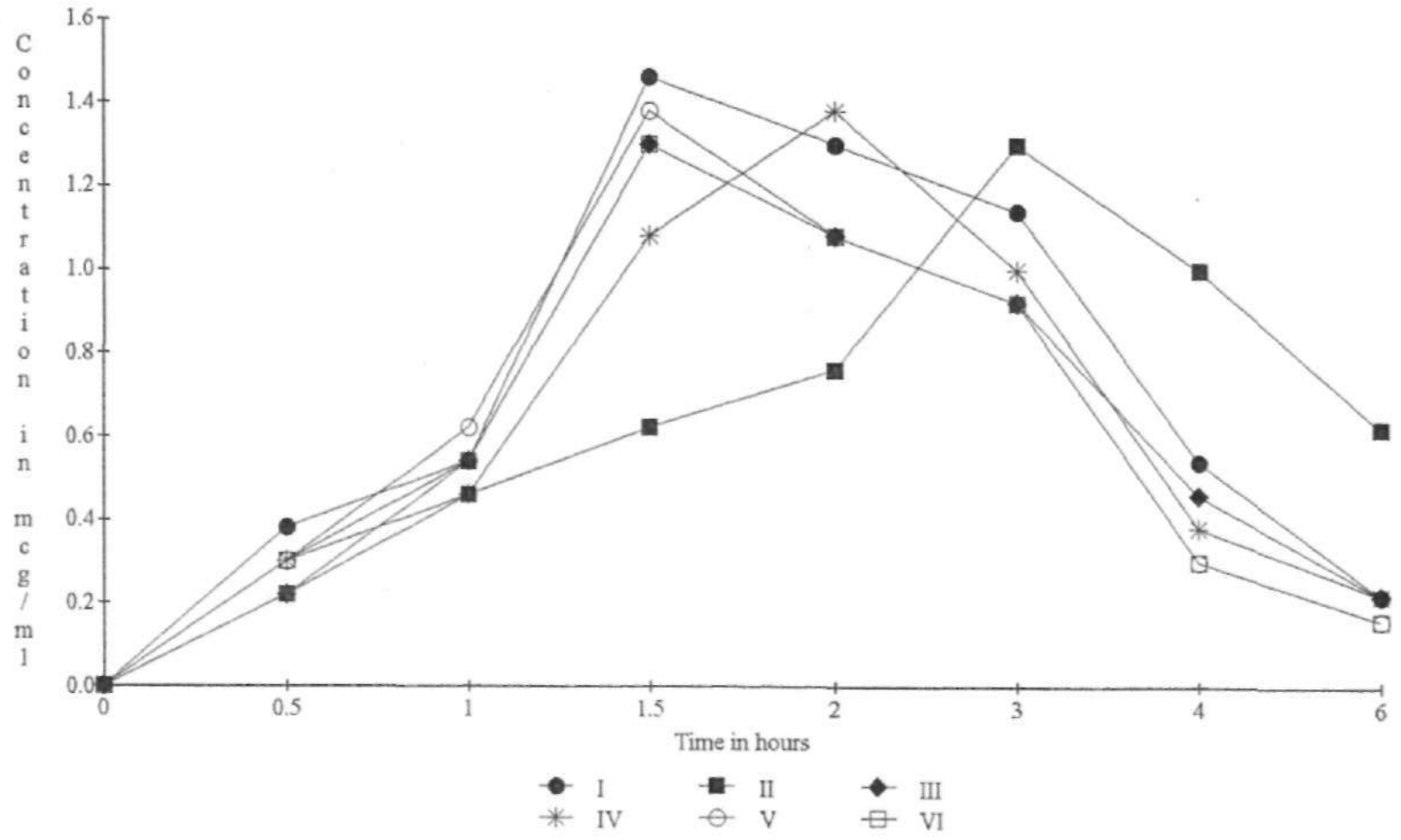
Table 4.3.2 Plasma concentrations of praziquantel, 40mg /kg one hour after a single dose of ranitidine 150mg

Time (h)	Concentration, µg/ml.						Mean ± SEM
	I	II	III	IV	V	VI	
0	0	0	0	0	0	0	0
0.5	0.38	0.38	0.3	0.22	0.38	0.22	0.3133 ± 0.0321
1.0	0.64	0.62	0.46	0.36	0.62	0.38	0.5133 ± 0.0526
1.5	0.54	0.76	0.76	1.62	0.68	0.62	0.6633 ± 0.356
2.0	0.46	0.62	0.54	1.76	0.54	0.76	0.6133 ± 0.0508
3.0	0.38	1.46	0.46	1.54	0.38	0.46	0.4467 ± 0.0246
4.0	0.30	1.38	0.30	0.30	0.30	0.30	0.3133 ± 0.0133
6.0	0.16	0.22	0.11	0.16	0.16	0.16	0.1617 ± 0.0142

SEM = Standard Error of the mean.

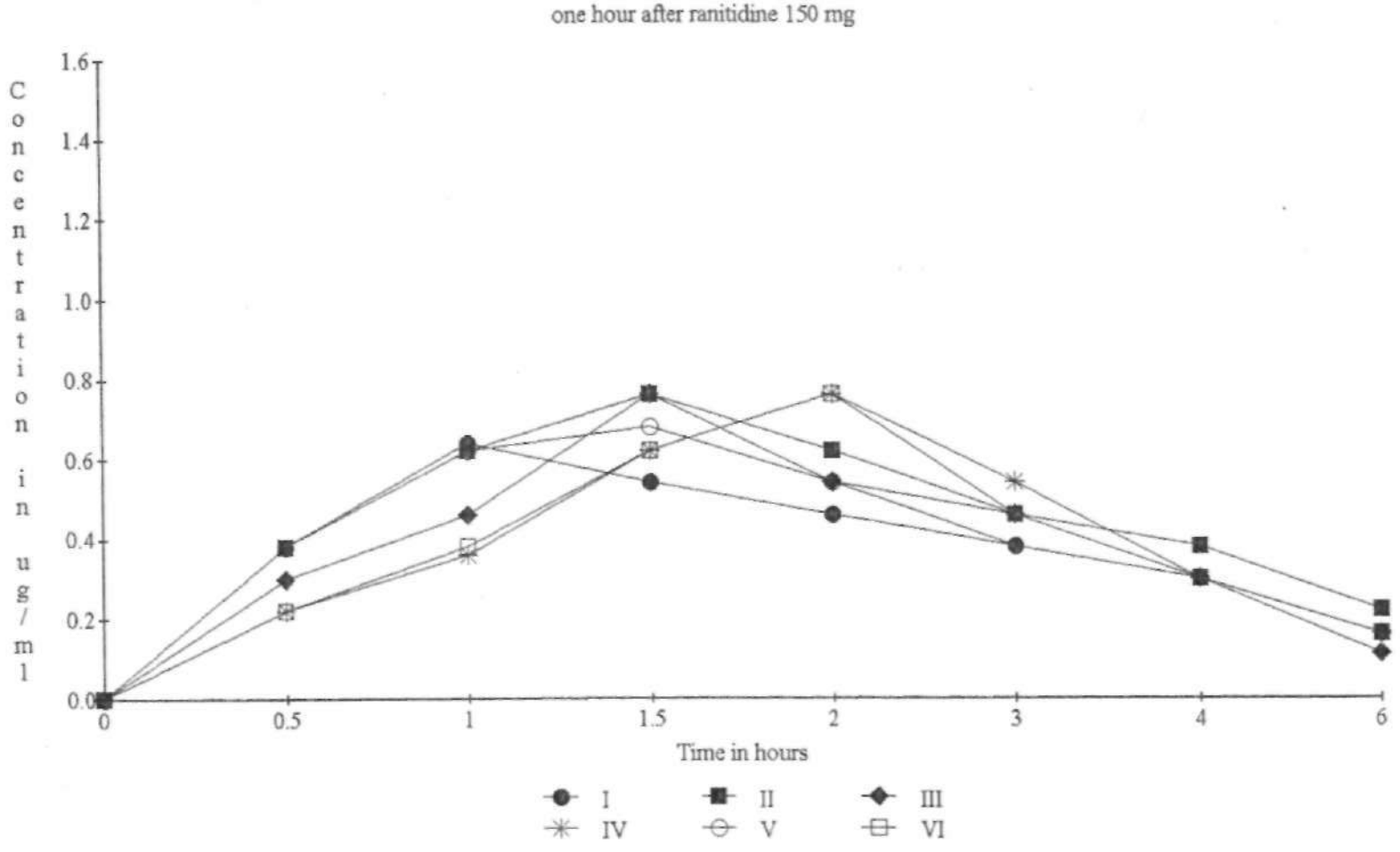
n = 6

Figure 4.3.1. Plasma praziquantel concentration - time profile of subjects following single dose of praziquantel 40mg/kg



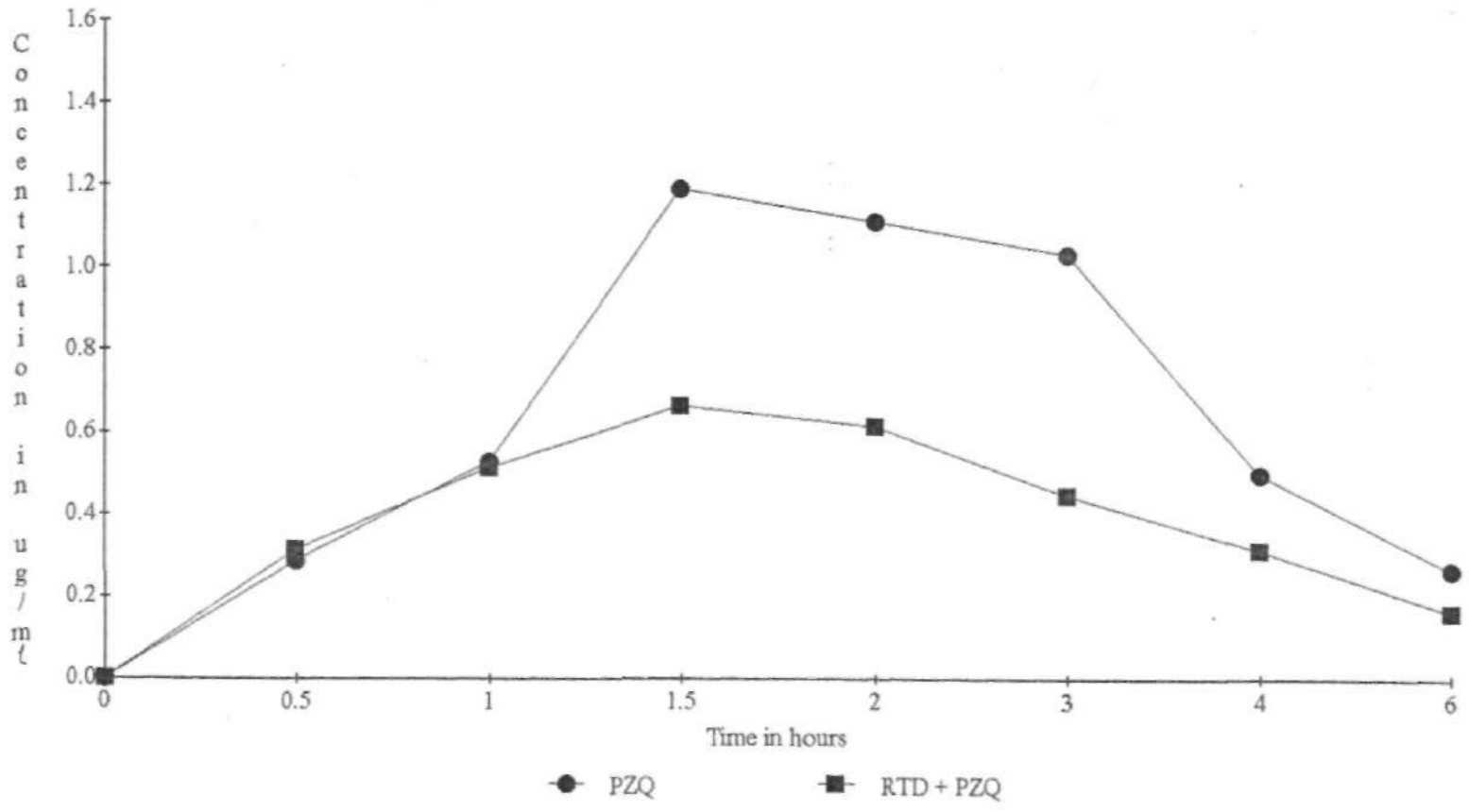
45

Figure 4.3.2. Plasma praziquantel concentration - time profile of subjects following single dose of praziquantel 40mg/kg



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Figure 4.3.3. Mean plasma concentration - time profile for praziquantel 40mg/kg alone and for praziquantel 40mg/kg one hour after a dose of ranitidine 150 mg



PZQ = Praziquantel 40mg/kg RTD = Ranitidine 150mg

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4.3.2 PHARMACOKINETIC PARAMETERS.

The pharmacokinetic parameters obtained from the plasma concentrations of subjects are presented in tables 4.3.3, 4.3.4 and 4.3.5.

Table 4.3.3. Pharmacokinetic parameters of praziquantel following single dose of praziquantel 40mg/kg.

Pharmacokinetic parameter (Unit)	Subjects						Mean ± SEM
	I	II	III	IV	V	VI	
Vd (L/kg)	12.85	14.97	14.42	11.22	11.89	12.37	12.95 ± 0.59
Clearance L/h/kg	6.81	7.55	7.11	6.39	6.47	6.82	6.85 ± 0.17
Tmax (h)	1.5	3	1.5	3	1.5	1.5	2 ± 0.32
Cmax(ug/ml)	1.46	1.3	1.3	1.38	1.38	1.3	1.35 ± 0.03
Ka(µg/ml/h)	0.62	0.60	0.93	0.67	1.04	0.69	0.75 ± 0.08
Lag time (h)	0.097	0.096	0.044	0.097	0.041	0.099	0.079 ± 0.01
Ke (µg/ml/h)	0.53	0.469	0.57	0.53	0.62	0.56	0.5465 ± 0.02
T½ (h)	1.297	1.48	1.22	1.32	1.12	1.24	1.2795 ± 0.05
AUC (0-6) (µg/ml/h)	3.7198	4.1725	3.1517	3.4554	2.7218	2.686	3.3179 ± 0.24
AUC (0–∞) (µg/ml/h)	5.37	4.77	4.17	4.71	3.91	4.12	4.5083 ± 0.22

SEM = Standard Error of the mean.

n = 6

Table 4.3.4. Pharmacokinetic parameters of praziquantel following single dose of praziquantel 40mg/kg one hour after ranitidine 150mg

Pharmacokinetic parameter (Unit)	Subjects						Mean ± SEM
	I	II	III	IV	V	VI	
Vd (L/kg)	48.37	34.37	32.33	23.49	23.99	31.3	32.31 ± 3.69
Clearance L/h/kg	13.54	11.08	11.13	6.30	10.53	10.56	11.02 ± 0.57
Tmax (h)	1.0	1.5	1.5	2.0	1.5	2.0	15837 ± 0.15
Cmax(ug/m)l	0.64	0.76	0.76	0.68	0.46	0.76	0.6767 ± 0.047
Ka(µg/ml/h)	2.5	2.02	1.02	0.84	2.4	0.88	1.61 ± 0.32
Lag time (h)	0.18	0.25	0.1	0.096	0.23	0.1	0.1593 ± 0.03
Ke (µg/ml/h)	0.28	0.3	0.44	0.37	0.3	0.39	0.3467 ± 0.026
T½ (h)	2.46	2.33	1.58	1.87	2.31	1.8	2.0583 ± 0.14
AUC (0-6) (µg/ml/h)	2.1167	2.596	1.9945	2.2973	2.2378	2.3677	2.2683 ± 0.09
AUC (0-∞) (µg/ml/h)	2.7	3.25	2.58	3.01	2.84	2.85	2.8717 ± 0.096

SEM = Standard Error of the mean.

n = 6

Table 4.3.5. Effect of a single dose of ranitidine 150mg on the pharmacokinetics of praziqunatel 40mg/kg administered one hour after ranitidine.

Pharmacokinetic Parameter (Units)	Mean ± SEM		Percentage Change (%)	P-value (T-test)
	Control 1	Cross over		
Vd (L/kg)	12.95 ± 0.59	32.31 ± 3.69	+ve 161	P<0.05
Clearance (L/h/kg)	6.85 ± 0.17	11.02 ± 0.57	+ve 60	P<0.01
T_{max} (h)	2.0 ± 0.32	1.5837 ± 0.15	-ve 21	P<0.5
C_{max} (µg/ml)	1.35 ± 0.03	0.6767 ± 0.047	-ve 50	P<0.001
Ka (µg/ml/h)	0.75 ± 0.08	1.61 ± 0.32	+ve 115	P<0.05
Lag time (h)	0.079 ± 0.01	0.1593 ± 0.03	+ve 102	P<0.05
Ke ((µg/ml/h)	0.5465 ± 0.02	0.3467 ± 0.026	-ve 37	P<0.01
T½ (h)	1.2795 ± 0.05	2.0583 ± 0.14	+ve 61	P<0.01
AUC (0-6) (µg/ml/h)	3.3179 ± 0.24	2.2683 ± 0.09	-ve 32	P<0.01
AUC (0-∞) (µg/ml/h)	4.5083 ± 0.22	2.8717 ± 0.096	-ve 36	P<0.001

SEM = Standard Error of the mean.

n = 6

+ve = positive

-ve = negative

CHAPTER FIVE

5.1 DISCUSSIONS

The pharmacokinetics of praziquantel in *Schistosoma haematobium* - infected subjects has been reported (Ofori - Adjei *et al*, 1988). The drug is about 80% absorbed, however extensive first - pass hepatic hydroxylation occurs and probably accounts for considerable inter individual variation in plasma levels and pharmacokinetics (Ofori - Adjei *et al*, 1988, Leopold *et al*, 1978, Jung *et al*, 1990 and Westhoff and Blaschke, 1992).

The pharmacokinetic parameters obtained in this study also show that there were varying interindividual differences in the plasma levels and pharmacokinetics of praziquantel. All the pharmacokinetic parameters derived in our study compare well with previously published values for praziquantel.

The mean peak concentration, Cmax was 1.35 μg/ml which is consistent with the range of 1.00μg/ml to 1.62 μg/ml reported for the same dose of 40mg/kg in eight healthy volunteers (Kaojerern *et al*, 1989). Putter and Held (1979) reported a mean Cmax of 1.36μg/ml in five lactating women receiving 50mg/kg of the drug. A higher mean value of 3.12 μg/ml has been reported by Ofori - Adjei *et al*. (1988) using a lower dose of 30mg/kg in *Schistosoma haematobium* - infected subjects in an endemic locality.

The correlation coefficient of the terminal ß-slope indicated linearity in the pharmacokinetics of praziquantel. The mean elimination t½ observed in the present study was 1.28h. Ofori-Adjei *et al*. (1988) reported a mean t½ of 1.7h while Westhoff and Blasckhe (1992) reported an apparent elimination t½ of 1.3h. Kaojerern *et al* (1989) reported a shorter t½ of 0.94h.

The time to peak concentration, t_{max} has been reported to occur between 1 and 3 hours (Leopold *et al*., 1978) The t_{max} obtained in the present study was 2h and was similar to a t_{max} of 1.9h reported by Ofori - Adjei *et al*. (1988).

The total area under the plasma concentration time curve (AUC) was 4.51 μg/ml/h as compared to a value of 4.83 μg/ml/h reported by Kaojerern *et al*. (1989) after a similar dose.

The administration of ranitidine in a crossover design one hour before praziquantel caused statistically significant changes in the mean pharmacokinetic parameters of the subjects. The potential of ranitidine-drug interaction has been subject of extensive investigations. Pharmacokinetic interactions of ranitidine may occur at the site of absorption, metabolism or renal excretion (Klotz *et al.*, 1992). Suppression of gastric acid secretions are generally not achieved until at least one hour after administration (Garty *et al.*, 1980) and might be avoided for concomitantly administered, rapidly absorbed drugs.

The absorption kinetics of drugs are usually measured by the following pharmacokinetic parameters; Kabs, t½ abs, Tlag, C_{max}, and T_{max} and mainly relate to the rate and extent of drug absorption. The other pharmacokinetic parameters are indices of metabolism and/or excretion and include t½, Kel, Vd, AUC and total body clearance (Cl_t)

Significant ranitidine - drug interactions which suggest that ranitidine may affect the bioavailability of certain drugs through pH-dependent effect on absorption or change in fluid volume had been reported (Piscitelli *et al*, 1991). In the present study, the prior administration of ranitidine 150mg, one hour before praziquantel has been shown to significantly affect the mean absorption and pharmacokinetic parameters.

The AUC (0-∝) after ranitidine pretreatment was significantly different from that of praziquantel alone ($P<0.001$). This result is consistent with the work of Piscitelli *et al.* (1991) on the effect of ranitidine on the bioavailability of ketoconazole: another minimally water soluble antifungal agent, the absorption of which had been shown to be dependent upon gastric acidity (Vander Meer *et al*, 1980). The AUC) (0-12h) was found to be significantly different for Ketoconazole alone.

A similar reduction in the relative bioavailability of praziquantel tablets ($P<0.017$) has been attributed to the unsatisfactory dissolution and subsequent reduction in the amount of praziquantel in the area of the upper gastro-intestinal tract (Kaojerern *et al*, 1989) where the most rapid absorption takes place (Kaplan, 1973). A similar reduction in the in-vitro dissolution rate of praziquantel was observed at pH 6.8 (simulated intestinal fluid) in the present study.

However, the dissolution rate of praziquantel tablets may not be a rate-limiting step in the absorption of praziquantel. The prior administration of ranitidine increased the Kabs from 0.75µg/ml/h

to 1.61 µg/ml/h, an increase of about 115% which is statistically significant (P<0.05). It has been reported (Hogben *et al*, 1957) that a drug absorbed primarily from the intestines could have gastric emptying rates as its rate-determining step. It is worth noting therefore that high acidity inhibits, while slight alkalinity enhances gastric emptying rate (Thomas, 1957). Thus even for weakly acidic drugs, the rate of absorption will be much greater from the small intestine due to the large surface area of the small intestine and greater blood flow compared to the stomach. The rate of gastric emptying will therefore have a pronounced effect on the speed of drug absorption.

Regardless of the significant increase in Kabs, the C_{max} decreased from 1.35µg/ml to 0.6767µg/ml, a decrease of about 50%, which is statistically significant (P<0.oo1). The decrease in t_{max} (by 20%) was however not significant (P<0.5).

Pharmacokinetic parameters indicative of metabolism and/or excretion also exhibited statistically significant changes. The elimination t½ increased from 1.2795h to 2.0583, i.e. by 60% (P<0.01) while the elimination rate constant, Kel, decreased from 0.5465µg/lml/h to 0.3467 ug/ml/h (a decrease of 36 %, P< 0.01). These changes are statistically significant and may suggest possible inhibition of hepatic praziquantel metabolism by ranitidine. However, the elimination t½ and elimination rate constant are often in appropriate expressions of the elimination characteristics of a drug because they only describe the rate of removal of drug from the plasma and not necessarily from the body.

The total systemic clearance increased from 6.85L/h/kg to 11.02L/h/kg, an increase of about 60% (P<0.01) which is significant.There is no obvious explanation for this observation except that there was ranitidine - mediated increase in the volume of distribution, Vd from 12.95L/kg to 32.3/L/kg. (P<0.05) and decrease in AUC. The change in Vd may be due to delayed on set of absorption, (Tlag increased by about 102%, P<0.05) or because of the reduced extent of absorption caused by prior ranitidine administration. Such large difference in Vd had previously been reported (Kaojerern *et al*, 1989). A standard praziquantel product gave a Vd of 11.88L/kg while a generic brand which exhibited poor dissolution rates and reduced extent of absorption gave a Vd of 25.74L/kg. These values for the distribution volume are however estimates since an intravenous product is necessary to fully characterise the distribution of praziquantel into body fluids and tissues.

On the other hand, the clearance of praziquantel observed in the present study may permit the speculation that, the liver may not be the only site of praziquantel presystemic elimination. Cimetidine which has been proved to inhibit hepatic cytochrome P-450 drug metabolism has also been observed to inhibit Cytochrome P-450 from gastric microsomes (Bonn *et al*; 1981). Therefore the observed decrease in AUC in spite of significant increase in absorption rate may be due to increased presystemic elimination in the gut lumen and/or gut wall. This will limit the amount of unchanged drug reaching the site of action.

The AUC is therefore a useful and direct measure of the exposure of the body to a drug after administration; its value depends on both the dose administered and systemic clearance of the drug.

5.2 CONCLUSION

The praziquantel tablets (Biltricide, Bayer) used in the in-vivo studies passed the official tests for assay and dissolution (USP XXI, supplement 8) and test for disintegration (B.P. 1988).

The plasma extraction method was a modification of the procedure developed by Westhoff and Blaschke (1992). The recovery of praziquantel from plasma was high; 92.2% for a nominal concentration of 0.4µg/ml. The method of analysis gave a reproducibility for repeat injections of the order of 5% on absolute quantity. The internal standard method could not be used because of lack of appropriate reference compound. These values compare well with published data (Xiao *et al*; 1983).

The mean pharmacokinetic parameters of praziquantel were similar to the values obtained by Ofori - Adjei *et al*. (1988). As shown in previous studies, there were interindivudual variations in plasma levels and pharmacokinetic parameters.

The prior administration of ranitidine has been shown to significantly affect the mean absorption and pharmacokinetic parameters of praziquantel. Although the clinical significance of such ranitidine - mediated pharmacokinetic interactions has not been established (Piscitelli *et al*; 1992), fatal ranitidine - induced theophylline toxicity had been reported (Roy *et al*; 1988).

Therefore a reduction of 36% in bioavailability observed in the present study caused by prior ranitidine administration may present crucial problems in the management of cerebral Cysticercosis with praziquantel in which the indication for the use of ranitidine also exists. The concentration of

praziquantel in the cerebrospinal fluid is about 14% to 20% of the total amount of praziquantel in the plasma (Thomas *et al*, 1982).

Ranitidine, unlike cimetidine is thought to be almost without inhibitory effect on the cytochrome p-450 enzyme system (Hande *et al*, 1986). However ranitidine is five times more potent at the H_2-receptor and deviations in linearity with respect to the absorption of a drug may be due to low drug solubility, low dissolution rate of the drug from the dosage form, changes in the gastrointestinal pH, and in the case of active transport processes, due to saturation of carriers (Ritschel, 1980). Nonlinearities in the pharmacokinetic behaviour must be recognised since they affect the outcome of pharmacological and toxicological studies (Clark et. al 1984).

Unfortunately systemic clearance determinations after oral administration are based on linear kinetics and assumptions concerning bioavailability (Wagner, 1967). Even though the elimination t½ values between treatments were measured, estimates for the volume of distribution were based on assumptions made regarding bioavailability. A drug may have a long elimination t½, because either it has a large Vd or it is slowly removed from the body by the eliminating organs or both.

Praziquantel is a broad spectrum schistosomicidal agent. The drug undergoes a pronounced first - pass metabolism in the liver, yielding preferentially monohydoxylated metabolites. In the light of current developments where multiple forms of cytochrome p-450 are known to exist with overlapping substrate specificities (Powell *et al*, 1984) considerable information can be obtained from measuring both drug and metabolites after drug administration. Unfortunately large intra individual and interindividual variations in drug metabolism mean that significant differences could not be found. Therefore using mean pharmacokinetic values may be misleading and should be cautiously interpreted especially where quantitative aspects of drug metabolism are concerned (Westhoff and Blaschke, 1992).

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APPENDIX I

Subject data

Subject	Age(yr)	Weight (Kg)	Dose of Praziquantel.(mg)
I	14	36	1200
II	16	40	1800
III	12	32	1200
IV	15	43	1800
V	18	52.5	2400
VI	12	35	1200
Mean ± SEM	14.5 ± 0.96	39.75 ± 2.99	1680 ± 204.89