EFFECTS OF CHLORPHENIRAMINE ON THE PHARMACOKINETICS OF ASCORBIC ACID IN HEALTHY MALE VOLUNTEERS

By

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DECLARATION

I declare that the work in the thesis entitled “Effects of chlorpheniramine on the pharmacokinetics of ascorbic acid in healthy male volunteers” has been performed by me in the Department of Pharmacology and Clinical Pharmacy under the supervision of Prof. I. Abdu- Aguye and Prof. (Mrs.) H.O. Kwanashie.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Mahdi, Mohammed Abubakar Signature: Date

CERTIFICATION

This thesis entitled "EFFECTS OF CHLORPHENIRAMINE ON THE PHARMACOKINETICS OF ASCORBIC ACID IN HEALTHY MALE VOLUNTEERS" by Mahdi, Mohammed Abubakar meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Ascorbic acid is a vitamin commonly used in the treatment of common cold and scurvy. It interacts with some drugs during absorption from the gastrointestinal tract, in the systemic circulation or during metabolism in the liver. However, interaction between ascorbic acid and chlorpheniramine has hitherto not been reported.

Six healthy adult male volunteers took part in this research. The average age of the volunteers is 24.33 ± 0.71 years and average weight of 64.83 ± 1.08 kg. The six healthy male volunteers were given 200 mg oral ascorbic acid only, during the control study. They received 200 mg ascorbic acid and 4 mg chlorpheniramine after a wash out period of two weeks through the same route. Urine samples collected in a plastic covered tube were analyzed for ascorbic acid levels by colorimetric method. A standard calibration curve was prepared by making five different concentrations of standard ascorbic acid each made to a total volume of 2mL. The concentrations were 0.01, 0.02, 0.03, 0.04, and 0.05 mg/mL. Absorbance was obtained using a colorimeter set at 520 nm. A plot of absorbance against standard ascorbic acid concentration resulted in a straight line with correlation coefficient of 0.98. The concentrations of the tests samples were obtained by interpolation of the calibration curve.

The pharmacokinetics parameters were generated from the urine levels of ascorbic acid excreted against time curve and rates of ascorbic acid excretion against average time intervals profile.

The apparent volume of distribution V_d, and total body clearance CL_T decreased significantly ($p<0.05$) in the tests when compared with the controls. However, the area under the curve $AUC_{0-\infty}$ increased significantly from values obtained in the control. In fact, the increase in $AUC_{0-\infty}$ was about 2-3 fold. The elimination half life $t_{1/2(\beta)}$ similarly increased ($p< 0.05$). There was little but

statistically insignificant increase in renal clearance CL_R, fraction of drug excreted f_e, and bioavailability fraction F.

In conclusion, it was observed that chlorpheniramine co-administration altered the volume of distribution, total body clearance, area under the curve, and elimination half-life of ascorbic acid tablet. The results from this study, implies that chlorpheniramine reduced the amount of ascorbic acid available to the body tissues for utilization and storage. Such concurrent administration should therefore be discouraged.

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ABBREVIATIONS

AUC	=	Area Under the Curve
B.P.	=	British Pharmacopoeia
^{O}C	=	degree centigrade (Celsius)
CL_T	=	total body clearance
CL_R	=	renal clearance
g	=	gram
μg	=	microgram
mL	=	milliliter
min	=	minutes
nm	=	nanometer
r	=	correlation coefficient
rpm	=	round per minute
SEM	=	Standard Error of Mean
$t_{1/2\beta}$	=	elimination half -life
pH	=	hydrogen ion concentration

CHAPTER 1 INTRODUCTION

1.1 Pharmacokinetics

Pharmacokinetics can be defined as the quantitative study of various kinetic processes of drug disposition in the body (Winters, 1994). Such processes are absorption, distribution, metabolism and excretion. A drug is absorbed from various sites of administration (oral, parenteral, inhalation, sublingual, e. t. c.); distributed by the blood to perfused tissues such as the liver, kidney, and lungs. Later it is excreted in the urine, feaces, expelled air, or sweat. However, the biological nature of drug distribution and disposition is complex, and drug events often happen simultaneously.

Drugs are in a dynamic state within the body. Simplifying assumptions are made to describe a complex biologic system concerning the movement of drugs. In this way, Pharmacokinetic models are used. A model is a hypothesis using mathematical terms to concisely describe quantitative relationships. Various mathematical models can be devised to simulate the rate processes of drug absorption, distribution, and elimination. These mathematical models make possible the development of equation to describe drug concentration in the body as a function of time.

1.2 Statement of Research Problem

Ascorbic acid (vitamin C) is used in the treatment of common cold and also in cases of scurvy in vitamin C- deficient subjects (Marcus and Coulston, 2001). This vitamin, owes its activity to the reducing property it has and hence functions in a number of biochemical reactions mostly involving oxidation. An extensive literature search reveals a number of interactions of some drugs with ascorbic acid. For example, ascorbic acid raises serum Cortisol concentrations depressed by etomidate infusions and restores the adrenocorticotropic hormone (ACTH)/Cortisol ratio to pre-

operative values (Boidin, 1985); ascorbic acid increases the half-life of paracetamol (acetaminophen) when it is metabolized in part to the sulphate and competes for the available substrate (Houston and levy, 1976). However, despite the report by Gershoff (1993), that ascorbic acid has an antihistamine activity, no observation has been made so far as to the possible interaction between ascorbic acid –a vitamin- and chlorpheniramine –a H_1 receptor antagonist.

The aim of the current study therefore, is to explore an ascorbic acid assay method and find out if chlorpheniramine can significantly affect the pharmacokinetics of ascorbic acid when both drugs are administered orally at the same time.

1.3 Justification for the Study

The work of Conney and Burns (1960) which states that 'compounds that cause enzyme induction also increase markedly the synthesis of ascorbic acid' and that of Gershoff (1993) which observed the antihistamine activity of the vitamin, can be used as justification for the study.

Vitamin C is commonly prescribed with chlorpheniramine for the treatment of common cold.

1.4 Theoretical Framework

For the purpose of this study some important parameters are highlighted:

1.4.1 One – Compartment Open Model

The body can be represented as a series, or systems, of compartments that communicate reversibly with each other. A compartment is not a real physiologic or anatomic region but is considered as a tissue or group of tissues that have similar blood flow and drug affinity. Within each compartment, the drug is considered to be uniformly distributed. In one –

compartment open model, elimination of drugs is a first – order process. The elimination rate constant K is a first – order elimination rate constant with units per time i.e. h^{-1} (Gibaldi and Nagaashima, 1969).

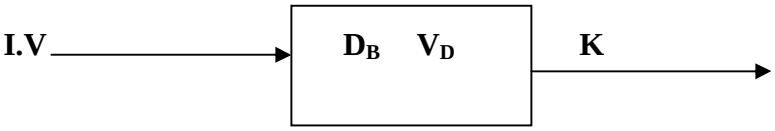


Figure 1.1 Pharmacokinetic model for a drug administered by rapid intravenous injection

A rate expression for figure 1.1 is $dD_B/ dt = - KD_B$

Where D_B = amount of drug in the body (dose)

Integration of the equation gives;

$\text{Log } D_B = - kt/ 2.3 + \text{Log } D_B^O$

Plotting $\text{Log } D_B$ against time (t) gives a straight-line graph

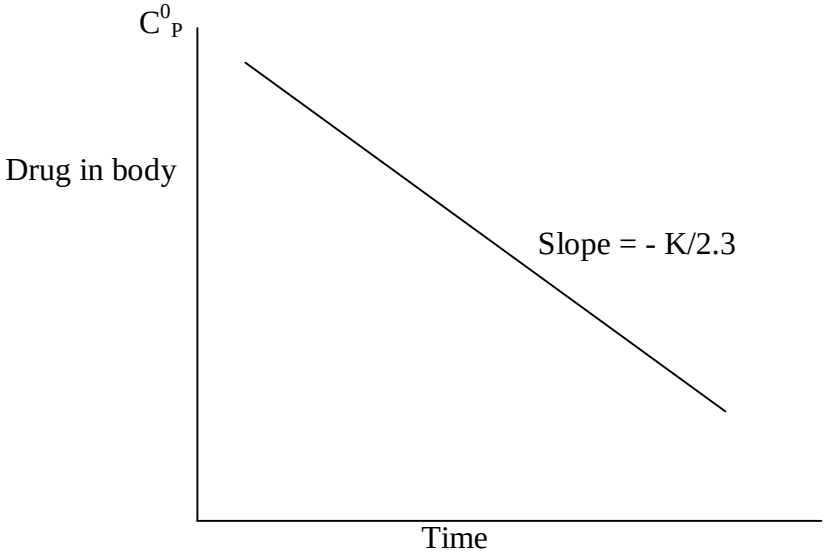


Figure 1.2 Log concentration versus time for one-compartment open model

1.4.1 Two – Compartment Open Model

The plasma level-time curve for a drug that follows a two – compartment model is as follows: -

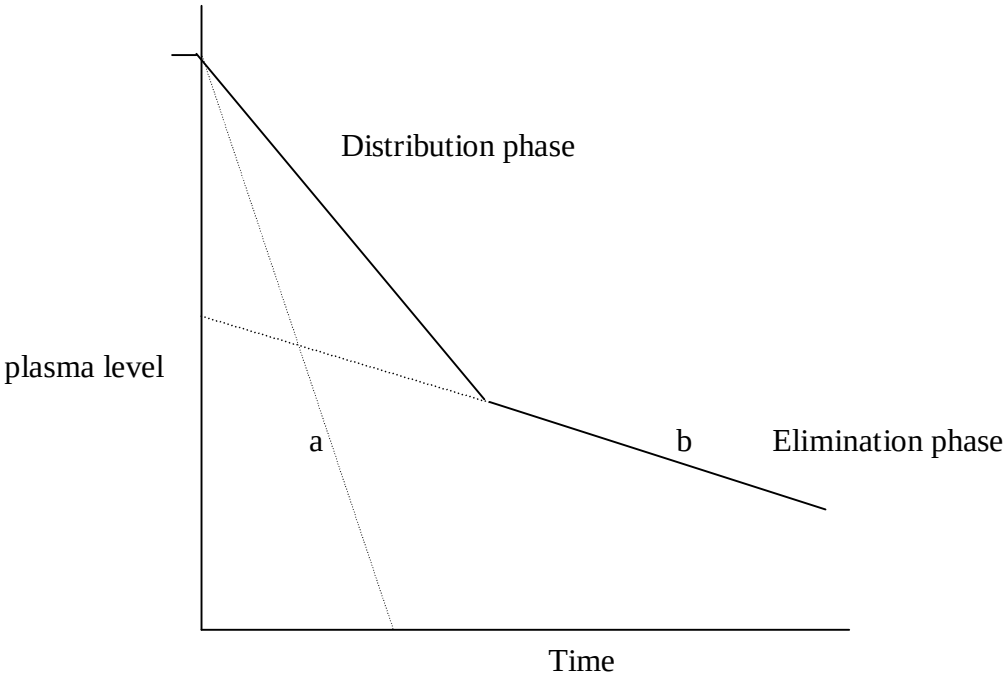


Figure 1.3 Plasma level versus time curve for two compartment open model (I.V.).

Where a = absorption rate constant

b = elimination rate constant

The plasma drug concentration decline biexponentially as the sum of two first – order processes i.e. distribution and elimination.

1.4.3 Renal Drug Excretion

Many Drugs are eliminated by renal excretion. Non-Volatile drugs, which are water-soluble and have a low molecular weight or are slowly biotransformed by the liver will be eliminated by renal excretion. The following processes are involved in drug excretion via the kidney:

i Glomerular filtration ii Active tubular Secretion iii Tubular reabsorption.

The amount of drug excreted in the urine, is directly related to the total amount of drug absorbed (Leon and Andrew, 1999).

The relationship between urine drug level versus time and the plasma level versus time curve is shown below:

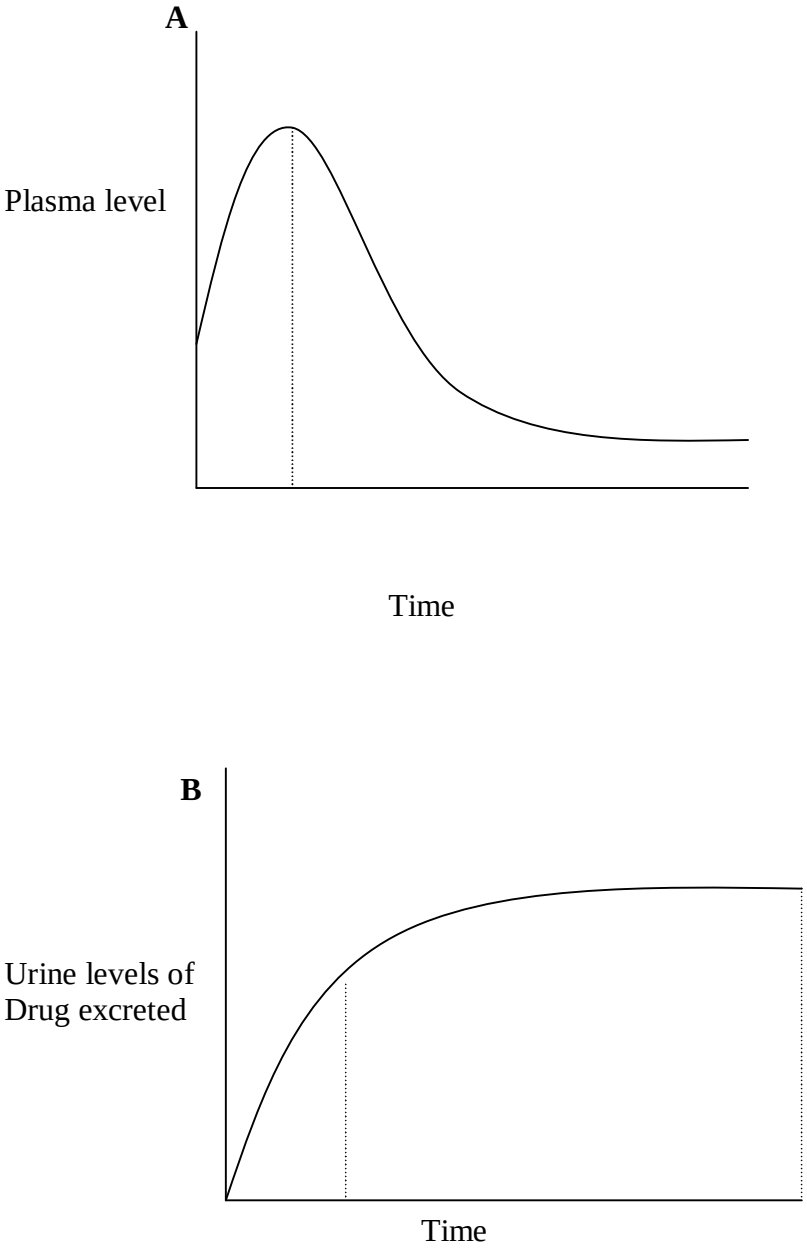


Figure 1.4 **A.** Plasma level versus time curve. **B.** Urine levels of drug versus time curve

Hence, a graph comparing the rate of drug excretion with respect to time should be similar in shape as the plasma level for that drug;

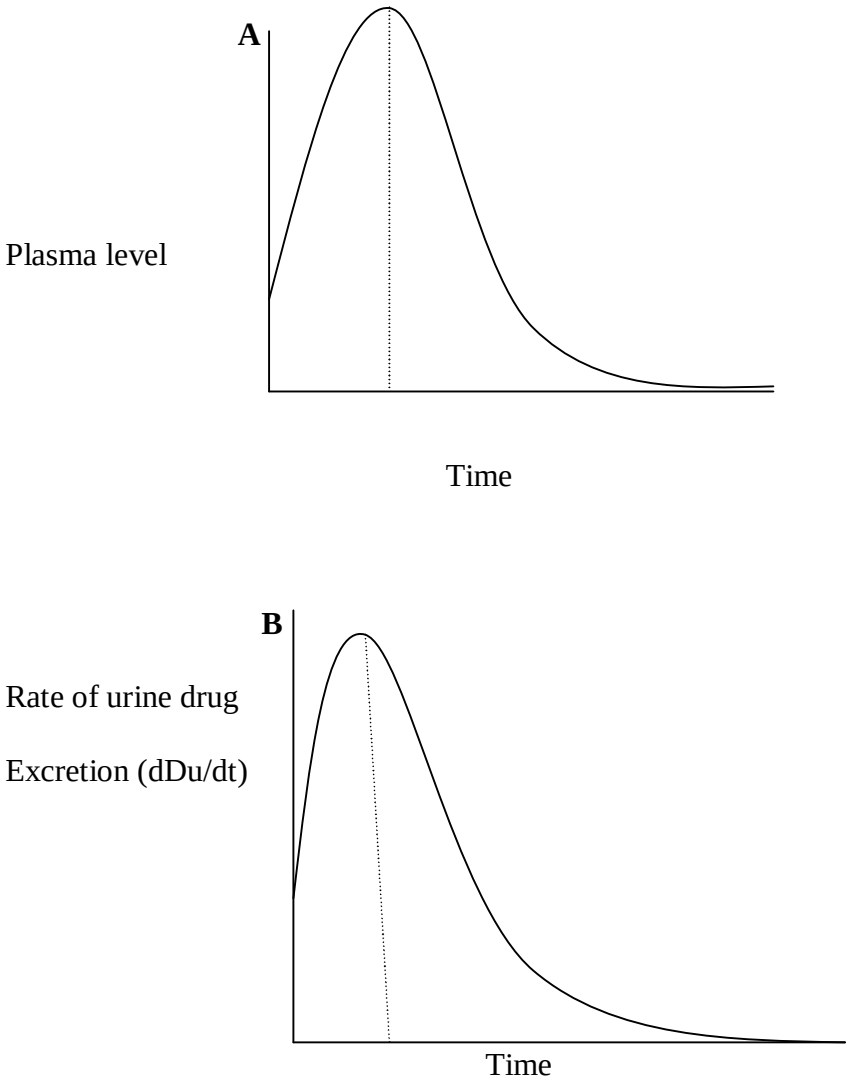


Figure 1.5 **A**. Plasma level versus time curve. **B**. Rate of urine drug excretion versus time

1.4.4 Drug Absorption from the Gastrointestinal Tract

A drug is absorbed by passive diffusion from all parts of the alimentary canal, including sublingual, buccal, gastrointestinal tract, and rectum. Most drugs are absorbed optimally from the upper portion of the small intestine, or duodenum region. Factors which influence the absorption of drugs from the gastrointestinal (GI) tract include: gastrointestinal motility, gastric emptying

time and perfusions of the GI tract disease condition, presence of other drugs, small intestine resident time, hepatic metabolism and formulation (Leon and Andrew, 1999).

1.4.6 Importance of Pharmacokinetics Study

The success of drug therapy is highly dependent on the dosage regimen design. A properly designed dosage regimen tries to achieve an optimum concentration of the drug at the receptor site to produce an optimal therapeutic response with minimum adverse effects (Evans *et al*. 1992). Individual variation in pharmacokinetics and pharmacodynamics makes the design of dosage regimens difficult. Therefore, pharmacokinetics is not only important in dosage regimen design but also in proper clinical evaluation and monitoring of drugs.

1.5 Objectives of the Study

The present study has the following specific objectives;

i To determine the ascorbic acid levels in urine using an adopted method (Roe and Kuether, 1943).

ii To compare the urinary data obtained from different individuals.

iii To ascertain the effect of coadministration of chlorpheniramine on the urinary concentrations of ascorbic acid.

iv To determine the pharmacokinetic variables of ascorbic acid following its excretion in urine.

1.6 Research Questions

Before concluding this research the following questions will possibly have answers:

i Is chlorpheniramine administration capable of altering the pharmacokinetics of ascorbic acid after oral ingestion of both drugs?

ii Is chlorpheniramine administration not capable of altering the pharmacokinetics of ascorbic acid after ingestion of both drugs?

CHAPTER 2 LITERATURE REVIEW

2.1 General

2.1.1 Name of Drugs

Vitamin C; ascorbate, L – ascorbic acid; 3 – keto –L – Gulofurolactone; 2,3 – dehydro – L – Threo – Hexono -1,4 – Lactone (FAO/WHO, 2004).

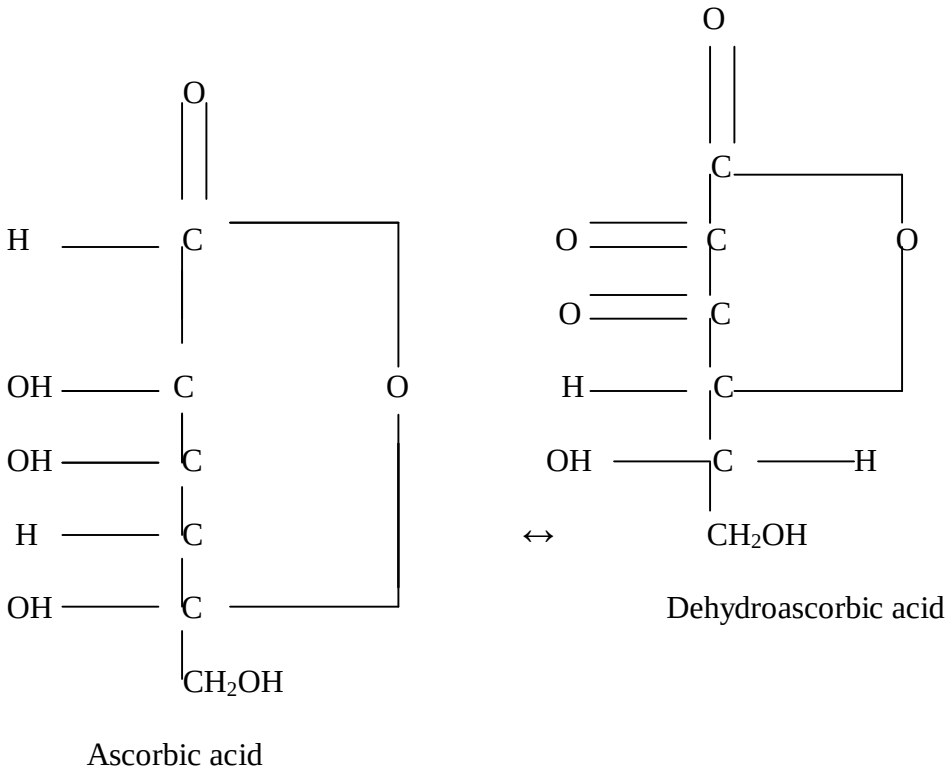
Chlorpheniramine Maleate; 1 – (N,N – Dimethylamino) – 3 – (Alpha – Pyridyl) Propane Maleate; 1 – P – Chloro – Alpha – (2 Pyridyl) – 3 – Dimethylaminopropane Maleate; Allerclor; Allergin;Chlor – trimeton; Piriton;Teldrin. (CAMEO Chemicals, 2007)

2.1.2 History and Chemistry

During the time of crusades in North Europe, Scurvy, the deficiency disease caused by lack of Vitamin C was prevalent. These populations subsisted on fresh fruits and vegetables. However, with the introduction of potato (a source of Vitamin C) in Europe in the seventeenth century, the incidence of scurvy was reduced (Cradon, *et al*., 1940). In 1953, a decoction was made from spruce leaves, and several subsequent ship captains prevented or cured scurvy by administration of lemon juice. In 1747, it was discovered that after a clinical trial on cases of frank scurvy, those who received citrus fruits recovered rapidly. Lemon juice was later introduced into the British Navy in 1800, which resulted in the reduction of scurvy (Marcus and Coulston, 2001). However, it was found that guinea pigs develop scurvy on a diet of oats and bran that is not supplemented with fresh vegetables. It was subsequently shown that most mammals synthesize ascorbic acid; human beings, non-human primates, the guinea pigs and Indian fruit bats are exceptions. The demonstration of the scurvy in the guinea pig allowed testing of fractions from citrus fruits for anti-ascorbic potency. A Hungarian biochemist in 1928, isolated a reducing agent in pure form

from cabbage and from adrenal glands (Csaba and Peter, 2006). Later this compound was identified as the active antiscorbutic factor in lemon juice. The chemical structure of this substance was then soon established in several laboratories, and the trivial chemical name was assigned to designate its function in preventing scurvy (Marcus and Coulston, 2001).

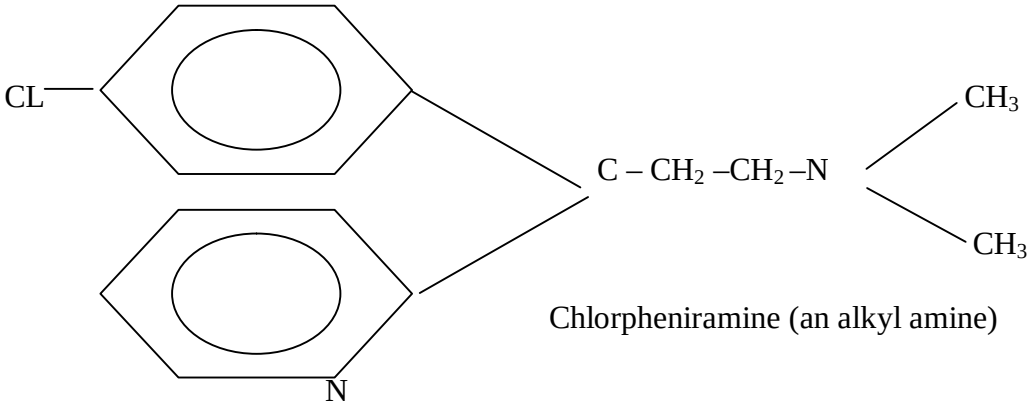
Ascorbic acid, a six-carbon ketolactone, is related structurally to glucose and other hexoses. It is reversibly oxidized in the body to dehydroascorbic acid, which also has full Vitamin C activity. The structural formulas of ascorbic acid are as follows:



Ascorbic acid has an optically active carbon atom, and antiscorbutic activity resides almost totally in the L-isomer.

Bovet and Staub in 1937 first detected Histamine-blocking activity in one of a series of amines with a phenolic ether function. The substance, 2- isopropyl-5-methylphenoxy diethyl-amine, was found to protect guinea pigs against several lethal doses of histamine, antagonized histamine-induced spasm of various smooth muscles, and lessened the symptoms of anaphylactic shock. But this drug was too toxic for clinical use. By 1944, Bo vet and his colleagues had described pyrilamine maleate, which is still one of the most specific and effective histamine antagonists of this category. In the 1980's non-sedating H_1 histamine-receptor antagonists were developed for treatment of allergic disease (Ganellin and Person, 1982).

Chlorpheniramine – a H_1 –receptor antagonist have the following chemical structure;



Chlorpheniramine (an alkyl amine)

2.1.2 Physical Properties

Ascorbic acid is a white crystalline powder and is about 33% soluble in water. It has a melting point of 192^0 C and decomposes at boiling point. The vitamin has a pH of 3 (5% solution). It is stable under ordinary condition and has an optical rotation between +20.5 to +21.5. Ascorbic acid

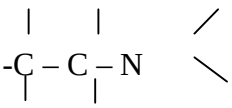
darkens gradually on exposure to air and light. It is sparingly soluble in alcohol, insoluble in chloroform, ether, and benzene (RxList, 2007).

Chlorpheniramine is an odorless white crystalline solid or white powder freely soluble in water, soluble in alcohol and slightly soluble in ether. It has a melting point of 132^0 C - 135^0 C (CAMEO Chemicals, 2007).

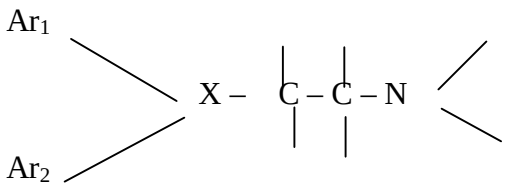
2.1.3 Biochemistry

Ascorbic acid (C_6, H_{12}, O_6) is a ketolactone with a molecular weight of 176.13g/mL. A basic identified biochemical role for ascorbic acid is to accelerate hydroxylation reaction in a number of biosynthetic pathways. In many of these reactions, ascorbate directly or indirectly provides electrons to enzymes that require prosthetic metal ions in a reduced form to achieve full enzymatic activity. The best-known biochemical role of ascorbate is that of co-factor for propyl and hydrolase's enzymes in the biosynthesis of collagen (Levine, 1986). The molecular structures of ascorbic acid are similar to that of glucose. Its structure is similar to glucose because of several hydroxyl groups (0H) that are next to each other.

Chlorpheniramine, (C_{16} H_{19} CLN_2) and all available H_1 receptor antagonists are reversible, competitive inhibitors of the interaction of histamine with H_1 receptors. Chlorpheniramine, like histamine contain a substituted ethylamine moiety,



But it differs with histamine in the sense that it has tertiary group linked by a two – or three-atom chain to two aromatic constituents and conform to the general formula.



Where Ar is aryl and a nitrogen or carbon atom or a C – 0 – either linked to the beta-amino ethyl chain.

2.1.4 Biological Functions

Ascorbate present in most biological settings (pKa = 4.2) is an essential vitamin for humans (Food and Nutrition Board, 1989). Scurvy, the deficiency disease arising from the lack of ascorbate can reach a life threatening level and even death (Guthrie, 1989). Most mammals synthesize ascorbate from glucose. However, humans and other primates' lack the enzyme (L – gulonolactone oxidase) required for its synthesis (Shils, 1994). It was Irwin stone who in 1965, proposed that a negative mutation might have occurred in these species so as to lose the ability to produce Vitamin C. In the cold-blooded amphibians and reptiles, the amounts of ascorbic acid that were produced in their small kidneys sufficed for their need. However, as soon as temperature regulatory means were evolved, producing the highly active, warm – blooded mammals; the biochemical crowded kidneys could no longer supply ascorbic acid in ample quantities (Levine,1986). Ascorbate is considered the most important antioxidant in extracellular fluid (Sies *et al*., 1992). Ascorbate is a water-soluble compound distributed throughout the body, with high concentration found in a number of tissues including the eye lens, white blood cells, adrenals, and pituitary glands (Levine, 1986). Normal plasma concentration of ascorbic acid is about 0.6 to 2.0mg/dL. Ascorbic acid is required in the synthesis of carnitine from lysine (Lebovitz and Mueller, 1993), neurotransmitter synthesis (Levine, 1986), cytochrome P –450

activities, cholesterol metabolism and detoxification of exogenous compounds (Shils, 1994; Komarova, *et al*., 2000), and as an antioxidant (Sies, *et al.*, 1992). In addition when given in large doses (mainly intravenous), ascorbate may function as an ergogenic agent. Ascorbic acid was postulated, 71 years ago, by Szent – Gyorgyi (Szent, 1960) to participate in electron transport reaction.

2.2 Pharmacokinetics and Metabolism

Ascorbic acid is readily absorbed from the intestine. It is accumulated in cells in part by sodium – dependent transporter with saturable kinetics. The transporter achieves Vmax at ≈70, μM (Washko, *et al.*, 1992), the same plasma concentration achieved by ingestion of 200mg daily. The absorption of dietary ascorbate is nearly complete (Kallner, *et al.*, 1977).

Ascorbic acid, when given in a single oral dose is observed to produce a decrease in absorption from 75% at 1 g to 20% at 5 g. The vitamin is present in the plasma and is ubiquitously distributed in the cells of the body. Ingestion of 100 mg vitamin C resulted in saturation of neutrophils, monocytes, and lymphocytes (Levine, *et al.*, 1996). Concentrations of ascorbic acid in leukocytes are sometimes taken to represent those in tissue and are less susceptible to depletion than is the plasma. In healthy adults concentrations in white blood cells reached about 27μg of ascorbic acid per 10^8 cells.

Concentration in plasma also varies with intake of the vitamin. Ascorbic acid concentration in plasma <0.15 mg/dL (8.5μM) resulted in frank scurvy (Marcus and Coulston, 2001). At this low plasma level, the body store of the vitamin approximates 300 mg. When the intake of ascorbic acid is raised, the concentration in plasma also increases at first linearly. The concentration deviates from linearity at about 200 mg/day and achieves saturation at 1000 mg/day. However, in

an earlier work (Levine, *et al.,* 1996), it was reported that linear kinetics for vitamin C occurs at doses >200 mg and that nonlinear kinetics occur at lower doses. When 60 mg (current adult RDA) of ascorbic acid is ingested, plasma concentration achieved is 0.8 mg/dL (45μM) and the body store is about 1500 mg. Higher doses beyond 200 mg/day tend to increase the body store to 2500 mg and concentration in plasma at 2 mg/dL (110μM). However, Levine (1990) suggested that based on these data and institute of Medicine criteria, the current RDA (Recommended Dietary Allowance) of 60 mg daily should be increased to 200 mg daily, which can be obtained from fruit and vegetables. Safe doses of vitamin C are less than 1000 mg daily, and vitamin C daily doses above 400 mg have no evident value. Bioavailability for vitamin C, according to Levine (1996) was 100% after a single oral dose of 200mg but decrease to ≈33% after a single oral dose of 1250 mg; < 50% of the 1250 mg dose was absorbed. The renal threshold for ascorbic acid is about 1.5 mg/dL of plasma (85μM), and increasing amount of ingested ascorbic acid are excreted when the daily intake exceeds 100 mg. Oxalate and urate excretion were elevated at 1000 mg of vitamin C compared to lower doses. Report has shown that there is an increased post – operative metabolic clearance of ascorbic acid in surgical patients (Rumelin, *et al*., 2005).

Ascorbic acid is oxidized to CO_2 in rats and guinea pigs, but considerably less conversion can be detected in human beings, the major route of metabolism of vitamin C is its conversion to oxalate and eventual excretion in the urine. Dehydroascorbic acid is presumably an intermediate; ascorbic acid – 2 – sulfate also has been identified as a metabolite of vitamin C in human urine.

Guinea pigs, human being and other primates as well as some bats are unable to synthesize ascorbic acid and require dietary vitamin C in the prevention of scurvy. Animals that do not require dietary vitamin C synthesize ascorbic acid from glucose through the intermediate formation of D- glucuronic acid, L- gulonic acid and L- gulonolactone. On-farm supplementation

of vitamin C was generally not effective in improving pork quality, which may be related to timing relative to slaughter (Pion *et al*., 2004). Ascorbic acid is usually administered orally. Parenteral solutions may be given in condition that prevents adequate absorption from the gastrointestinal tract.

The absorption of H_1-antagonist such as Chlorpheniramine from the gastrointestinal tract is adequate. Peak plasma concentration is achieved following oral administration, in 2 to 3 hours and effects usually last 4 to 6 hours. However, some of the drugs are much longer acting. On oral administration of diphenhydramine it reaches a maximal concentration in the blood in about 2 hours, and then falls exponentially with a plasma elimination half-life of about 4 to 8 hours. Distribution of the drug in the body is wide, including the CNS; little if any, is excreted unchanged in the urine. Most appears there as metabolites. Other first – generation H_1 –antagonist such as chlorpheniramine appears to be eliminated in much same way (Paton and Webster, 1985).

The H_1 -antagonist is eliminated more rapidly by children than adults and more slowly in those with severe liver disease. Among the many drugs that induce hepatic microsomal enzymes, are the H_1-receptor antagonists. They may facilitate their own metabolism (Paton and Webster, 1985; Simons and Simons 1994). Chlorpheniramine has a half-life of about 20 hours in adults and is eliminated by metabolism to immodesmethyl and didesmesthyl compounds. The half-life increases in renal dysfunction and exhibit wide individual variations in pharmacokinetics. Age, dialysis, urinary pH and blood flow influences elimination kinetics of chlorpheniramine.

2.3 Pharmacological properties

Vitamin C posses few pharmacological actions. Administration of the compound in amounts greatly in excess of the physiological requirements causes few demonstrable effects in the

scorbutic individual whose symptoms are alleviated rapidly (Marcus and Coulston, 2001). However, it has been reported by Gershoff (1993), that ascorbic acid acts as an antihistamine, so it can be helpful for allergic-type problems and helps to lower cholesterol level. Also for its antioxidant effect and immune stimulating function, vitamin C has been found to protect against the harmful effects of pollution, prevent cancer and protect against infection (West, *et al*., 1994). However, sporadic reports of the efficacy of vitamin C in curing cancer or the common cold have not been substantiated (Gershoff, 1993). Again, L – ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin (Regina, *et al*., 2001). In this study, ascorbate stimulates endothelial nitric oxide synthesis in a time – and concentration – dependent fashion without affecting nitric oxide synthase expression or L – arginin uptake. The evidences of a chemical stabilization of tetrahydrobiopterin by ascorbic acid are an increase in the half – life of tetrahydrobiopterin in aqueous solution.

Feng, *et al.* (2002), reported that gluthathione monoethyl ester (GSHme), but not ascorbic acid exerted protective effects against ischemia – reperfusion injury; and that the protective effects of GSHme were further enhanced by co- administration with ascorbic acid, suggesting a synergistic effect between GSHme and ascorbic acid. It is important to note that gluthathione and ascorbic acid are the two most important hydrophilic antioxidants in the body. Chronic oral vitamin C therapy lowers blood pressure in hypertensive patients. It also improves endothelial vasomotor functions. However, acute or chronic ascorbic acid treatment had no effect on brachial artery endothelium dependent flow-mediated dilation (Stephen, *et al.,* 2001). In a study involving twenty-type II diabetic patients chronic vitamin C administration improved whole body glucose disposal and nonoxidative glucose metabolism (Diplock, 1995). Pharmacological concentrations of vitamin C are required for the beneficial effects on endothelial function in hypertension (Debra, *et al.*, 2000). Chandrashekhar, *et al*. (2006), reported the antagonistic activity of ascorbic

acid on doperminergic modulation in apomorphine-induced stereotypic behavior in mice. The bioavailability and diuretic effect of azosemide was enhanced in ascorbic acid treated rats (Choi, *et al.*, 2006). Ascorbic acid antagonizes nicotine – induced place preference and behavioral sensitization in mice (Hedayat, *et al.*, 2007). The nutrient mixture of ascorbic acid, tea phenolics, and selected amino acids has potential in blocking the development of atherosclerotic lesions by blocking responces of vascular smooth muscles to pathologic stimuli (Ivanov, *et al.*, 2007).

Chlorpheniramine and other H_1 antagonists have similar pharmacological actions and therapeutic application and can be conveniently discussed together. Because of the knowledge of the histamine that involves interaction with H_1 receptors the effects of the H_1 -antagonist are largely predictable. In smooth muscles, H_1 antagonist inhibits most responses of smooth muscles to histamine. In guinea pigs, for example, death by asphyxia follows quite small dose of histamine, yet the animal may survive a hundred lethal doses of histamine if given an H_1 antagonist (Brown and Roberts, 2001). In the same species, striking protection also is afforded against antagonistic bronchospasm. This is not so in human beings, where allergic brochoconstriction appears to be caused by a variety of mediators such as leukotrienes and platelet activating factor. The H_1 antagonists, within the vascular tree, inhibit both the vasoconstrictor effects of histamine and to a degree, the more rapid vasodilator effects that are mediated by H_1 receptors on endothelial cells. Residual vasodilatation reflects the involvement of H_2 receptors on smooth muscles and can be suppressed only by the concurrent administration of an H_2 antagonist (Brown and Roberts, 2001). H_1 antagonists markedly block the action of histamine that results in increased capillary permeability and formation of edema and wheal. Again, they suppress both the component of the triple response and the itching caused by intradermal injection of histamine.

An H_1 antagonist suppresses histamine-evoked salivary, lachrymal, and other exocrine secretions with variable responses. However, they do not inhibit gastric secretions. The atropine-like properties of many of these agents may however contribute to loosen secretion in chorlinergically-innervated glands and reduce on going secretion in, for example, the respiratory tree.

During hypersensitivity reactions, histamine is one of many potential autacoids released, and its relative contribution to the ensuring symptoms varies widely with species and tissues. The protection afforded by histamine antagonists thus also varies accordingly. Some phenomena in human beings such as edema formation and itch are effectively suppressed. Others such as hypotension are less so. This may be explained by the existence of other mast-cell mediators, specifically prostaglandin D_2, also contributing to the vasodilatation (Roberts, *et al.*, 1980). Brochoconstriction is reduced little, if at all (Dahlen, *et al.,* 1983). Many at times, the first generation H_1 antagonists both stimulate and depress the central nervous system (CNS). In patients given conventional doses stimulation is occasionally encountered. The patients become restless, nervous and unable to sleep. Central excitation also is a striking feature of poisoning, which commonly results in convulsion particularly in infants. On the other hand, central depression is the usual accompaniment of therapeutic doses of the older H_1 antagonists. Diminished alertness, slowed reaction times, and somnolence are common manifestations. It is important to note that some of the H_1 antagonists counter motion sickness. Perhaps, due to their antichorlinergic properties. Quite a number of first-generation H_1 antagonists tend to inhibit responses to acetylcholine that are mediated by muscarinic receptors (Raymond, *et al*., 2004).

2.3.1 Mechanisms Of Action

Ascorbate is considered one of the strongest reductant and radical scavenger. Ascorbate reduces unstable oxygen, nitrogen and sulphur centered radicals; in addition it acts as primary defense against aqueous radicals in blood (Niki, 1991). In studies with human plasma ascorbate protected plasma lipids against detectable peroxidative damage induced by aqueous peroxyl radicals (Frei, *et al.,* 1989). Thus, efficiently trapping peroxyl radicals in the aqueous phase before they reach the lipid rich membranes and initiate lipid peroxidative damage. Also ascorbic acid can protect biomembranes against primary peroxidation due to its synergistic antioxidant function with vitamin E.

Ascorbic acid may enhance or reinstate the activity of tocopherol (Vitamin E), the principal lipid soluble antioxidant (Niki, 1991). Even though the occurrence of this action has been questioned in an *in vivo* setting (Frei, *et al.,* 1989); it seems reasonable when both vitamins are present in environments suffering enhanced oxidative stress.

Furthermore, ascorbic acid reacts with the tocopheroxyl (Chromanoxyl) radical that arises in cell membranes as a result of vitamin E antioxidant activity; and regenerate at the same time that it transfers the oxidative challenge to the aqueous phase. (Vandenberg, *et al.,* 1990). At this point, the less reactive ascorbate radical can be enzymatically reduced back to acid by an NADH – dependent system (Chan, 1993). The cellular uptake of ascorbic acid is regulated by both glucose and insulin (Wilson, 2005).

Chlorpheniramine and other H_1 antagonists act by competing with histamine for binding to H_I receptors on basolateral membranes of parietal cells. H_I receptors are coupled to phospholipase C, and their activation leads to formation of inositol – 1-4-5- trisphosphate (IP_3) and diacylglycerols

from phospholipids in the cell membranes; IP_3 cause a rapid release of Ca^{2+} from the endoplasmic reticulum. Diacylglycerols (and Ca^{2+}) activate protein kinase C, while Ca^{2+} /activates Ca+/ calmodulin dependent protein kinase and phospholipase A_2 in the target cell to generate the characteristics response. In a species dependent manner, adenosine receptors may interact with H_I receptors.

In the CNS of human beings activation of adenosine, receptors inhibit second messenger generation via H_I receptors. A possible mechanism for this is interaction between the G proteins to which the A_I and H_I receptors are coupled functionally (Dickenson and Hill, 1994).

2.4 Toxicity

Vitamin C is remarkably nontoxic at high levels (10 to 100 times the RDA when taken (per oral). However minor toxic effects include, oxaluria, acidosis, renal stone, glycosuria, renal tubular disease, gastrointestinal disturbances, sensitivity reactions, conditioned need, prothrombin and cholesterol disturbances, vitamin B_{12} destruction, fatigue and sterility (Barness, 1974).

The commonest and most prevalent of all these is gastrointestinal disturbances following the ingestion of large quantities of oral ascorbic acid since nausea, abdominal cramp and diarrheas are frequently mentioned as negative side effects. These effects are alleviated or eliminated by taking ascorbic acid as a buffered salt or immediately after meals. Stress or severity of an ailment determines the amount of oral ascorbic acid that can be tolerated by a patient without producing diarrhea (Cathcart, 1981). Bowel tolerance doses of ascorbic acid ameliorate the acute symptoms of many diseases. Smaller doses often have little effects on acute symptoms but assist the body in handling the stress of disease and may reduce the morbidity of the disease (Carthcart, 1981). Most toxic effects reported for taking large amounts of vitamin C in reality are insignificant, rare and of

minor consequences. Nevertheless, a word of caution should be given for patients with glucose-6-phosphate deficiency. These patients when given high dose of ascorbic acid may be at a risk of developing hemolysis (Rees, *et al.*, 1993). There is need for screening patient for vitamin C deficiency before applying ascorbic acid therapy. Caution must be taken during ascorbic acid therapy to avoid intake of inorganic selenium (Na Selenite). A possibility exist that ascorbic acid may reduce selenite and render it unavailable for tissue uptake (Gonzalez, 1990). In relation to kidney stones, these are formed mostly in alkaline urine calcium oxalate stones. High doses of ascorbic acid make the urine acidic, thus preventing stone formation. There are various studies that have addressed this issue (Heinz-Schmidt, *et al.,* 1981; Sutton, *et al.,* 1983; Wandzilak, *et al.*, 1997) and found no evidence of ascorbate increasing the risk of kidney stone formation. For intravenous ascorbic acid, no ill effects have been reported with dose as high as 150-200g over a 24hours period (Leung *et al.*, 1993; Punnonen, *et al.,* 1994; Jaruga and Olinste, 1994; Sun, *et al.*, 1993; Bozzi, *et al.*, 1979; Klenner, 1971; Cathcart, 1985). Reports indicated that ascorbic acid is more efficient when administered intravenously than when higher circulating levels are achieved for longer period of time. Moreover, intravenous ascorbic acid causes rapid tumor hemorrhage and necroses (Campbell and Jack, 1979). Ascorbic acid is one of the safest and most valuable substances available to the physician.

Acute poisoning with H_I antagonist cause central excitatory effects, which constitute the greatest danger. The syndrome includes hallucination, excitement, ataxia, in- coordination, athetosis, and convulsions. Fixed, dilated pupils with a flushed face, together with sinus tachycardia urinary retention, dry mouth, and fever, lend the syndrome a remarkable similarity to that of atropine poisoning. Terminally there is deepening coma with cardio – respiratory collapse and death, usually within 2 to 18 hours. Treatment is along general symptomatic and supportive lines.

2.5 Drug Interactions

Ascorbic acid (Vitamin C) is used primarily in the treatment of scurvy and is a powerful reducing agent and also function in a number of biochemical reactions mostly involving oxidation.

Ascorbic acid raises serum cortisol concentrations depressed by etomidate infusions. It restores the (ACTH/ cortisol ratio to pre-operative values. The free imidazol radical in etomidate binds to cytochrome P-450 and prevents the reconversion of dehydroascorbic acid necessary in steroid synthesis. Ascorbic acid provides the substrate for cortisol synthesis (Boidin, 1985).

Ascorbic acid increases the half-life of paracetamol (acetaminophen). It is metabolized in part to the sulphate and competes for the available substrate. Concomitant administration of ascorbic acid result in a significant decrease in the fraction of the dose of paracetamol (acetaminophen) excreted as the glucoronide (Houston and Levy, 1976).

Vitamin C like ethinylestradiol is metabolized to sulphate conjugates. There is the possibility of transient overload of sulphation in the intestinal wall and/ or the liver if vitamin C is co administered with a combined oral contraceptive. Ascorbic acid may be used in high dosage. Two small clinical studies in six and five subjects seemed to confirm this interaction with the bioavailability of ethinyl oestradiol being increased by about 50 percent when 1 g of vitamin C was given half hour before pill administration (Back and Orme, 1994).

A later study on 37 women suggested that this interaction is unlikely to be of clinical importance. Aspirin blocks the uptake of ascorbic acid into leukocytes *in vitro* and *in vivo* (Loh and Wilson, 1975). Administration of high aspirin doses to healthy subjects significantly reduced platelet ascorbic acid levels (Sahud and Cohen, 1971). As a result increased excretion of ascorbic acid has

been observed after aspirin administration in children (Daniel and Everson, 1936) and in healthy young adults (Loh, 1973). Ascorbic acid has been reported to alter blood coagulation of fat (Spittle, 1973). Ascorbic acid induced acidic urine, increases the possibility of amino salicylic acid and sulfonamide crystalluria (Meyers, 1970) and increases the tubular reabsorption of tricyclic antidepressants (Sjoquist, 1969). In addition large doses of ascorbic acid were reported to destroy substantial amounts of dietary cynocobalamin (Herbert and Jacob, 1974) and increase the absorption of elemental iron (Forth and Rummel, 1973). Conney and Burns (1960) discovered that compounds, which cause enzyme induction, also increase markedly the synthesis of ascorbic acid in the rat. This implies that wide variety of chemicals of markedly different structures and pharmacological activity can stimulate the activity of drug metabolizing enzymes in liver microsomes. Examples of these stimulators include; drugs (Phenobarbital, phenylbutazone 3,4-benzpyrene, e. t. c.) and insecticides (DDT and chloretone). It is important to note that human lack the enzyme system required for the conversion of L- gulonolactone to L- ascorbic acid. Hence, increased excretion of L- ascorbic acid does not occur following administration of the vitamin in man. However, increased excretion of D – glucaric acid (a metabolite of glucuronic acid) is observed. Interestingly enough, D- glucuronic acid excretion has been used as a measure of the ability of drug to cause enzyme induction in man. Decreased activity of drug – metabolizing enzyme occurs in guinea pigs fed ascorbic acid deficient diet. Ascorbic acid has been reported recently to prevent the formation of nitrosamines, which come from the interaction of nitrites with secondary and tertiary amines. For example, Mirvish (1972), have reported that ascorbate in the offspring of rats administered ethyl urea and sodium nitrite on days 10 and 11 of pregnancy inhibited formation of nitroso-ethylurea. It was reported that L -ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin (Regina, *et al.,* 2001). Thus, pretreatment of human umbilical vein endothelial cells with ascorbate (1μM – 1mM/24h) led to an up to 3- fold increase of intracellular tetrahydrobiopterin

levels that was concentration dependent and saturable at 100μM. Nitric oxide synthesis (NOS) expression or L- arginine uptake was not affected in myocardial reperfusion injury. Ascorbic acid enhanced gluthathione cardio- protection (Feng, *et al.*, 2002). Gluthathione and ascorbic acid are the two most important hydrophilic antioxidants in myocardial ischemia –reperfusion injury. An effort to study the effects of administration on competitive drug biotransformation interaction involving salicylamide ascorbic acid interaction in rats revealed that with intravenous salicylamide, intravenous ascorbic acid caused a modest decrease of salicylamide sulfate formation and thereby of salicylamide body clearance. Orally administered vitamin had a somewhat more pronounced effect. The vitamin had no effect on the apparent volume of distribution of salicylamide and the renal clearance of its conjugates. With orally administered salicylamide, oral ascorbic acid causes a very pronounced decrease of salicylamide body clearance, whereas the intravenous vitamin has little or no effect (Houston and Levy, 1976). These observations are consistent with the assumption that the magnitude of competitive drug biotransformation interactions, being a function of concentration, is most pronounced when the interactants are co-administered orally, due to their high concentration in the intestinal wall and during the first pass. Vitamin C taken with aspirin a nonsteroidal anti-inflammatory drug (NSAID) may protect the stomach and intestines against injury from these medications. Also it may affect the levels of furosemide, a diuretic, and propranolol, a beta-blocker; therefore, concurrent administration with these medications should be avoided, cyclosporine, used for the treatment of cancer, may reduce blood levels of vitamin C. Coadministration of vitamin C with nitroglycerine and nitrate (isosorbide dinitrate and isosorbide mononitrate), used to treat heart disease, reduced the effect of nitrate tolerance, an effect by which the body become accustomed to the medicine and then requires a brief break from it for it to work properly (Eberhard, 1998).

Ascorbic acid acts as an antihistamine so it can be helpful for allergic- type problems and helps to lower cholesterol (Gershoff, 1993). For its antioxidant effect and immune stimulation function, ascorbic acid has been found to protect against the harmful effects of pollution, prevent cancer, and protects against infection (Diplock, 1995). In twenty patients, vitamin C administration improved whole body glucose disposal and non-oxidative glucose metabolism. Percent increase in vitamin C in plasma correlated with decrease in plasma low-density lipoprotein (LDL), cholesterol and insulin level. Chronic vitamin C administration has beneficial effects upon glucose and lipid metabolism in aged non-insulin dependent (type II) diabetic patients (Diplock, 1995).

In animals chlorcyclizine, chlorpheniramine, cyclizine, diphehydramine and hydroxyzine induce hepatic microsomal enzyme that metabolize certain drugs. Increased rates of metabolism of carisoprodol, hexobarbital and zoxazolamine occurred in liver microsomal preparations of rats pretreated with antihistamines. The effect on anticoagulant drug metabolism was not tested (Conney and Burns 1960; Humminghake and Azarnoff, 1968). *In vitro* test showed that antihistamine can impair platelet function but this effect is probably not clinically significant (Deykin, 1971). Chlorpheniramine increases antimuscarinic side effects and enhance sedative effects of anxiolitic and hypnotic drugs (BNF, 1997). Chlorpheniramine could theoretically prevent the β- adrenergic blocking effect of propanolol and enhance its quinidine like effects (Calson and Linquist, 1969; Johnson and Khan, 1966).

2.6 Assay Methods

In this study, the method of Roe and Kuether method (1943), for the determination of ascorbic acid in whole blood and urine using the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid with absorbance measured at 520 nm was used. This method was adopted because it requires

the utilization of Colorimeter which though less sensitive than High Performance Liquid Chromatography (HPLC), is readily available in most Nigerian Laboratories.

Earlier, Harry and Kathryn (1939) used a blue dye, 2,6-dinitrophenol-indophenol (DCPIP) an oxidizing agent against ascorbic acid solution until a faint pink color persists for 15 seconds. Clayden, *et al* (2001), reported other titration methods such as the use of iodine and a starch indicator. Iodine reacts with ascorbic acid and when all the ascorbic acid has reacted, the iodine is then in excess, forming a blue/black complex with the starch indicator. This indicates the end point of the titration. Alternatively, ascorbic acid is reacted with iodine in excess, then back titration against sodium hydroxide (using starch as an indicator). A second titration method reported by Clayden, *et al*. (2001), was the use of a common oxidizing agent, N-bromosuccinamide, (NBS). In this titration, the NBS oxidizes the ascorbic acid (in the presence of potassium iodide and starch). When the NBS is in excess i.e. the reaction is complete, the NBS liberates the iodine from the potassium iodide which then form the blue/black complex with starch, indicating the end point of the titration.

Winnie, *et al* (1997), introduced a new procedure that permits the automated determination of plasma ascorbic acid (AA) concentration with a Roche Farer centrifugal analyzer. After the deproteinization of plasma with metaphosphoric acid, AA is oxidized to dehydroascorbic acid by AA oxidase. The product is coupled to 0-phenylenediamine to produce a chromophore for which the absorbance is measured at 340 nm. Linearity extends to beyond the reference range of 24.1-84.6μmol/L, and severe hemolysis is the only interference identified. Again, Aslam, *et al (*2000), reported a spectrophotometer determination of ascorbic acid in pharmaceuticals by flow injection analysis using brown mono 1,10-phenanthroline-iron (iii) complex as an oxidant. Thus, the

method was based on the on-line reduction of mono (1,10-phenanthroline)-iron (iii) complex to tris (1,10-phenanthroline)-iron (ii) complex by reducing action of dienol group of ascorbic acid and the absorbance of the resultant tris chelate was monitored at 510 nm. Saleh, *et al.* (1994), described an on-line preparation of tris (1,10-phenanthroline)-iron (iii) (ferroin) complex in two-channel manifold followed by injection of sample ascorbic acid and subsequent measurement of absorbance of resultant product tris (1,10-phenanthroline)-iron (ii) (ferroin) complex.

Determination of vitamin C by analytical voltammetry was also reported. Analytical voltammetry is an electrochemical method in which the changes of electrolysis current are measured when a gradually increasing voltage is applied to the cell. Conditions are adjusted so that the analyte is oxidized or reduced selectively at one of the electrodes in the cell. During the electrolysis, the ascorbic acid donates electrons to the indicator electrode and the Volta metric experiment exhibits an oxidation (anodic) current step. The quantity of electricity involved in the oxidation peak is directly proportional to the concentration of ascorbic acid. Therefore an unknown concentration of ascorbic acid can be determined.

CHAPTER 3 MATERIALS AND METHODS

3.1 Drugs

Ascorbitone® brand of vitamin C manufactured by Achy Pharmaceutical Nigeria Limited was used. Chlorpheniramine maleate manufactured by Vitabiotic Nigeria Limited was also used in the study.

Ascorbitone® brand of vitamin C (chewable)

100 mg of ascorbic acid USP

Manufacturer: Achy Pharmaceutical Nig. Ltd.

Batch number: VCC 5053

Manufactured date: 11/2005

Expiry date: 10/2007

NAFDAC Registration number: 04-5070

Storage: Ascorbitone® was stored in a plastic covered container and placed in a cool dry place.

Chlorpheniramine maleate, each tablet contain 4 mg of

Chlorpheniramine CMP

Manufacturer: Vitabiotic Nigeria Limited

Batch number: T5905R

Manufactured date: 09/2005

Expiry date: 08/2008

NAFDAC Registration Number: 04-0071

Storage: chlorpheniramine maleate was stored in a plastic covered container protected from light.

3.2 Equipment

Refrigerators

Water bath

Centrifuge machine - Centrifuge 72 Tuttligyen West Germany, Hetlich Universal

Colorimeter- Corning 253, Corning Ltd, Hallstead

Weighing balance -Metler instruments, Switzerland

Thermometer

Pyrex glassware.

3.3 Reagents

The chemicals used for *in vitro* analysis were products of BDH Poole, London, United Kingdom. They are of high quality grade and were used in preparing the following reagents;

Acid-Washed Norit: Prepared by placing 5 g activated charcoal in a 100 mL volumetric flask and adding 5% Trichloroacetic acid (TCA). The mixture was well shaken for five minutes and allowed to settle. The supernatant was decanted and the procedure was repeated until the supernatant had no fine particle in suspension. After the last washing it was made up to 100 mL with 5% TCA. The essence of using activated charcoal was to prevent interference with bilirubin.

2,4-Dinitrophenylhydrazine: Two grams of 2,4-dinitrophenylhydrazine (DNPH) was placed in a 100 mL Volumetric flask, 25 mg thiourea was added and the mixture was shaken thoroughly to dissolve the DNPH and stored in a brown bottle. It was then made up to 100 mL with 9N Sulphuric acid. The DNPH produce a chromophore with ascorbic acid.

9N Sulphuric acid: Prepared by diluting 24.5 mL in 75.5 mL of distilled water. The acid was slowly added with constant agitation and this was used to make 2,4-DNPH reagents.

65% Sulphuric acid: Prepared by diluting 65 mL of concentrated Sulphuric acid in 35 mL of distilled water. The oxidation of ascorbic acid is favorable in a strongly acidic medium.

Thiourea (10%): 10 gram of thiourea was dissolved in 50 mL absolute ethanol and made up to 100 mL with distilled water. Here thiourea prevents the effect of interfering compounds on DNPH giving elevated results.

5% Trichloroacetic acid (TCA): 5 grams of TCA crystals were dissolved in enough water and made up to 100 mL with distilled water. The TCA chelates proteins.

Ascorbic acid Standard (stock solution): 5 milligrams of ascorbic acid powder (BDH Poole, London) dissolved in 100 mL 5% TCA.

Principle: The ascorbic acid in the presence of copper is oxidized to dehydroascorbic acid which in a strongly acidic medium (Sulphuric acid), reacts with 2,4- DNPH to form corresponding hydrazine. The hydrazone when treated with dilute Sulphuric acid (65% in this study), develop an orange- red colour which is measured spectrophotometrically at 520 nm.

3.4 Methodology

3.4.1 *In vitro* Study

The following tests were carried out to ascertain the quality of Ascorbitone® brand of ascorbic acid tablet. A calibration curve was also prepared.

3.4.1.1 Identification Test

Ascorbic Acid: 20 tablets of 100 mg Ascorbitone® were powdered. Into a 250 mL volumetric flask, 100 mg of the powdered ascorbic acid was placed and 100 mL of distilled water was added. The mixture was then filtered. The filtrate was tested with litmus paper and its ability to decolorize 2,6 chlorophenolendophenol solution.

3.4.1.2 Test for uniformity of weight

Twenty tablets of 100 mg Ascorbitone® tablets were weighed using a Metler sensitive weighing balance. The average weight and percent weight of the average was calculated. The test was done in accordance with B.P. 2002 specification of test for uniformity of weight for uncoated tablets.

3.4.1.3 Preparation of standard Curve

Five milligrams of standard ascorbic acid (BDH, Poole, London) was weighed and dissolved in 100 mL of 5% trichloroacetic acid to make a stock solution. Then 0.4, 0.8, 1.2, 1.6, 2.0 mL of the stock solution was placed into five different test tubes. These were diluted serially with 5% TCA i.e. 1.6, 1.2, 0.8, 0.4 and 0 mL to make a total volume of 2mL. By implication, each of the tubes contained 0.01, 0.02, 0.03, 0.04, and 0.05 mg/mL of standard ascorbic acid.

All samples were analyzed by the method of Roe and Kuether (1943). The absorbances were taken at 520 nm against a reagent blank. A graph of absorbance against ascorbic acid concentration was plotted. Although the concentrations of test samples were obtained from the standard curve, they can also be calculated from the following formular;

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard} = \text{Concentration of test } (\mu g/mL)$$

3.5 *IN VIVO* STUDY

3.5.1 Study Design

Six healthy young adult male volunteers with average age of 24.33 years and average weight of 64.83 kg took part in the study. Before taking part in the research, a written consent was obtained from all the six volunteers and the protocol of the study complied with the declaration of Helsinki (1989). No drugs including alcohol and cigarette were allowed two weeks before their participation in the study.

3.5.2 Test Protocol

The volunteers fasted overnight before taking part in the study the following morning. Each volunteer attended the trial room and was allowed a resting period of 5 minutes. Urine was collected in 5 mL sample tubes from all subjects (0 minute). Following this 2x 100 mg of ascorbic acid tablets was administered orally with 200 mL of water. Urine was then sampled at 0.25 hour, 0.5 hour, 1 hour, 1.5 hour, 2 hours, 3, 4, and 6 hours. All the volunteers remained in the room and no food or drink was allowed throughout the sampling period (6 hours).

A washout period of two weeks was allowed for all the six volunteers. Then 200 mg ascorbic and 1 x 4 mg tablets of chlorpheniramine was co-administered to all the subject The drugs were ingested by each volunteer with a glass of water (200 mL). Urine samples were collected from each volunteer as above. The samples collected were covered and stored in the fridge compartment until the time of analysis .The samples were analyzed for ascorbic acid levels using the method of Roe and Kuether, (1943).

3.5.3 Analysis of Urine Samples

In this study, the method of Roe and Kuether, (1943) for the determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid, adopted by Mahdi and Umar (1999) was used. 1 mL of the sample was added to 4 mL of acid-washed Norit. After mixing, the tube was centrifuged at 1,500 rpm for 5 minutes and the clear supernatant decanted and filtered through Whatmann number 42 filter paper. Into a test tube 2 mL of the clear filtrate was placed. Into another test tube, 2 mL of distilled water was placed. This served as the blank. 0.3 mL of 10% thiourea was added to all the test tubes. Then 0.4 mL of 2,4-dinitrophenylhydrazine was added to the test. Then all tubes were placed into a water bath with temperature at 37^{o}C. The tubes remained in the water bath for exactly 3 hours.

After removing the tubes they were placed into a crushed ice bath with constant agitation. Then 0.3 mL of 65% sulphuric acid was added and well shaken. The tubes were removed after 10 minutes and 0.4 ml 2,4-dinitrophenylhydrazine was added to the blank. After 30 minutes of standing the tubes were wiped dry and their absorbance read at 520 nm using a colorimeter. The samples were assayed by reference to standard calibration curve.

3.6 Data Handling and Statistics

Results obtained in the study were recorded carefully. Graphs and tables were drawn**.** The rate of ascorbic acid excretion in urine for each collection period was calculated by dividing the urinary levels obtained at that period by the average times interval for sample collection. The line of best – fit was drawn through the rate of excretion of ascorbic acid against time data (manually). Hence the elimination rate constant was calculated from the slope (β) of this graph. Area under the curve AUC $_{0\text{-}\alpha}$ was obtained by the trapezoidal method from the urinary levels of ascorbic acid

excreted against time curve with extrapolation to infinity. The elimination half live was obtained from the ratio 0.693/β.

The total body clearance CL_T was obtained from the ratio of dose to area under the drug concentration curve assuming bioavailability is equal to one. Apparent volume of distribution V_d is the ratio of total body clearance to elimination rate constant. The bioavailability fraction was obtained from the ratio of the product of CL_T and AUC to dose. Fraction of drug excreted, Fe, was obtained from the ratio of the total amount of drug excreted to the product of the bioavailability fraction F, and dose. Finally, the renal clearance, CL_R in this study was generated from the product of the excretion rate constant Ke, and $V_{d.}$ Student's paired t test was used. P values less than 0.05 were considered to be statistically significant.

3.6.1 Pharmacokinetic analysis

Concentrations of ascorbic acid in urine of control and test subjects were plotted against time on an arithmetic scale. The AUC_{0-6} and $AUC_{6-\infty}$ were calculated by the trapezoidal method and other variables obtained by standard relationships (Leon and Andrew, 1999);

$\{AUC\}_0^{\infty} = \sum \{AUC\}^{tn}_{tn-1} + Cu/\beta$

Where AUC= area under the curve, t= time, Cu= terminal urine concentration, β= elimination rate constant

Other parameters were calculated from the following;

$\beta = 0.693/Slope$

where β = elimination rate constant

$t_{1/2} = 0.693/\beta$

where $t_{1/2}$ = elimination half-life

$CL_T = Dose/AUC_{0-\infty}$

Where Dose = 200 mg, CL_T = total body clearance, $AUC_{0-\infty}$ = Area Under the Curve 0-infinity

$V_d = CL_T / \beta$

Where V_d = Volume of distribution

$f_e = D^{\infty}u / FD_O$

where f_e = fraction of drug excreted unchanged, $D^{\infty}u$ = cumulative amount of urine excreted, F = bioavailability fraction, D_O = Dose administered.

$CL_R = f_e\ CL_T$

where CL_R = renal clearance, f_e = fraction of drug excreted unchanged, CL_T = total body clearance.

CHAPTER 4 RESULTS

4.1 Identification Test

The filtrate obtained from the solution of 100% ascorbic acid was acidic to litmus paper and decolorized 2,6-dichlorophenolindophenol solutions. Hence, Ascorbitone® brand of ascorbic acid passed the identification test of ascorbic acid tablets in compliance with B.P. (2002) specification.

4.2 Test for Uniformity of weight

The results after weighing twenty tablets of 100mg Ascorbitone® brand of ascorbic acid tablet are shown in table 4.1. From the data it can be seen that no two tablets deviated from the average weight i.e. 100.1 mg by 7.5% or $\pm$ 2 mg. Thus, Ascorbitone® brand of ascorbic acid has passed the test for uniformity of weight as specified by B.P. (2000)

4.3 Standard Curve

The absorbance readings of known concentration of ascorbic acid (mg/mL) in urine is indicated in table 4.2. A plot of absorbance against concentration resulted in a straight line with correlation coefficient r = 0.98 as shown in figure 4.1 below.

4.4: *In vivo* Study

The mean age of the volunteers who participated in the study was 24.33 ± 0.71 years while the mean weight was 64.83 ± 1.08 Kg as shown in table 4.3 below.

Table 4.1 Weight and Percent Weight of Mean of 100 mg Ascorbic Acid Tablets

S/No	Weight of tablet (mg)	Percent weight of mean
1	100	100.1
2	99.5	99.6
3	100	100.1
4	100	100.1
5	100.7	100.8
6	100	100.1
7	99.9	100.0
8	100.3	100.4
9	99.8	99.9
10	100	100.1
11	100	100.1
12	100	100.1
13	99.7	99.8
14	100	100.1
15	100	100.1
16	100.8	100.9
17	101	101.1
18	100	100.1
19	100	100.1
20	100.4	100.5
	Mean = 100.1 mg	SD = 0.37

TABLE 4.2: Absorbance of Known Concentrations of Ascorbic Acid in Human Urine

Ascorbic acid conc. (mg/mL)	Absorbance reading
0.01	0.22
0.02	0.34
0.03	0.43
0.04	0.67
0.05	0.78

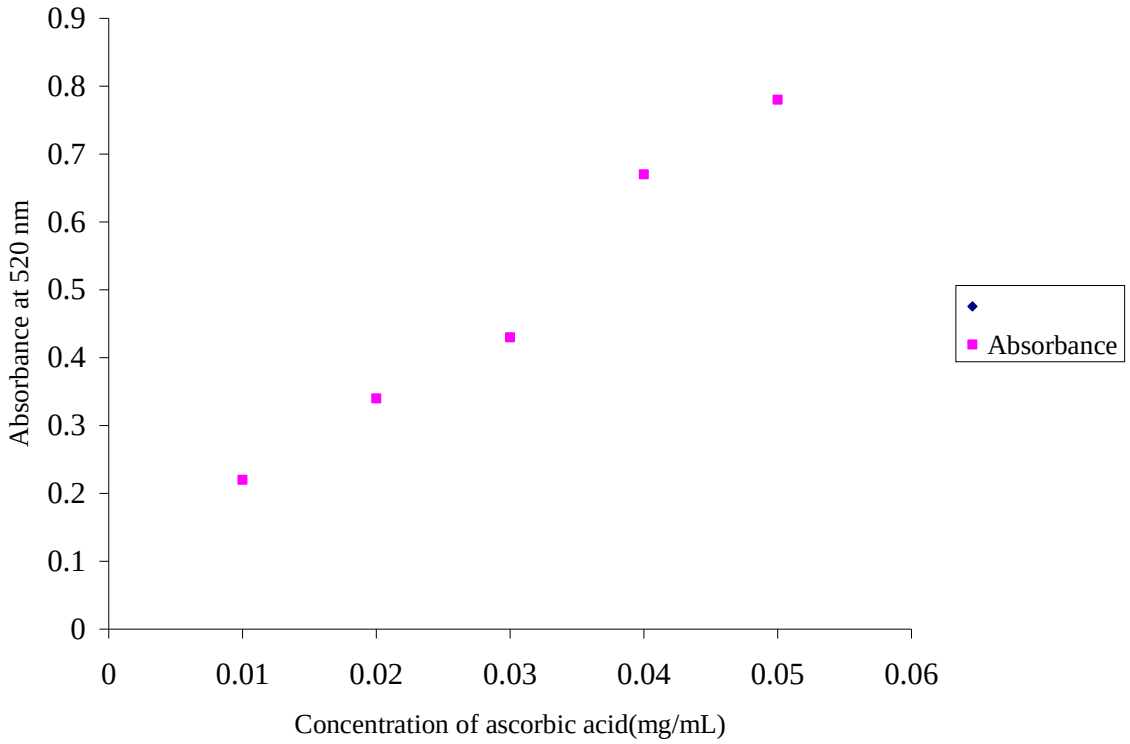


Figure 4.1 Absorbance against ascorbic acid concentration (mg/m L)

TABLE 4.3: The Ages and Weight of Six Volunteers that Ingested Vitamin C/Chlorpheniramine

Volunteer	Sex	Age (Years)	Weight (Kg)
1	Male	24	66
2	"	25	63
3	"	22	68
4	"	27	67
5	"	25	64
6	"	23	61
Mean ± SEM		24.33 ± 0.71	64 ± 1.08

The concentrations of ascorbic acid in the volunteers urine after oral administration of 200 mg of the drug orally is shown in table 4.4. The minimum concentration was 1.5 ± 0.22 µg/mL detected after 0.25 hours in the urine sample of the control volunteers while a higher concentration but statistically insignificant (2.92 ± 0.27 µg/mL) was detected in of the test at the corresponding time. The concentration thereafter increases gradually with time up to 1.5 hours where the concentration was 5.69 ± 0.74 µg/mL in urine of controls and declined gradually again until it slightly increased at 6 hours.

Concentrations in the test volunteers continue to increase and achieved maximum level (14.32 ± 0.19 µg/mL) after 6 hours. The difference in ascorbic acid concentrations in urine of controls and tests were statistically significant between 0.5 and 6.0 hours.

Table 4.4 Mean ± SEM Urine Levels of Ascorbic Acid in Controls and Tests (n=6)

Time (h)	Control (μg/ml)	Test (μg/ml)	t-test
0.25	1.5 ± 0.22	2.92 ± 0.27	NS
0.5	3.1 ± 0.38	6.0 ± 0.58	($P < 0.05$)
1.0	4.9 ± 0.75	10.5 ± 0.68	($P < 0.05$)
1.5	5.69 ± 0.74	11.52 ± 0.14	($P < 0.05$)
2.0	4.73 ± 1.02	12.13 ± 0.11	($P < 0.05$)
3.0	4.68 ± 0.84	10.8 ± 0.51	($P < 0.05$)
4.0	4.73 ± 0.76	13.12 ± 0.06	($P < 0.05$)
6.0	5.98 ± 0.80	14.32 ± 0.19	($P < 0.05$)

Calculations: The area under the curve (AUC), was calculated by the trapezoidal rule from urine levels of ascorbic acid collected at corresponding times (Figure 4.2) as follows;

Control: [AUC] t 0 – t 0.25 = 0 + 1.5/2 (0.25 – 0) = 0.1875 µgh/mL

[AUC] t 0.25 – t 0.5 = 1.5 + 3.1/2 (0.5 – 0.25) = 0.575 µgh/mL

[AUC] t 0.5 – t 1.0 = 3.1 + 4.9/2 (1.0 – 0.5) = 2 µgh/mL

[AUC] t 1.0 – t 1.5 = 4.9 + 5.69/2 (1.5 – 1.0) = 2.6475 µgh/mL

[AUC] t1.5 – 2.0 = 5.69 + 4.73/2 (2 – 1.5) = 2.605 µgh/mL

[AUC] t 2.0 – 3.0 = 4.73 + 4.68/2 (3.0 – 2.0) = 4.705 µgh/mL

[AUC] t 3.0 – 4.0 = 4.68 + 4.73/2 (4.0 – 3.0) = 4.705 µgh/mL

[AUC] t 4.0 – t 6.0 = 4.73 + 5.98/2 (6.0 – 4.0) = 10.71 µgh/Ml

[AUC] t 0 – t 6.0 = 28.135 µgh/mL

[AUC] t 6.0 – t ∞ = Cpn/β = 5.98/0.193078623 = 30.97183887 µgh/mL

TOTAL [AUC] t 0 – t ∞ = 0.1875 + 0.575 + 2 + 2.6475 + 2.605 + 4.705 + 4.705 + 10.71 + 30.97183887 = 59.10683887 µgh/mL

Test: [AUC] t 0 – t 0.25 = 0 + 2.92/2 (0.25 – 0) = 0.365 µgh/mL

[AUC] t 0.25 – t 0.5 = 2.92 + 6.0/2 (0.5 – 0.25) = 1.115 µgh/mL

[AUC] t 0.5 – t 1.0 = 6.0 + 10.5/2 (1.0 – 0.5) = 4.125 µgh/mL

[AUC] t 1.0 – t 1.5 = 10.5 + 11.52/2 (1.5 – 1.0) = 5.505 µgh/mL

[AUC] t 1.5 – t 2.0 = 11.52 + 12.13/2 (2.0 – 1.5) = 5.9125 µgh/mL

[AUC] t 2.0 – t 3.0 = 12.13 + 10.8/2 (3.0 – 2.0) = 11.465 µgh/mL

[AUC] t 3.0 – t 4.0 = 10.8 + 13.12/2 (4.0 3.0) = 11.96 µgh/mL

[AUC] t 4.0 – t 6.0 = 13.2 + 14.32/2 (6.0 – 4.0) = 27.44 µgh/mL

[AUC] t 0 – t 6.0 = 67.8875 µgh/mL

[AUC] t 6.0 – t ∞ = Cpn/β = 14.32/0.1139509731 = 125.66808 µgh/m

The rate of ascorbic acid excretion, dDu/dt, cannot be determined experimentally, hence

urine level is divided by the mid time interval at each collection time (Leon and Andrew, 1999). The difference in rates of ascorbic acid excretion between controls and test increased significantly ($p < 0.05$) at all collection times as shown in figure 4.5 below. Maximum excretion rates were detected after 0.375 hours in controls (10.7 ± 0.67 µg/h) and tests (24.00 ± 2.31 µg/h). The excretion rates declined in both control and test until 2.5 hours where it increased to 4.68 ± 0.84 µg/h in control and 13.12 ± 1.38 µg/h in the test volunteers. The rates of excretion increased further and thereafter reduced to minimum rates of 2.99 ± 0.40 µg/h in controls and 7.16 ± 0.49 in tests after 5 hours. A plot of rates of excretion against average times resulted in an elimination curve (figure 4.2) with slope equal to $-\beta/2.03$.

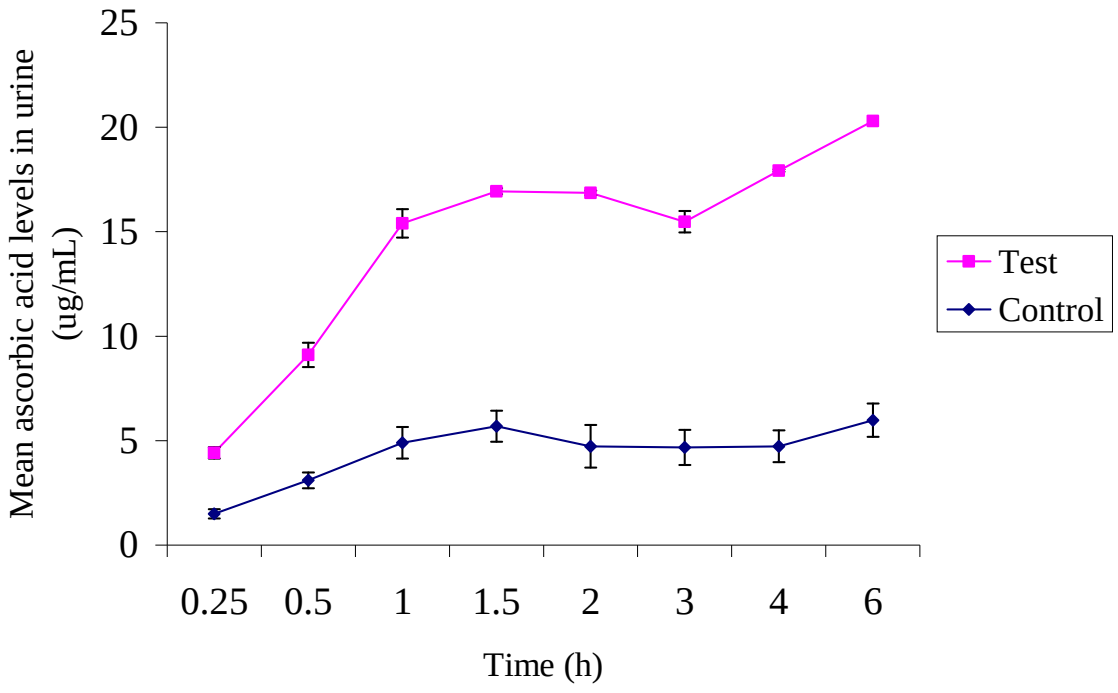


Figure 4.2 Mean urine levels of ascorbic acid against time curves

Table 4.5 Mean ± SEM Rates of Ascorbic Acid Excretion in Urine of Controls and Tests Against Average Times (n = 6)

Time* (h)	Control (μg/h)	Test (μg/h)	t-test
0.125	6 ± 0.89	11.67 ± 1.31	(P < 0.05)
0.375	10.7 ± 0.67	24.00 ± 2.31	(P < 0.05)
0.75	6.05 ± 0.82	14.00 ± 1.21	(P < 0.05)
1.25	4.55 ± 0.60	9.22 ± 0.58	(P < 0.05)
1.75	3.50 ± 0.59	6.92 ± 0.21	(P < 0.05)
2.5	4.68 ± 0.84	10.8 ± 0.92	(P < 0.05)
3.5	4.73 ± 0.76	13.12 ± 1.38	(P < 0.05)
5.0	2.99 ± 0.40	7.16 ± 0.49	(P < 0.05)

*- Average times for sample collection

SEM – Standard error of mean

Calculations: A plot of rates of ascorbic acid excretion against average time resulted in the curve shown in figure 4.3. The slope in figure 4.3 of controls and tests and other pharmacokinetic variables were obtained as follows;

Control: Slope = ln 3 – ln 2/5.6h – 3.5h = 1.098612289 – 0.6931471806/2.1 =

0.193078623

$t_{½}$ = 0.693/slope = 0.693/0.193078623 = 3.589211427 h

β = 0.693/$t_{½}$ = 0.693/3.589211427h = 0.193078623/h

CL_T = Dose/[AUC] t 0 – t ∞ = 200000 μg/59.10683887 μgh/mL

= 3383.703203 mL/h

V_d = CL_T/β = 3383.703203/0.193078623 = 17525.00173 mL

F = CL_T x [AUC] t 0 – t ∞/Dose = 3383.703203 x 59.10683887/200000

= 200000/200000 = 1

$f_e = D^{\infty} u/FDo = 35.31/1 \times 200000 = 0.00017655 = \underline{0.018\%}$

$K_e = f_e \times \beta = 0.00017655 \times 0.193078623 = 0.00003408803089/h$

$CL_R = Ke \times V_d = 0.00003408803089 \times 17525.00173 = \underline{0.5973928003\ mL/h}$

Test: Slope = ln 6 – ln 5/6.6h – 5h = 1.791759469 – 1.609437912/1.6 = <u>0.1139509731</u>

$t_{½} = 0.693/0.1139509731 = \underline{6.081562809\ h}$

$\beta = 0.693/t_{½} = 0.693 / 6.081562809\ h = \underline{0.1139509731/h}$

CL_T = Dose/[AUC] t 0 – t ∞ = 200000/193.55558 = <u>1033.294933 mL/h</u>

$V_d = CL_T/\beta = 1033.294933/0.1139509731 = \underline{9067.890379\ mL}$

$F = CL_T \times$ [AUC] t 0 – t ∞/Dose = 1033.294933 x 193.55558/200000 = 1

$F_e = D^{\infty} u/F \times Do = 81.31/ 1 \times 200000 = 0.00040655 = \underline{0.04\%}$

$K_e = Fe \times \beta = 0.00040655 \times 0.1139509731 = \underline{0.00004632676811/h}$

$CL_R = K_e \times V_d = 0.00004632676811 \times 9067.890379 = \underline{0.4200860548\ mL/h}$

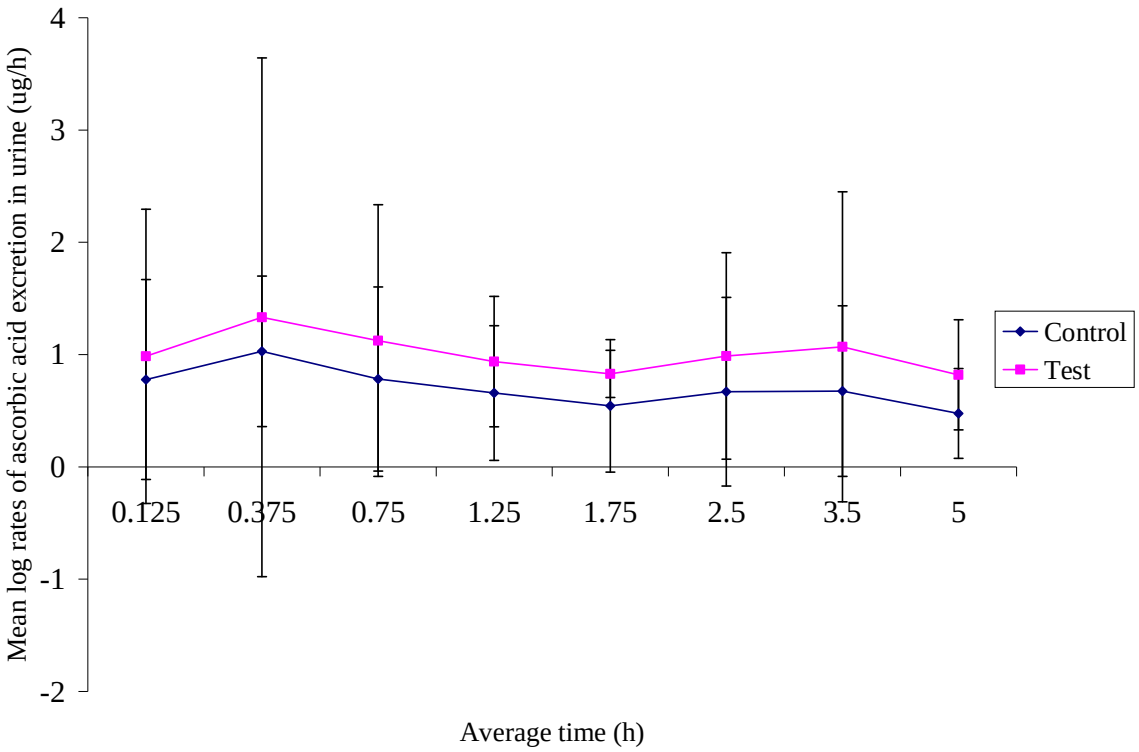


Figure 4.3 Log mean rates of ascorbic acid excretion in urine against average times

The elimination rate constant β was calculated from the slope. Half-life of elimination and other parameters were obtained from formulars presented in section 3.1.6 above. The values and statistics of the pharmacokinetic parameters are shown in table 4.6 below. The result shows that the volume of distribution V_d, decreased significantly($p<0.05$) when controls (16929.30 ± 1819.96) were compared to tests (8902.14 ± 295.18). Similarly, the total body clearance CL_T decreased significantly ($p<0.05$) from 3843.31 ± 609.61 in controls to 1071.35 ± 60.99 in tests. Renal clearance CL_R, elimination rate constant β, and excretion rate constant decreased in the tests when compared with controls but the decrease were statistically insignificant. The elimination half-life of ascorbic acid in controls was 3.33 ± 0.46 hours and increased significantly ($p<0.05$) in the tests (5.90 ± 0.5). Both the AUC_{0-6} and $AUC_{6-\infty}$ significantly increased in the tests (67.861 ± 0.46; 121.84 ± 10.43) about 2-3 times above the controls (29.64 ± 3.87; 29.80 ± 6.22). Bioavailabity F, and fraction of ascorbic acid excreted unchanged fe recorded a statistically insignificant increase in tests volunteers compared to controls. All the values and the t-test of the pharmacokinetic parameters observed are presented in table 4.6.

The rates of ascorbic acid against average times for the six volunteers is shown in appendices C and D. Plots of ascorbic acid excretion rates against average times for the volunteers were presented in figures 4.4 – 4.9 below. The slope of each graph is equal to $-\beta / 2.303$. From the slope, the elimination rate constant β, and other pharmacokinetic parameters were calculated using formulars outlined in section 3.6.1 above.

Table 4.6 Mean ± SEM Pharmacokinetic Parameters obtained from Periodic Urine Data of Controls and Tests (n=6)

S/No	Parameter	Control	Test	t-test
1	V_d(mL)	16929.30 ± 1819.96	8902.14 ± 295.18	(P < 0.05)
2	CL_T(mLh^1)	3843.31 ± 609.61	1071.35 ± 60.99	(P < 0.05)
3	CL_R (mLh^{-1})	0.6608 ± 0.50	0.44 ± 2.40	NS
4	K_{el} (h^{-1})	0.2298 ± 0.03	0.122 ± 0.01	NS
5	K_e ($h\text{-}^{1}$)	$4.09x10^{-5}$ ± $4.16x10^{-6}$	$4.96x10^{-5}$ ± $4.45x10^{-6}$	NS
6	$t_{1/2\beta}$ (h)	3.33 ± 0.46	5.90 ± 0.50	(P < 0.05)
7	AUC ($\mu ghmL^{-1}$)$_{0-6}$	29.64 ± 3.87	67.861 ± 0.46	(P < 0.05)
8	AUC ($\mu ghmL^{-1}$)$_{6-\alpha}$	29.80 ± 6.22	121.84 ± 10.43	(P < 0.05)
9	f_e (%)	0.02 ± 0.00	0.04 ± 0.00	NS
10	F	0.98 ± 0.02	1 ± 0.00	NS

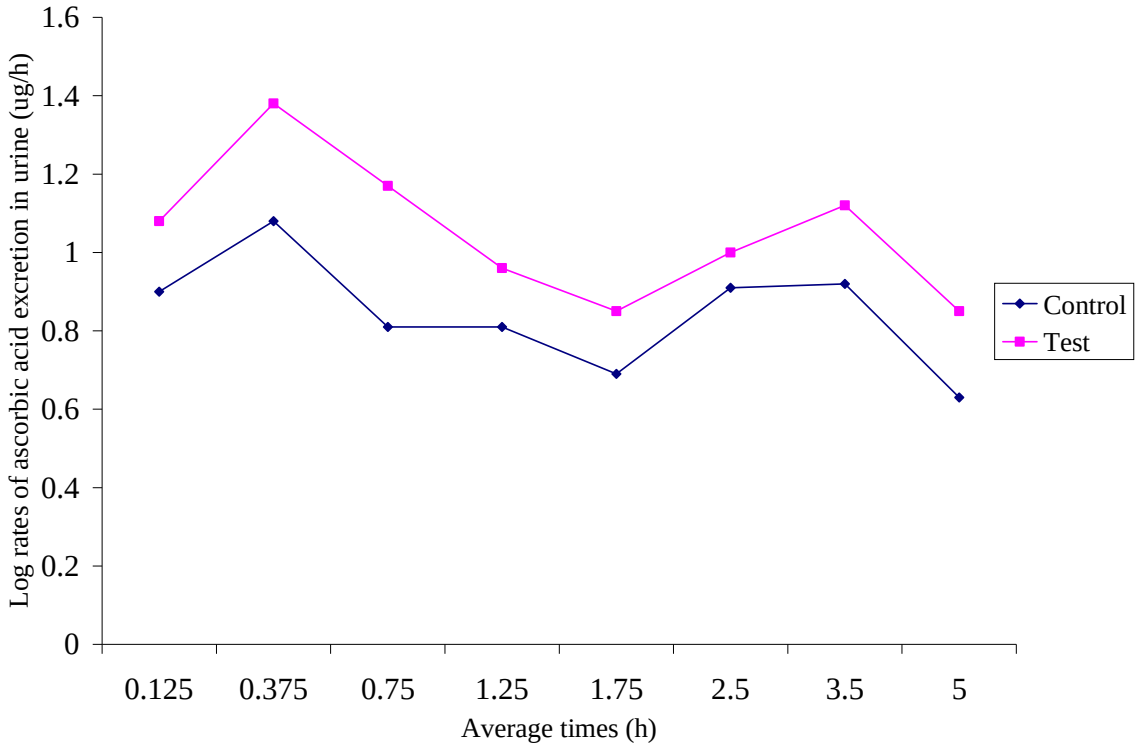


Figure 4.4 Log rates of ascorbic acid excretion in urine against average times (volunteer 1)

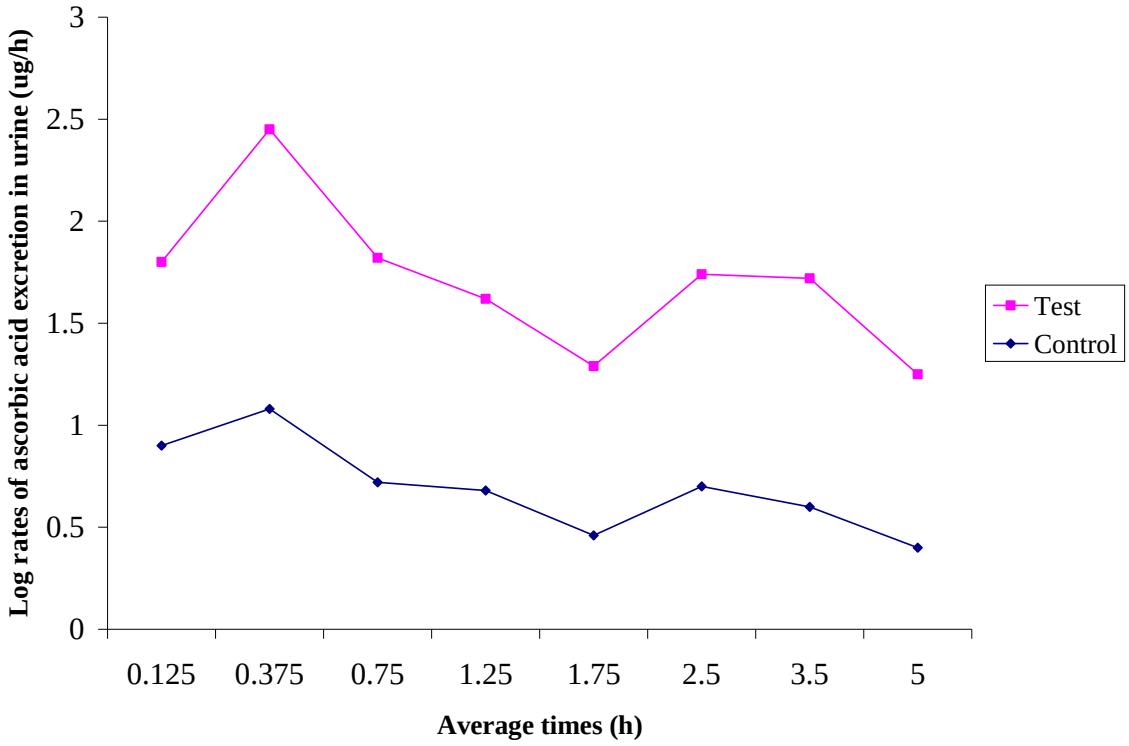


Figure 4.5 Log rates of ascorbic acid excretion in urine against average times (volunteer 2)

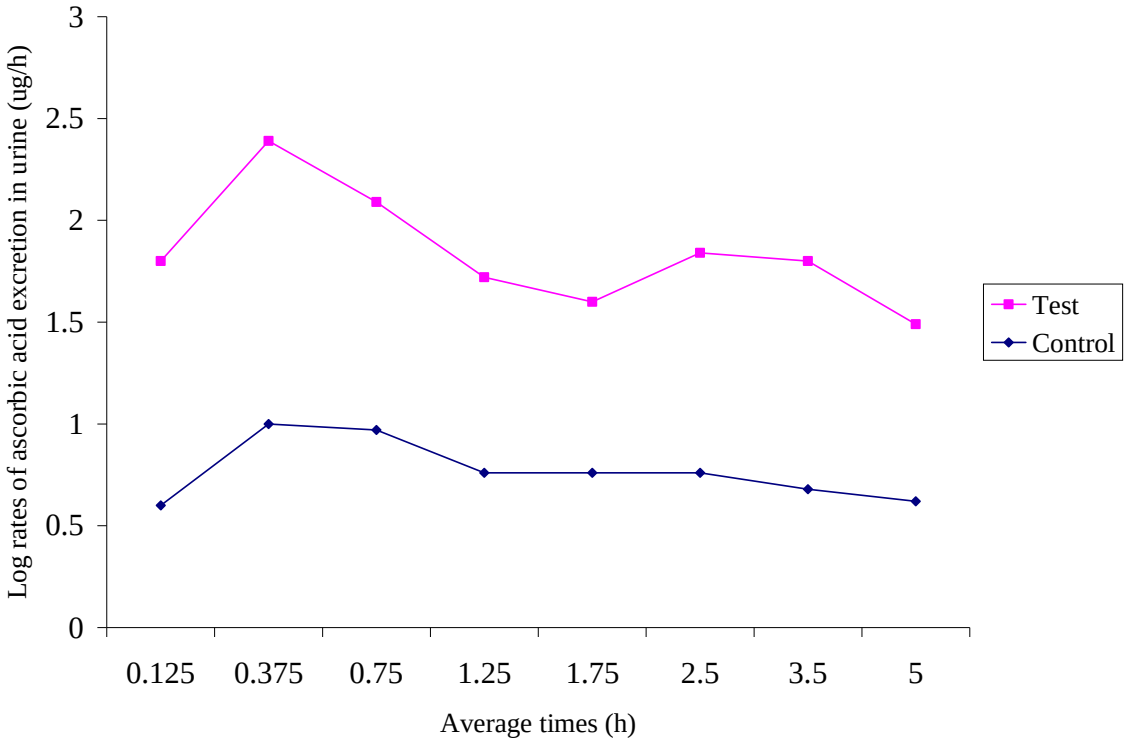


Figure 4.6 Log rates of ascorbic acid excretion in urine against average times (volunteer 3)

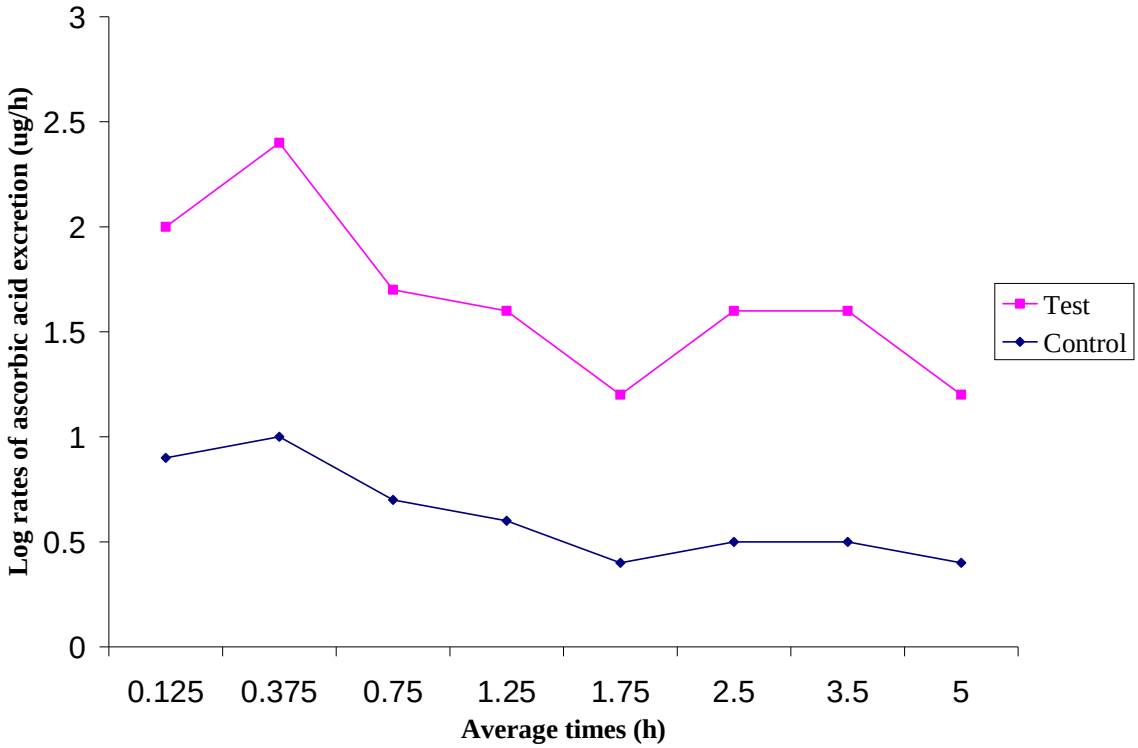


Figure 4.7 Log rates of ascorbic acid excretion in urine against average times (volunteer 4)

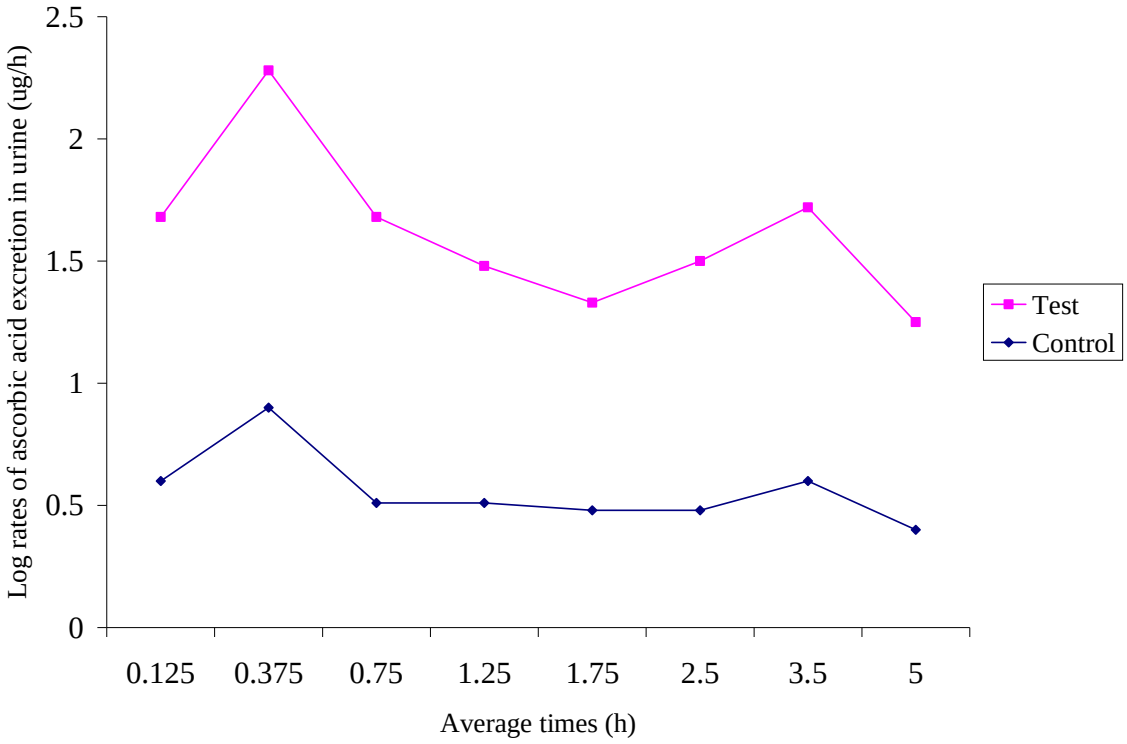


Figure 4.8 Log rates of ascorbic acid excretion in urine against average times (volunteer 5)

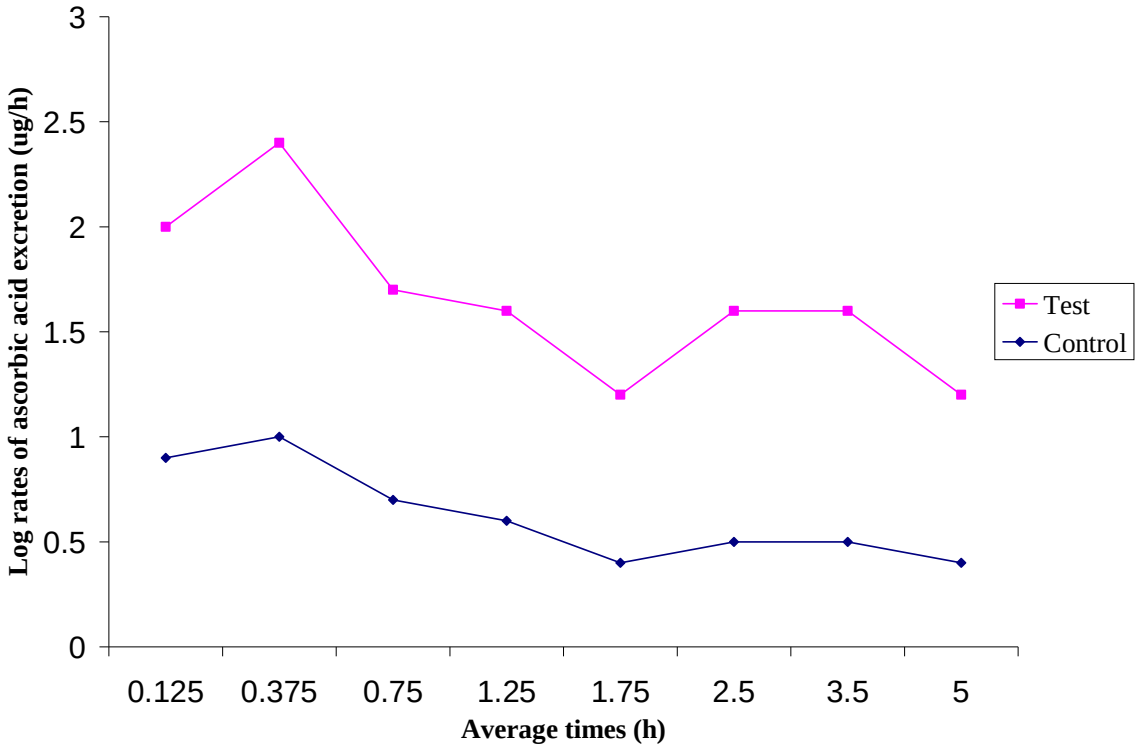


Figure 4.9 Log rates of ascorbic acid excretion in urine against average times (volunteer 6)

CHAPTER 5 DISCUSSION

5.1 Quality Control Analysis

The ascorbic acid tablet used in this study satisfactorily passed the identification test for ascorbic acid tablet specified by the B.P. 2002, since the tablet in solution was acidic to litmus and decolorizes 2,6-dichlorophenolindophenol for about fifteen seconds. Also from the result obtained by weighing twenty tablets of 100 mg ascorbic acid, not more than two tablets deviated from the average mean by $\pm$ 2 mg or 7.5%. This confirmed that the tablet has passed the test for uniformity of weight for tablets and capsules between 80 – 250 mg as described in B.P. 2002 specifications. This analysis was conducted to certify the quality of the ascorbic acid tablet used. This is necessary for precise and reliable results.

5.2 *In* v*ivo* Study

Administration of ascorbic acid and chlorpheniramine tablets concurrently showed a decrease in the apparent volume of distribution V_d (mL) from 16929.30 $\pm$ 1819.96 in the control to 8902 $\pm$ 295.18 in the test and the difference was statistically significant ($P < 0.05$). Decrease in volume of distribution implies a decrease in amount of drug available in various tissues of the body. Hence , more ascorbic acid was excreted or eliminated from the body. The volume of distribution represents a volume that must be considered in estimating the amount of drug in the body from the concentration of drug found in the sampling compartment. (Gibaldi, *et al*., 1969) Some literature reported an inverse relationship between V_d and Kel; a direct proportional relationship between V_d and total body clearance CL_T (Leon and Andrew, 1999). In an effort to determine the effect of age and intake on ascorbic acid disposition in females, it was reported that the volume of distribution and total body clearance decreased after supplementation (Blanchard, *et al*., 1990).Under normal physiological condition, ascorbic acid is distributed to highly perfused

tissues such as the brain, the pituitary gland, adrenal glands, the various white blood cells, intestinal cells, liver cells, and kidney cells. This may account for the large V_d observed in the control. Histamine receptor antagonists induce hepatic microsomal enzymes and they may facilitate their own metabolism (Paton and Webster, 1985; Simons and Simons, 1994). Therefore chlorpheniramine might have caused microsomal enzyme induction thereby increasing the metabolism and excretion of ascorbic acid into urine. Ascorbic acid is excreted by tubular reabsorption and reabsorption of weak acids and weak bases is influenced by the pH of the fluid in renal tubule and the PKa of the drug and this determines the percentage of dissociated and undissociated drug (Cafruni, 1977). Perhaps chlorpheniramine, a weak base, increased the pH of urine dissociation of ascorbic acod and its eventual excretion in urine. A significant increase ($P < 0.05$) in the amount of ascorbic acid excreted in urine of tests volunteers between 0.5-6 hours is good evidence. By implication, the apparent volume of distribution and subsequently total body clearance has decreased.

Total body clearance CL_T (mLh^{-1}) of ascorbic acid markedly decreased from 3843.4 ± 609.61 in the control group to 1071.35 ± 60.99 in the tests. The difference was statistically significant ($P < 0.05$). For a first-order rate process, total body clearance is the product of Kel and V_d (Levine, 1990). Thus, the decrease in CL_T might be due to a corresponding decrease in apparent volume of distribution V_d.

Renal clearance, CL_R (mLh^{-1}), in the control group i.e. 0.6608 ± 0.50 did not differ ($P > 0.05$) statistically from CL_R of the tests i.e. 0.44 ± 2.40. Ascorbic acid is excreted from the body via the kidney by active tubular reabsorption involving a carrier-mediated system that is saturable and dose-dependent (Evans, *et al.*, 1992). Thus, the slight but insignificant decrease in renal

clearance could be due to partial saturation of the carrier system for weak acids or perhaps a decrease in V_d.

There was no significant difference in elimination rate constant β (hr^{-1}) between the control and tests. The difference of the slope of mean rate of ascorbic acid excretion in urine between control and test is only 0.9.

From the results obtained, the ascorbic acid excretion rate constant K_e (h^{-1}) did not change significantly from a value of $4.09x10^{-5} \pm 4.16x10^{-6}$ in the controls to $4.96x10^{-5} \pm 4.45x10^{-6}$ in the tests. The little increase in K_e, though insignificant can be ascribed to a corresponding decrease in the volume of distribution V_d in the tests. Elimination half-life, $t_{½}$ β (h) increased significantly from 3.33 ± 0.46 hours in control to 5.90 ± 0.5 hours in the test. The increase can be ascribed to a significant decrease ($P < 0.05$) in both CL and V_d due to increase metabolism of ascorbic acid in the liver and excretion into urine. Nielsen (1994) reported a long elimination half-life (67hours) for tenoxicam with corresponding low V_d (9.6L) and CL_T (0.106L/h). Dollery (1991) reported an elimination half-life of 3.4hours of ascorbic acid when the body is saturated with the vitamin.

Area under the curve AUC_{0-6} increased from 29.64 ± 3.87 in the control to 67.861 ± 0.46 $\mu ghmL^{-1}$ in the test($P < 0.05$). Similarly, $AUC_{6-\infty}$ ($\mu ghmL^{-1}$) increased ($P < 0.05$) from 29.80 ± 6.22 to 121.84 ± 10.43 in the test. In a study to find a new recommended dietary allowance for ascorbic acid, Levine (1996) observed a complete bioavailability at 200 mg of the vitamin. Chlorpheniramine may have caused the increase of the AUC by inducing the liver to synthesize more ascorbic acid oxidase, which was reported to have been found on the gastrointestinal enterocytes (Dollery, 1991). The enzyme oxidizes ascorbic acid to dehydroascorbic acid. The dehydroascorbic acid is transported via the glucose transporters and subsequently reduced by

gluthathione to ascorbic acid, which enters the systemic circulation. However, the increase in $AUC_{6-\infty}$ was greater than AUC_{0-6}. The reason for this may be due to the fact that the onset of action of chlorpheniramine is about 30 – 60 minutes with peak concentration occurring in about 2 – 6hours and peak effect in about 6 hours (Clinical Pharmacology editorial team, 2006).

The fraction of drug excreted into urine increased ($P > 0.05$) from 0.02 to 0.04 percent. This increase can be associated to a slight increase in Ke.

The bioavailability fraction recorded little increase ($P > 0.05$) when the control and the test were compared. The value obtained was almost unity. This is an indication that ascorbic acid tablets used in the study escaped first-pass elimination and or gastrointestinal metabolism.

CHARPTER 6

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

6.1 Summary

This study briefly elucidated the basic concept of Pharmacokinetics in general and the relationship between plasma and urinary levels of ascorbic acid in particular. Earlier on, various researchers have reported Interaction studies but data was inadequate about the interaction of ascorbic acid and chlorpheniramine and that is the theme of this study. The study can be justified by the routine use of ascorbic acid and chlorpheniramine in the treatment of common cold, and the work of Gershoff (1993), which reported an antihistamine activity of ascorbic acid. The implication is that chlorpheniramine may probably have a synergistic effect with ascorbic acid. Quantitative relationship can be described using one, two, multi-compartments or non-compartmental models (Leon and Andrew, 1999). Such mathematical models can be devised to simulate the rate processes of drug absorption, distribution, and elimination.

Ascorbic acid, a six- carbon ketolactone, was discovered and identified as the active antiscorbutic factor in lemon juice in 1932. The vitamin plays a number of biochemical and biologic functions. However, it has few pharmacological properties and administration of the compound in amounts greatly in excess of the physiologic requirements causes few demonstrable effects in the scorbutic individual whose symptoms are alleviated rapidly (Marcus and Coulston, 2001).

Various methods have been used to determine ascorbic acid levels in biologic samples (Harry and Kathryn, 1939; Saleh *et al.*, 1994; Winnie, *et al.*, 1997 Aslam, *at el.*, 2000; and Clayden, *et al.*, 2001).

The method adopted in this study is based on the principle of the oxidation of ascorbic acid to dehydroascorbic acid within a strongly acid medium which forms a reddish complex with 2,4 dinitrophenylhydrazine and absorbs maximally at 520 nm. A colorimeter set at 520mm was used. Prior to this, a standard calibration curve was prepared using various concentrations of standard ascorbic acid (BDH Poole, London) ranging from 0.01 – 0.05 mg/mL. A plot of absorbance against standard ascorbic acid concentration resulted in a straight line passing through the origin with correlation coefficient r equal to 0.98.

6.2 Conclusions and Recommendations

Coadmistration of 200 mg ascorbic acid and 4 mg chlorpheniramine have increased the V_d, $t_{½}$ and $AUC_{0-\alpha}$ of urinary ascorbic acid in healthy male volunteers and the increase was statistically significant ($P<0.05$) when compared to the control . On the contrary, the CL_T decreased significantly ($P<0.05$) from a value obtained in the control group. Other parameters like, K_e, β, F_e, CL_R, and F recorded little change but the changes were statistically insignificant.

Despite the efforts made to study the Pharmacokinetic interaction of ascorbic acid and chlorpheniramine, further research is required to overcome the limitations of this work. The concurrent administration of ascorbic acid and chlorpheniramine should be discourage or delay administration emphasized. We therefore wish to recommend simultaneous assay of ascorbic acid in plasma and urine. The samples are to be taken from the same individual and six or more volunteers can be incorporated to give room for individual variation in metabolism.

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Appendix A

Urine Levels of Ascorbic Acid (μg/mL) in Controls Ingesting 200 mg Ascorbic Acid Only

Time	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6	Mean	SEM
0.25	2.0	2.0	1.0	1.0	1.0	2.0	1.5	0.22
0.5	3.0	3.0	2.5	3.0	5.0	2.5	3.1	0.38
1.0	7.0	4.0	7.0	5.0	2.4	4.0	4.9	0.75
1.5	8.0	6.0	7.25	5.0	2.9	5.0	5.69	0.74
2.0	8.0	5.0	10.0	4.0	5.25	4.0	4.73	1.02
3.0	8.2	5.0	5.7	3.0	3.0	3.2	4.68	0.84
4.0	8.4	4.0	4.8	4.0	4.0	3.2	4.73	0.76
6.0	8.5	5.0	8.4	4.0	5.0	5.0	5.98	0.80

n = 6
SEM = Standard Error of Mean

Appendix B

Urine Levels of Ascorbic Acid (μg/mL) in Tests Ingesting 200 mg and 4 mg Chlorpheniramine

Time (h)	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6	Mean	SEM
0.25	3.0	2.0	4.0	2.5	3.0	3.0	2.92	0.27
0.5	6.0	5.8	6.2	5.9	6.0	6.1	6.0	0.58
1.0	11.0	9.5	10.0	13.2	11.0	8.3	10.5	0.68
1.5	11.5	11.0	11.4	11.5	11.7	12.0	11.52	0.14
2.0	12.3	11.9	12.0	11.8	12.5	12.3	12.13	0.11
3.0	10.0	11.0	12.1	9.0	10.5	12.2	10.8	0.51
4.0	13.2	13.1	13.2	13.0	13.3	12.9	13.12	0.06
6.0	14.0	14.0	14.8	15.0	14.1	14.0	14.32	0.19

n = 6

SEM = Standard Error of Mean

Appendix C

Rates of Ascorbic Acid Excretion (μg/h) Against Average Times in Urine of Controls

TIME * (h)	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6	Mean	SEM
0.125	8.0	8.0	4.0	4.0	4.0	8.0	6.0	0.89
0.375	12.0	12.0	10.0	12.0	8.0	10.0	10.7	0.67
0.75	6.5	5.3	9.3	6.76	3.2	5.3	6.05	0.82
1.25	6.4	4.8	5.8	4.0	2.32	4.0	4.55	0.6
1.75	4.86	2.86	5.7	2.29	3.0	2.28	3.50	0.59
2.5	8.2	5.0	5.7	3.0	3.0	3.2	4.68	0.84
3.5	8.4	4.0	4.8	4.0	4.0	3.2	4.73	0.76
5.0	4.25	2.5	4.2	2.0	2.5	2.5	2.99	0.4

Time * = Average times for collection sample

n = 6

SEM = Standard Error of Mean

Appendix D

Rates of Ascorbic Acid Excretion (µg/h) Against Average Times in Urine of Test

Time(h)*	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6	Mean	SEM
0.125	12.0	8.0	16.0	10.0	12.0	12.0	11.67	1.31
0.375	24.0	23.2	24.8	23.6	24.0	24.4	24.00	2.31
0.75	14.67	12.7	13.3	17.6	14.7	11.0	14.00	1.21
1.25	9.2	8.8	9.12	9.2	9.4	9.6	9.22	0.58
1.75	7.03	6.8	6.86	6.74	7.1	7.0	6.92	0.21
2.5	10.0	11.0	12.1	9.0	10.5	12.2	10.8	0.92
3.5	13.2	13.1	13.2	13.0	13.3	12.9	13.12	1.38
5.0	7.0	7.0	7.4	7.5	7.05	7.0	7.16	0.49

Time* = average times for collection of urine samples

n=6

SEM = Standard Error of Mean

Appendix E

Pharmacokinetic Parameters Obtained from Urine Data of Controls Ingesting 200 mg Ascorbic Acid Only

Parameter	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6	Mean	SEM
V_d (mL)	12223.81	17530.23	12938.10	18360.48	24544.03	15979.14	16929.30	1819.96
CL_T (mL/h)	2145.04	3857.47	2460.63	5656.23	3402.53	5537.95	3843.31	609.61
CL_R (mL/h)	0.5749	0.6558	0.5739	0.8202	0.5396	0.8002	0.6608	0.5
β (h^{-1})	0.1755	0.2200	0.1902	0.3081	0.1386	0.3466	0.2298	0.03
K_e (h^{-1})	$4.7x10^{-5}$	$3.7x10^{-5}$	$4.4x10^{-5}$	$4.5x10^{-5}$	$2.2x10^{-5}$	$5.0x10^{-5}$	$4.0x10^{-5}$	$4.16X10^{-6}$
$t_{1/2\beta}$ (h)	3.95	3.15	3.64	2.25	5.00	2.00	3.33	0.46
AUC_{0-6} (µgh/mL)	44.8	29.13	37.11	22.38	22.71	21.69	29.64	3.87
$AUC_{6-\infty}$ (µgh/mL)	48.44	22.72	44.17	12.98	36.07	14.43	29.80	6.22
f_e (%)	0.027	0.017	0.023	0.015	0.016	0.014	0.00	0.00
F	1	1	1	1	0.99	1	0.98	0.02

n = 6
SEM = Standard Error of Mean

Appendix F

Pharmacokinetic Parameters Obtained from Urine Data of Tests Ingesting 200 mg Ascorbic Acid and 4 mg Chlorpheniramine

Parameter	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6	Mean	SEM
V_d (mL)	9045.75	9463.89	9316.99	7943.81	9587.42	8054.96	8902.14	295.18
CL_T (mL/h)	1090.44	1017.03	890.76	1197.00	949.36	1283.51	1071.35	60.99
CL_R	0.4416	0.3982	0.3727	0.4902	0.3897	0.5185	0.44	2.40
β (h^{-1})	0.12	0.11	0.10	0.15	0.10	0.16	0.122	0.01
K_e (h^{-1})	$4.9x10^{-5}$	$4.2x10^{-5}$	$4.0x10^{-5}$	$6.2x10^{-5}$	$4.1x10^{-5}$	$6.4x10^{-5}$	$4.96x10^{-5}$	$4.45X10^{-6}$
$t_{1/2\beta}$ (h)	5.75	6.45	7.25	4.60	7.00	4.35	5.90	0.50
AUC_{0-6} (µgh/mL)	67.28	66.38	69.73	67.54	68.28	67.96	67.861	0.46
$AUC_{6-\infty}$ (µgh/mL)	116.14	130.28	154.80	99.55	142.39	87.86	121.84	10.43
f_e (%)	0.041	0.039	0.042	0.041	0.041	0.040	0.04	0.00
F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00

n = 6

SEM = Standard Error of Mean