Assessment of the absorption profile of Ascorbic Acid as a biomarker for Moringa oleifera Lam absorption after a bolus oral dose

By

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Declaration

I, Isaac Chipako, do hereby declare that this dissertation is the result of my investigation and research and that this has not been submitted in part or full for any degree or for any other degree to any other university.

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I. Chipako Date

Supervisors

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

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Dedication

I dedicate my dissertation work to my wife Faustina, and our children Natalia and Erik. Thank you for your prayers and being there for me when the journey was rough.

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Abstract
Moringa oleifera Lam is a medicinal plant used in the management of various ailments in many countries. Despite being used widely, the rate and extent of absorption of these compounds from the ingested Moringa leaf powder is unknown. As a result there is no a standard dosing regimen for Moringa oleifera Lam leaf powder. There is also potential for herb-drug interactions if Moringa is taken concomitantly with conventional medicines.

Aim. The aim of the study was to estimate the absorption profile of Moringa oleifera Lam leaf extract after oral dosing using ascorbic acid as a bio-marker.

Methods:
Extract preparation. Harvested Moringa oleifera Lam leaves were dried under shade and kept away from direct sun light before being ground into powder and macerated with a water and methanol mixture followed by drying.

Experimental animals. Six male rabbits of about 6 months were used.

Feeding and blood sample collection. The rabbits were fasted overnight preceding dosing. Three rabbits receiveda weighed amount of the Moringa oleifera Lam extract at a dose of 300mg/kg body weight. Three rabbits received de-ionised water only, and were used to obtain control plasma. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4 and 8 hrs after dosing. Plasma was taken and assayed by uv/visible spectrophotometry.

Results. The Moringa extract percentage yield was 16%. The content of vitamin C in the extract was 1.371g per 100g of extract. The average bioavailability was 33%.The average C_{max} was 0.91mg/ml.This occurred at an average T_{max} of 1.04 hours. The average time to reach plasma was 0.37 hours.

Conclusion. Moringa oleifera Lam is a good source of vitamin C. Usingthe absorption profile of the vitamin C it was estimated that Moringa compounds may reach the systemic circulation after37.33 minutes and reaches maximum concentration in plasma after about one hour following oral ingestion. It was also estimated that by the end of eight hours following oral ingestion all the accessible vitamin C in Moringa will have been absorbed.

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CHAPTER ONE: INTRODUCTION

1.1 Introduction

Moringa oleifera Lam is a highly valued plant, distributed in many countries across the world. It is being used as a herbal supplement due to its high nutritional value (Fayeh, 2005). Different parts of this plant contain many important minerals, and also provide protein, vitamins, β-carotene, amino acids and various phenolics needed by the body for well being (Mehta, 2011). Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods find use as cardiac and circulatory stimulants; they also possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, diuretic, antihypertensive, cholesterol lowering, antioxidant, anti-diabetic, antibacterial and antifungal activities. Thus the plant is employed for the treatment of different ailments, particularly in Asia and Africa (Rani, 2013).

1.2 Background to the problem

In Africa, Moringa finds its use mainly as a nutritional supplement in the management of HIV and AIDS. In Zimbabwe, the prevalence of herbal medicine use in HIV-infected people is as high as 79%, with Moringa among the five most commonly used herbal supplements (Sebit et al, 2000). It is documented that the majority of HIV/AIDS patients use at least one herbal drug together with their antiretroviral therapy. In light of this high rate of concomitant use of herbal therapy and antiretroviral therapy, there is a potential of altered antiretrovirals (ARVs) pharmacokinetics (Sebit et al, 2000).

Some herbal formulations have been identified as having pharmacokinetic interactions with ARVs, for example, leaf extracts of Moringa oleifera Lam have been established as having *in-vitro* CYP3A4 inhibitory activity (Monera et.al, 2008). Extracts of other plants such as Sutherlandia and grapefruit juice have also been found to inhibit CYP3A4 (Greco, 1995).

Antiretroviral therapy in a resource-limited setting where herbal use is high needs to make efforts for active monitoring of herbal medicine use and the potential effects it can have on therapy (Bepe et.al 2011). Investigating the pharmacokinetic profiles of specific herbal medicines that are taken concomitantly with ARVs thus potentially influencing adverse drug reactions can form a key step in exploring the mechanism of action through which they elicit their action (de Maat, 2003).Bioavailability is one of the principal pharmacokinetic properties of drug used to determine drug exposure in the body (FDA, 2013).

Oral bioavailability, that is the rate and extent a drug taken orally reaches the systemic system, is an essential parameter for determining the efficacy and adverse effects of new and developing medications, as well as finding an optimal dosing regimen (HU, 2011). The term *bioavailability* (*F*) is used to indicate the fraction of an orally administered dose that reaches the systemic circulation as intact drug, taking into account both absorption and local metabolic degradation (Rang and Dale, 2007).

1.3 Problem statement

Although relatively high concentrations of vitamins, β -carotene, amino acids and various phenolics have been reported in the leaves, the rate and extent of absorption of these compounds from the ingested Moringa leaf powder is unknown. As a result there is no a standard dosing frequency for Moringa oleifera Lam leaf powder. Secondly concomitant use of herbs with conventional drugs may lead to herb-drug interactions. Therefore there is need for an appropriate dosing schedule in relation to other conventional medicines such as antiretrovirals. Such information can be deduced by estimating the pharmacokinetic profile of Moringa oleifera Lam after oral ingestion.

1.4 Study significance

The assessment of the rate and extent of absorption of Vitamin C as a bio-marker will enable the estimation of the pharmacokinetic profile of Moringa oleifera Lam leaf after oral

ingestion. The study will enable the estimation of the peak concentration and the time to peak information of Moringa oleifera Lam after an oral dose. This information would be valuable in accumulating evidence towards coming up with a more appropriate dosing frequency and more appropriate dosing schedules in relation to concomitant conventional medicines such as ARVs to avoid possible interactions. Ascertaining the pharmacokinetic profile of Moringa oleifera Lam would also help in the determination of its safety index particularly in patients on antiretroviral therapy, who are the main users of the herbal preparation.

1.5 Research question

What is the absorption profile of Moringa oleifera Lam leaf extract after an oral dose as estimated by the use of vitamin C as a biomarker?

1.6 Aim

To estimate the absorption profile of Moringa oleifera Lam leaf extract after oral dosing using ascorbic acid as a bio-marker.

1.7 Specific objectives

- To determine the concentration of ascorbic acid in Moringa oleifera Lam leaf extract.
- To determine the time taken for ascorbic acid to reach the systemic circulation after a bolus oral dose of Moringa oleifera Lam leaf extract.
- To determine the plasma concentrations of ascorbic acid at defined time intervals after oral dosing.
- To determine the fraction of ascorbic acid that reaches the systemic circulation after a bolus oral dose of Moringa oleifera Lam leaf extract.

1.8 Study design

An experimental study design was employed using a rabbit model to determine bioavailability.

CHAPTER TWO: LITERATURE REVIEW

2.1 Herbal medicine

Herbal medicine which is also referred to as botanical medicine or phytomedicine refers to the use ofa plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes (Zafar, 2009). The World Health Organization estimated that 80% of people worldwide use herbal medicines for some part of their primary health care (WHO, 2005). Herbal medicines are usually classified as dietary supplements e.g. by the U.S. Dietary Supplement Health and Education Act of 1994, implying that herbal medicines, unlike conventional medicines can be sold without any tests for safety and efficacy (FDA, 2010). The most commonly used herbal medicines the world over are echinacea, St. John's wort, ginkgo, garlic, ginseng, feverfew, ginger, Moringa oleifera Lam and milk thistle (Kraft, 2009).

In Africa, the use of Moringa is widespread, particularly as a nutritional supplement in the management of HIV and AIDS. In Zimbabwe, the prevalence of herbal medicine use in HIV-infected people is as high as 79% (Sebit et al, 2000). In a study carried out by Mudzviti et.al, in 2012 the majority (98.2%) of participants used at least one herbal medicine together with their antiretroviral therapy. The most common herbal remedies used were Allium sativum (72.7%), Bidenspilosa (66.0%), Eucalyptus globulus (52.3%), Moringa oleifera Lam (44.1%), Lippia javanica (36.3%), and Peltoforum africanum (34.3%) (Mudviti et al, 2012). Evidently Moringa oleifera Lam is among the most commonly used herbal medicines both locally and the world over.

2.2 Moringa oleifera Lam

Moringa oleifera Lam is a small size tree with approximately 5 to 10 m height. It is cultivated allover the world due to its multiple utilities (Mahmood et al, 2010). Every part of Moringa is

used for certain nutritional and/or medicinal proposes. In addition to being a good source of protein, vitamins, oils, fatty acids, mineral elements and various phenolics, Moringa is used as an antioxidant, anticancer, cardiovascular, hepatoprotective, anti-ulcer, diuretic, antiurolithiatic and antihelmintic agent. Its anti-inflammatory, antimicrobial, antibacterial and antifungal activitiesare employed for the treatment of various ailments in some parts of Asia and Africa (Farooq et.al, 2012).

Its leaves (weight per weight) have more calcium content than that of milk, more vitamin C content than that of oranges, more potassium than that of bananas, more iron than that of spinach, more vitamin A than that of carrots and more protein than that in milk (Kamal, 2008). Besides, Moringa is also suggested as a viable supplement of dietary minerals. The pods and leaves of Moringa contain high amounts of Ca, Mg, K, Mn, P, Zn, Na, Cu, and Fe (Aslam et al., 2005), although minerals content of Moringa shows variation in composition with changes in location (Anjorin et al., 2010). Easy cultivation of Moringa within adverse environmental conditions and wide availability attract attention for economic and health related potential in resource limited developing countries.

2.3 Nutritional content of fresh Moringa leaves

Moringa oleifera Lam leaves belong to the family of dark green leafy vegetables, a food group particularly rich in nutrients (Aslam et al, 2005). In addition, Moringa oleifera Lam leaves have a high dry matter content (around 20-25%) compared to most other plant food sources (generally around 10%). This makes iteven more beneficial as a fresh vegetable since 100 grams of fresh leaves will bring twice as much nutritive material as 100 grams of most other vegetables (http://www.greatnaturallife.com/momitr.html).

Table 1 below gives the mean nutritional values of fresh Moringa oleifera Lam leaves. These values can vary according to many factors such as environmental conditions, farming

methods, maturity of the leaves (dark green, mature leaves are generally richer than light green, young ones), harvesting season, and the genetic background of the trees (Armelle, 2010).

Table 1: Mean nutritional values of 100 grams fresh Moringa oleifera Lam leaves

Dry matter	20-25%
Proteins	5-7 grams
Total ash (= total minerals)	2-3 grams
Minerals	
Calcium (Ca)	350-550 mg
Potassium (K)	200-500 mg
Magnesium (Mg)	80-120 mg
Phosphorus (P)	50-120 mg
Iron (Fe)	5-8 mg
Manganese (Mn)	1,2-2,5 mg
Zinc (Zn)	0,4-0,6 mg
Copper (Cu)	0,2-0,3 mg
V itamins	
Vitamin C	120-200 mg
Vitamin A (as ß-carotene)	1500-4000 µg eq. retinol
Vitamin E (as □-tocopherol)	150-200 mg

Source: Moringanews- Moringa Association of Ghana 2010

2.4 Nutritional content of dry Moringa leaf powder

Moringa oleifera Lam leaves can be consumed after drying and reducing them into powder, making it easier to store and use at any time. In order to preserve the nutrients and reduce microbiological growth, its water content has to be lower than 7%, the drying time as short as possible and the drying temperature not more than 50-55°C. Although some nutrients and vitamins are lost during drying and storage, the leaf powder will still remain a very rich nutritional supplement, since it is a concentrate of the leaves (Anjorin, 2010) *(see Table 2 below)*.

Table 2: Mean nutritional values of 100 grams Moringa oleifera Lam leaf powder.

Dry matter	90-95%
Proteins	20-26 grams
Total ash (= total minerals)	8-11 grams
Minerals	
Calcium (Ca)	1600-2200 mg
Potassium (K)	800-1800 mg
Magnesium (Mg)	350-500 mg
Phosphorus (P)	200-600 mg
Iron (Fe)	18-28 mg
Manganese (Mn)	5-9 mg
Zinc (Zn)	1,5-3 mg
V itamins	
Vitamin C	15-100 mg
Vitamin A (as ß-carotene)	4000-8000 µg retinol eq.
Vitamin E (as α-tocopherol)	80-150 mg

Source: Moringanews- Moringa Association of Ghana 2010

One hundred grammes of fresh Moringa oleifera leaves are enough to cover 30 to 100% of the daily recommended intake of calcium (Igbal, 2006). Also 100 grams fresh Moringa oleifera Lam leaves are enough to cover 25 to 80% of the daily recommended intake of iron. As for the vitamins, the recommended daily intake for vitamin A varies from 400 µg retinol equivalents (young children) to 1,000 µg retinol equivalents (breastfeeding women) (Anjorin, 2010). Therefore, 100 grams of fresh Moringa oleifera leaves could theoretically cover 100%

of daily needs, although this is highly variable depending on storage. Similarly, 100gof fresh Moringa oleifera Lam leaves could cover 100% of the vitamin C requirements, but this vitamin degrades quickly with time and during cooking (Armelle, 2010).In storage, the protein and mineral contents will be preserved for up to six months, whereas a loss of up to 50% of vitamins can be reached after six months of storage. Table 3 below compares the nutritional value of the two states of the plants.

Table 3: Vitamin &mineral content of Moringa (Values per 100g of edible portion).

	Fresh Leaves	**Dried Leaves**
Carotene (Vit. A)*	6.78 mg	18.9 mg
Thiamin (B1)	0.06 mg	2.64 mg
Riboflavin (B2)	0.05 mg	20.5 mg
Niacin (B3)	0.8 mg	8.2 mg
Vitamin C	220 mg	17.3 mg
Calcium	440 mg	2,003 mg
Calories	92 cal	205 cal
Carbohydrates	12.5 g	38.2 g
Copper	0.07 mg	0.57 mg
Fat	1.70 g	2.3 g
Fiber	0.90 g	19.2 g
Iron	0.85 mg	28.2 mg
Magnesium	42 mg	368 mg
Phosphorus	70 mg	204 mg
Potassium	259 mg	1,324 mg
Protein	6.70 g	27.1g
Zinc	0.16 mg	3.29 mg

Fuglie, 2000: Combating malnutrition with Moringa.

*Figures shown for vitamin A are carotene content for fresh leavesand beta-carotene content for dried leaves.

2.5 Key nutrients in Moringa and disease prevention

Moringa supplies a wide variety of nutrients in a nontoxic and easy to digest form. Moringa also contains these nutrients in combinations that are easy for the body to assimilate and digest. No wonder why Moringa is considered a “miracle tree” with the ability to save life (Farooq, 2006).

By providing vitamin A, Moringa helps prevent blindness, maternal mortality, pregnancy and lactation production problems, weak immunity and inability to fight infections. Approximately 250,000 to 500,000 malnourished children in the developing world go blind each year from a deficiency of vitamin A, approximately half of which die within a year of becoming blind (WHO, 2008). Over 100 million children around the world may go blind simply because they are not getting enough vitamin A, although in many of the countries where this is a problem, Moringa often grows wild and just adding a few spoonfuls onto the children’s diet could easily save them from going blind. Moringa is a good source of vitamin C, and therefore helps prevent scurvy. Scurvy leads to the formation of spots onthe skin, spongy gums, and bleeding from themucous membranes. Enough vitamin C also helps to prevent high blood pressure, weakness lassitude and swollen gums and nose bleeds (Pullakhandam and Failla, 2007).

Moringa is also a good source of iron. Iron deficiency can cause anaemia and the resultant signs and symptoms e.g. fatigue, irritability, weakness, shortness of breath etc(McDowell, 2006). Iron deficiency is one of the most commonly known forms of nutritional deficiencies especially in children and pre-menopausal women (Fahey, 2005).

Protein deficiency is also a problem particularly in developing countries. Protein deficiency canresult in general weakness and lethargy,slowness in healing wounds, cuts, reduced intelligence or mental retardationand bruises. In countries that suffer from widespread protein deficiency, food is generally full of plant fibers, which makes adequate energy and protein

consumption very difficult. Thus the problem of protein deficiency can be alleviated by adding Moringa to one's diet (Richardson, 2009).

2.6 Moringa in the management of various diseases

Moringa preparations have been cited in the scientific literature as having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypo-cholesterolemic, and hypoglycaemic activities, as well as having considerable efficacy in water purification (Ara et al, 2008; Richardson, 2009).

2.7 Antibiotic activity

Documented evidence indicates that Moringa has been used for its antibacterial activities for over 50 years (Fahey, 2005). Components of Moringa that have been shown to have antibacterial activity include 4-(4'-*O*-acetyl-α-L-rhamnopyranosyloxy)benzyl isothiocy-anate**,** 4-(α-Lrhamnopyranosyloxy) benzyl isothiocy-anate**,** niazimicin, benzyl isothiocyanate**,** and 4-(α-Lrhamnopyranosyloxy) benzyl glucosinolate (Saadabi and Zaid, 2011).

2.8 Malnutrition

The body is able to prevent disease as well as fight disease as long as it has the nutrients it needs to do this work (Fuglie, 2000). The body, its organs and its immune system need these nutrients in the appropriate amounts in order to function properly. If these nutrients are lacking the full and most efficient functioning of the body deteriorates or even lost. For example, many children in developing countries suffer from night blindness and other eye diseases and afflictions because of lack of vitamin A (Saadabi and Abu Zaid, 2011). This problem of nutrient poor diets can be alleviated by adding Moringa leaf powder to their diets (Fahey, 2005).

2.9 Diabetes

Moringa oleifera Lam has been shown to help in maintaining normal blood sugar levels (Gupta et al, 2012). The preparation has been shown to naturally boost the immune system, which usually becomes compromised in those who suffer from type 1and type 2 diabetes mellitus. Moringa oleifera Lam has also been shown to possess many key anti-inflammatory benefits; diabetes often causes circulatory problems which can be managed through anti-inflammatory supplements (Ndong et al., 2007). Thus Moringa oleifera Lam use is considered a safe and natural way for people to manage their blood sugar and care for their diabetes symptoms (Fuglie, 2001).

2.10 Moringa as antioxidants source

Moringa is said to have approximately 46 antioxidants and is one of the most powerful sources of natural anti-oxidants (Chumark et al., 2008). Anti-oxidants supply the free atoms needed by the human body in mitigating the effect of free radicals. Moringa leaves are rich in flavonoids, a class of anti-oxidants, and beta carotene, which also acts as an antioxidant (Mahmood, 2010). A combination of antioxidants is more effective than a single antioxidant on an equal weight basis due to antioxidant cascade mechanism. This is why Moringa tea acts as an effective source of antioxidants than any other herbal tea (Schwarz, 2008).

2.11 Moringa and HIV/AIDS

Seventy percent of all HIV positive people live in sub-Saharan Africa where malnutrition is rife (UNAIDS, 2013). Certain elements and vitamins, for example vitamin C, E and A, elements zinc and iron, can stimulate the immune system and therefore improve the quality of life (Burger, et al. 2014). Dried leaf powder from the Moringa oleifera Lam is an excellent nutrient source and can easily supplement basic food intake as it contains almost a full RDA

of most nutrients required for a healthy lifestyle (Andrew, 2012). Enhanced nutrition through consuming Moringa could benefit a person with AIDS. While Moringa's antioxidant and nutritional benefits cannot directly compete with antiretroviral therapy, it shows promise in providing reduced mortality rates and improved health for HIV/AIDS patients in less developed areas(Burger, et al. 2014).

In a study carried out in Zimbabwe to determine the prevalence of Moringa use, sixty-eight percent (68%) of the study participants consumed Moringa oleifera. Of these, 81% had already commenced antiretroviral drugs. Most (80%) consumed Moringa oleifera Lam to boost the immune system. The leaf powder was mainly used, either alone (41%) or in combination with the root and/or bark (37%). Moringa oleifera Lam supplementation is common among HIV positive people. Because it is frequently prescribed by non-professionals and taken concomitantly with conventional medicine, there is potential risk for herb-drug interactions. Further experimental investigations into its effect on drug metabolism and transport would be useful in improving the clinical outcome of HIV positive patients on HAART (Monera and Maponga, 2010).

2.12 Drug-herb interactions

More often herbal medicines are taken concomitantly with other conventional medicines. It is documented that some herbal medicines may interact with some of these conventional medicines (WHO, 2012). Examples include St John's wort which is a CYP450 inducer and lowers blood concentrations of drugs such ascyclosporin, amitriptyline, digoxin etc. and causes intermenstrual bleeding when used concomitantly with oral contraceptives ethinylestradiol/desogestrel) (Firenzuoli, 2007). Ginkgo biloba interactions include bleeding when combined with warfarin and raised blood pressure when combined with a thiazide diuretic. Ginseng lowers blood concentrations of alcohol and warfarin, and induces mania if

used concomitantly with phenelzine. Garlic changes pharmacokinetic variables of paracetamol and decreases blood concentrations of warfarin (Izzo, 2001). Thus interactions between herbal medicines and synthetic drugs exist and can have serious clinical consequences (Smith, 2000).

2.13 Bioavailability

To get from the lumen of the small intestine into the systemic circulation, a drug must penetrate the intestinal mucosa and run the gauntlet of enzymes that may inactivate it in gut wall and liver. B*ioavailability* (*F*) is used to indicate the fraction of an orally administered dose that reaches the systemic circulation as intact drug, taking into account both absorption and local metabolic degradation. *F* is measured by determining the plasma drug concentration versus time curves in a group of subjects following oral administration. By definition, the fraction absorbed following an intravenous dose is 1. The areas under the plasma concentration time curves (AUC) are used to estimate *F* as $AUC_{oral}/AUC_{intravenous}$. AUC is estimated using the 'trapezoidal rule', by calculating the area under each pair of data points as a trapezoid (i.e. a rectangle with a triangle on top). The areas of all the trapezoids are summed, and the area from the last point to infinite time is estimated as C_{last}/k, where C_{last} is the last measured concentration and k is the elimination rate constant of the slowest elimination phase (Rang and Dale, 2007).

For dietary supplements, herbs and other nutrients taken orally, bioavailability is generally defined simply as the fraction of the ingested dose that is absorbed (Heaney, 2001). In case of the intake of nutrients and non-drug dietary ingredients, the concept of bioavailability lacks the well-defined standards associated with the pharmaceutical industry. The classical definition cannot apply to these substances because cellular uptake and absorption depends on the nutritional status and physiological state of the individual. This results in greater

variation from individual to individual (Heaney, 2001). Thus, bioavailability for dietary supplements is defined in terms of the proportion of the administered amount of substance capable of being absorbed and available for use or storage (Srinivasan, 2001).

2.14 Factors affecting bioavailability

The absolute bioavailability of a drug, when administered by an extravascular route, is usually less than one (i.e., F<100%). Various physiological factors reduce the availability of drugs and affect their entry into the systemic circulation. Taking a drug with or without food can affect absorption, some drugs taken concurrently may affect absorption and first-pass metabolism, intestinal motility may alter the dissolution of the drug and thereby affecting the degree of chemical degradation of the drug by intestinal microflora. Some disease states may also affect liver metabolism and/or gastrointestinal motility or function(Washington, 2001). All these actors may vary from patient to patient or within the same patient over time(Smith, 2000).

2.15 Assessing bioavailability

The most reliable measure of a drug's bioavailability is the area under the curve (AUC) of the plasma concentration of the substance plotted against time. AUC is directly proportional to the total amount of unchanged drug that reaches systemic circulation. Plasma drug concentration increases with extent of absorption; the maximum (peak) plasma concentration is reached when drug elimination rate equals absorption rate. Peak time (when maximum plasma drug concentration occurs) is the most widely used general index of absorption rate; the slower the absorption, the later the peak time (Midha, 2005; Rang and Dale, 2007).

2.16 Bioavailability using animal models

Compounds can be transported across the intestinal epithelium by four different routes: passive transcellular transport, passive paracellular transport, carrier-mediated transport or transcytosis (Shah et al, 2006). Thus any experimental approach geared towards the investigation of intestinal absorption of bioactive molecules should incorporate a barrier membrane separating the donor compartment, that is, the gastrointestinal lumen, from the receiver compartment, that is, blood circulation, through which the molecule has to pass. For bioavailability and pharmacokinetic studies, where humans cannot be used for ethical reasons, animals that are either phylogenetically related, such as dogs, pigs, and rabbits, are the next best choice (Krishnan, 1994).The *in vivo* evaluation is more relevant than the *in vitro* models of oral bioavailability (Stoner et al, 2004). The in vivo model using a rabbit was deemed appropriate for this study as it more closely approximates the human system.

More often Moringa oleifera Lam is taken concomitantly with other conventional medicines. It is documented that Moringa oleifera Lam may interact with some of these conventional medicines. In view of the above possible interactions of Moringa, a study of the absorption profile of Moringa oleifera Lam after oral ingestion may allow us to predict some of these interactions, thereby allowing a dosing schedule that prevent or minimises these possible adverse effects. This type of information may strengthen our knowledge about Moringa oleifera Lam concerning its use as commercial medicinal and nutritional supplement.

The pharmacokinetic profile of Moringa oleifera Lam after oral ingestion of Moringa can be studied by "following" some of the constituents of the extract as a biomarker after an oral dose using an animal model. Thus this study assesses the absorption profile of vitamin C as a biomarker for Moringa oleifera Lam leaf extract absorption after a bolus oral dose using a rabbit model.

2.17 Vitamin C pharmacokinetics

Ascorbic acid, an essential vitamin, influences tyrosine metabolism, conversion of folic acid to folinic acid, carbohydrate metabolism, resistance to infections, and cellular respiration. Restoring adequate ascorbic acid intake completely reverses symptoms of ascorbic acid deficiency (Padayatty, 1996). After oral administration, ascorbic acid is absorbed readily. After very large doses, absorption may be limited because absorption is an active process. Absorption also may be reduced in patients with diarrhoea or GI diseases. Normal plasma levels of ascorbic acid in humans are about 10 to 20 mcg/ml. Plasma levels below 1.5 mcg/ml are linked to scurvy. About 1.5 g of ascorbic acid is stored in the body. Within 3 to 5 months of ascorbic acid deficiency, clinical signs of scurvy become evident (Padayatty, 2004).

Vitamin C is distributed widely in the body, with large concentrations found in the liver, leukocytes, platelets, glandular tissues, and lens of the eye. Vitamin C is metabolized in the liver. It is reversibly oxidized to dehydroascorbic acid. Some is metabolized to inactive compounds that are excreted in urine. The renal threshold is about14 mcg/ml. When the body is saturated and blood levels exceed the threshold, unchanged ascorbic acid is excreted in urine. Renal excretion is directly proportional to blood levels (Duconge et al,2008; Padayatty, 2004).

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Collection of plant material and extraction procedure

The leaves of Moringa oleifera Lam were collected in the month of July 2014 from Herbal Health Centre, a Moringa oleifera Lam plantation in Waterfalls, Harare. The leaves were kept away from high temperatures and direct sun light to avoid denaturation of active compounds. They were then ground into powder in preparation for the extraction of active compounds.

3.2 Experimental animals

Male rabbits species of about 6 months were obtained from animal house of the University of Zimbabwe. The animals were acclimatised for about seven days under standard environmental conditions, with 12 hours light/dark cycle maintained on a regular vital feed and water.

3.3 Equipment, instruments, chemicals and reagents

All equipment and instruments used were from the Department of Clinical Pharmacology, University of Zimbabwe except a Freeze dryer and the uv/visible machine which were obtained from the Department of Veterinary Sciences and School of Pharmacy respectively. All the chemicals and reagents used were from the Department of Pharmacology except the Vitamin C standard which was obtained from Varichem Pharmaceuticals.

3.4 Preparation of Moringa oleifera Lam extract

3.4.1 Stage one- Moringa oleifera Lam extraction

Apparatus: *6l round bottom flask, Buchii waterbath equipment, capillary stand, Satorius top loading balance model, amber bottle, 1l measuring cylinder, methanol, deionised water*

Method

1. 150g of Moringa oleifera powder were weighed to 2 decimal places.
2. 750ml of methanol and 750ml of water were measured separately.
3. The Moringa oleifera powder was poured into the round bottom flask and the methanol and water were added into the flask.
4. Water was put into buchii water-bath and allowed to heat to 60°C.
5. The flask was dipped into the water-bath and held by its neck with the capillary stand.
6. The flask was shaken at 15minutes intervals until two hours elapsed.
7. The resulting extract was poured into an amber bottle and allowed to cool.

3.4.2 Stage two- Drying of Moringa oleifera extract

Apparatus: *6l Rotavapour Machine, and its accessories, 1l measuring cylinder, filter paper, round bottomed flask*

Method

1. A water-bath was created for the rotavapour machine at 70°C.
2. About 200ml of the extract was poured into the round bottom flask on the rotavapour machine.
3. The machine was left to vapourize the methanol until no visible change in volume of the solute (signifying an end of the vapourisation).
4. The final Moringa extract was then filtered into a measuring cylinder.
5. The procedure was repeated with the remaining quantity of the Moringa extract.
6. The final filtrate collected was measured and made ready for freeze drying.

3.4.3 Stage three- Freeze drying of Moringa oleifera Lam filtrate

Apparatus: *Freeze dryer Modulyo, Gallenkemp Super 85 dryer, drying pans, spatula*

Method

1. The above filtrate was freeze dried using the above named freeze dryer.
2. The resultant extract was weighed and kept in air tight bottle in a refrigerator (-4^{o}c) until used.

3.5 Dosing procedure

Material: *6 rabbits, rabbit pellets, deionised water, Moringa oleifera Lam extract, lignocaine spray, heparin, glass centrifuge tubes, 1ml syringes, stop watch, centrifuge machine, thermometre and fridge.*

3.5.1 Moringa stock solution preparation

12g of Moringa extract were weighed and dissolved to make 100ml of solution using deionised water. The resultant solution was 120mg of Moringa extract per ml of solution.

3.5.2 Feeding and blood sample collection

- Six adult male rabbits from the University of Zimbabwe Animal House were used.
- The rabbits were fed with standard rabbit pellets for seven days for them to acclimatise to the environment until the time of the experiment.
- The rabbits were fasted overnight preceding dosing.
- Three rabbits received, by oral gavage, a bolus dose of weighed amount of the Moringa oleifera Lam extract at a dose of 300mg/kg body weight as shown in table 4 below.
- Three rabbits received deionised water only, and were used to obtain control plasma
- Blood samples (0.5ml) were collected through the marginal vein of the rabbits (with the aid of topical lignocaine spray) into heparinised glass centrifuge tubes using

sterilized disposable plastic syringes at 0, 0.25, 0.5, 1, 2, 4 and 8 hours after the drug administration.

- Blood samples were centrifuged at 5,000 rpm for 10 min using a high-speed centrifuging machine, and plasma samples were withdrawn and stored at −18°C.
- Plasma (0.25ml) was taken for analysis and assayed within 2 days.

Table 4: Rabbit weighing and dosing - Intervention group

Rabbit ID	Rabbit weight (Kg)	Moringa extract dose (Dose = 300mg/kg weight) (mg)	Moringa extract sol (120mg/ml) amt (ml)
1	1.45	435	3.625
2	1.85	555	4.625
3	1.85	555	4.625

Table 5: Rabbit weighing and dosing - Control group

Rabbit ID	Rabbit weight (Kg)	Moringa extract dose (mg)	Deionised water (mls)
4 (control)	1.70	0	4.250
5 (control)	1.85	0	4.625
6 (control)	2.00	0	5.000

3.6 Vitamin C in plasma extraction

Chemicals and materials

Methanol, deionized water, EDTA, micropipettes, blood tubes

- Plasma protein was precipitated with 60% methanol and 1mM EDTA.

- Plasma (100µl) was mixed with 400µl of 60% methanol/EDTA and incubated for 10min at 4ºC before centrifuging at 12,000 rpm for 8min.
- The clear phase was transferred to another polypropylene tube and evaporated to dryness using a freeze dryer.
- The absorbance of the extracts solutions were measured at 525nm against a reagent blank

3.7 Assay of Vitamin C

Chemicals and materials: *Vitamin C, methanol, deionized water, EDTA, ethanol, Uv/visible spectrophotometer Model UV-1601 (SHIMADZU)*

Vitamin C concentrations were measured in the plasma by the UV/VISIBLE spectrophotometer. A standard calibration curve was constructed by solutions with known concentrations of vitamin C.

3.7.1 Preparation of standard solution

Vitamin C (250mg) standard was dissolved in a 250 ml of methanol to give a concentration of 1mg/ml.

3.7.2 Preparation of calibration curve

Portions (of 25ml each) of different concentrations were made from the vitamin C stock solution by appropriate dilutions as shown in table 6 below. To each flask KMnO4 solution was added to obtain a 40ppm concentration of KMnO4 in each flask. The absorbance of the solutions were measured at 525nm against reagent blank. A standard calibration curve of was prepared by plotting absorbance versus concentration.

Table 6: Standard curve determination

Sample no.	**Vitamin C Conc adjusted for potency (potency =96.91%)**	**Absorbance**
1	0.001	0.0008
2	0.0019	0.0012
3	0.0029	0.0055
4	0.0039	0.0099
5	0.0048	0.0156
6	0.0058	0.0201
7	0.0068	0.0229
8	0.0078	0.0302
Sample no.	**Vitamin C Conc adjusted for potency (potency =96.91%)**	**Absorbance**

3.8 Analysis of results

The data were analysed by plotting concentration and log concentration versus time curves of ascorbic acid for the rabbits in both groups. The total amount of the ascorbic acid was determined by calculating the area under the curve (AUC) using the trapezoidal method. To get the amount that reached the circulation system, an average of baseline vitamin C amount was deducted from the AUCs obtained for the three rabbits in the control group. The baseline was obtained by averaging the baseline plasma vitamin C content for the rabbits in the control group. T_{max} and C_{max} and time to reach plasma values of the ascorbic acid were obtained from the graphs.The percentage content of vitamin C in Moringa oleifera Lam extract was used to calculate the amount of vitamin C contained in the amount of extract that

was fed to each rabbit. This in turn was used to calculate the bioavailability of the vitamin C for the rabbits in the intervention group.

3.9 Ethical considerations

This study followed the standard research protocols and was approved by the Joint Research Ethical Committee of Parirenyatwa Group of Hospitals and University of Zimbabwe College of Health Sciences (JREC) (approval number: JREC/130/14).

CHAPTER 4: RESULTS

4.1 Calculation of Moringa extract yield

Moringa extract yield was calculated by weighing the amount of extract produced from 150g of freshly dried and ground Moringa oleifera Lam leaf powder. Table 7 below shows the calculation for Moringa yield extract.

Table 7: Calculation of Moringa extract yield

Input	**Determination of percentage yield**
Weight of Extract = 24.11g Weight of pulverized leaves = 150g	% Yield = Weight of extract × 100 Weight of pulverized leaves % Yield = 24.11 150.00 = 16%

4.2 Assay of Vitamin C standard

The vitamin C standard used to construct the standard curve was assayed by titration method to determine its potency in order to more accurately determine the concentration of vitamin C in the plasma samples (British Pharmacopia, 2009). Table 8 below shows the results of the titration.

Table 8: Assay of Vitamin C standard (By Titration)

Sample No.	Weight (grammes)	Vol of 0.05M iodine sol used (ml)
1	0.150	16.9
2	0.150	16.50
3	0.150	16.50

Using the above tabulated results of titration to calculate potency an average volume of 0.05M Iodine solution of 16.50 ml was found as per the calculations below:

Table 9: Determination of potency of vitamin C standard

Input	Determination of potency of vitamin c standard
Average volume of 0.05M Iodine solution $= \frac{16.50 + 16.50}{2}$ $= 16.50\ ml$ Where: 1ml of 0.05M iodine solution is equivalent to 8.81mg of Vitamin C ($C_6H_8O_6$)	Amount of Vitamin C in 0.150g = 16.50ml x 0.00881/g = 0.145g Therefore potency = $\frac{0.145}{0.150}$ 96.91%

4.3 Assay of Vitamin C Moringa oleifera Lam extract

The amount of vitamin c in the Moringa extract obtained was determined by titration using the assay method according to the British Pharmacopia of 2009. The results of the titration are shown in table 10 below:

Table 10: Titration results (amount of vitamin C in the moringa extract)

Sample No.	Weight (grammes)	Vol of 0.001M iodine sol used (ml)
1	0.150	12.10
2	0.150	11.50
3	0.150	11.40

The results of the titration in table 10 above were then used to calculate the amount of vitamin C in the Moringa extract as per the calculations in table 10 below:

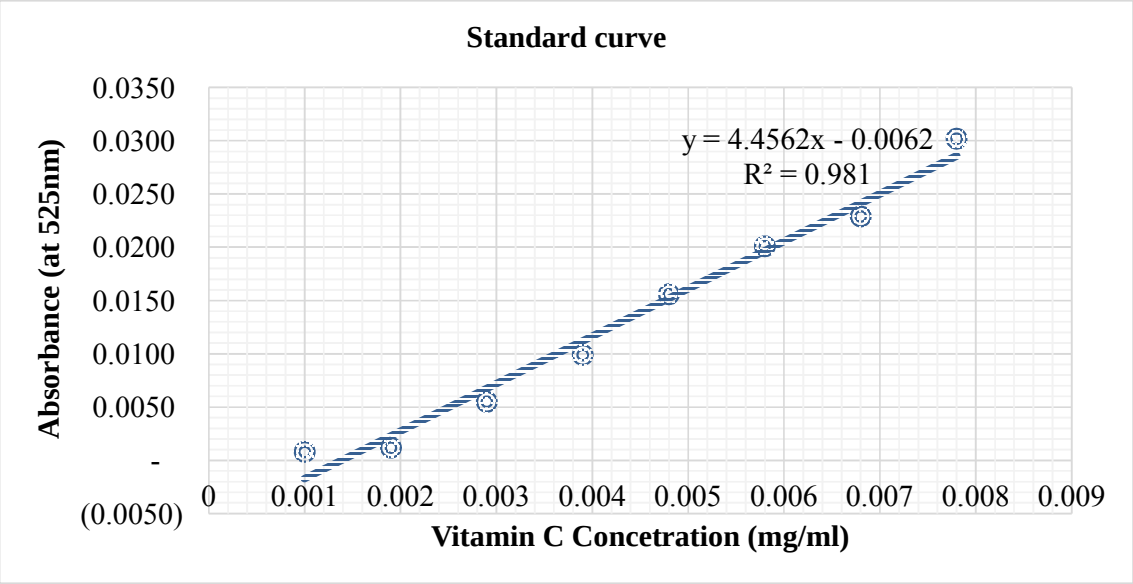
Table 11: Determination of % content of vitamin C in Moringa extract

Input	Determination of % content of vitamin C in Moringa extract
Average volume of 0.001M Iodine solution = 11.67ml Equivalent volume for 0.05M iodine solution = 0.2334ml Where: 1ml of 0.05M iodine solution is equivalent to 8.81mg of Vitamin C ($C_6H_8O_6$)	Amount of Vitamin C in 0.150g $= 0.2334 \times 0.00881$ = 0.002056254g % Vitamin C content = 0.002056254g / 0.150 = 1.370836% **1.371g per 100g of extract**

4.4 Standard curve determination

The potency of the Vitamin C standard that was used to produce the standard curve was determined by titration and found to be 96.91 % as shown in table 9 above. This standard curve is the plot of known concentrations of vitamin C against their respective absorbance using a uv/visible spectrophotometer. The vitamin C concentrations were corrected by multiplying by the potency. The results were then plotted on a X Y scatter as shown in figure 1 below.

Figure 1: Standard curve (Absorbance versus Concentration)



4.5 Calculation of vitamin C concentration in plasma samples

Figure 1 above was then used for calculating the vitamin C concentrations in the plasma samples in both the intervention and control groups using the equation shown below:

Y = 4.4562x - 0.0062

x = (Y+0.0062)/4.4562

Where : Y = Absorbance

X = Concentration (mg/ml)

4.5.1 Intervention group (Rabbit 1, 2 and3)

The results of the concentration in plasma for rabbit 1, 2 and 3 were calculated using tables 12, 13 and 14 respectively as shown below.

Table 12: Rabbit one Concentration of vitamin C in plasma sample against time

Sample(Wt = 1.45kg, dose = 5.964mg)	Absorbance	Conc (mg/ml)	Vol Dissolved (ml)	Amt in plasma (mg)	Plasma vol used (ml)	Conc in plasma (mg/ml)	Time (Hrs)
a	0.05	0.0126	3	0.0378	0.1	0.3784	0.0
b	0.0518	0.0130	3	0.0390	0.1	0.3905	0.3
c	0.0546	0.0136	3	0.0409	0.1	0.4093	0.7
d	0.179	0.0416	3	0.1247	0.1	1.2468	1.3
e	0.144	0.0337	3	0.1011	0.1	1.0111	3.3
f	0.095	0.0227	3	0.0681	0.1	0.6812	5.1
g	0.078	0.0189	3	0.0567	0.1	0.5669	6.8

Table 13: Rabbit two Concentration of vitamin C in plasma sample against time

Sample (Wt = 1.85kg, dose = 7.609mg)	Absorbance	Conc (mg/ml)	Vol Dissolved (ml)	Amt in plasma (mg)	Plasma vol used (ml)	Conc in plasma (mg/ml)	Time (Hrs)
a	0.0681	0.0167	3	0.0500	0.1	0.5002	0.00
b	0.1227	0.0289	3	0.0868	0.1	0.8678	0.32
c	0.1538	0.0359	3	0.1077	0.1	1.0772	0.73
d	0.1086	0.0258	3	0.0773	0.1	0.77286	1.10
e	0.0723	0.0176	3	0.0528	0.1	0.5285	2.07
f	0.078	0.0189	3	0.0567	0.1	0.5669	4.38
g	0.039	0.0101	3	0.0304	0.1	0.3043	7.52

Table 14: Rabbit three Concentration of vitamin C in plasma sample against time

Sample (Wt = 1.85kg, dose = 7.609mg)	Absorbance	Conc (mg/ml)	Vol Dissolved (ml)	Amt in plasma (mg)	Plasma vol used (ml)	Conc in plasma (mg/ml)	Time (Hrs)
a	0.0104	0.0037	3	0.0112	0.1	0.1118	0.00
b	0.037	0.0097	3	0.0291	0.1	0.2908	0.32
c	0.0509	0.0128	3	0.0384	0.1	0.3844	0.73
d	0.0535	0.0134	3	0.0402	0.1	0.4019	1.10
e	0.0236	0.0067	3	0.0201	0.1	0.2006	2.07
f	0.1853	0.0430	3	0.1289	0.1	1.2892	4.38
g	0.1682	0.0391	3	0.1174	0.1	1.1741	7.52

Figure 3: Rabbit two - Concentration in plasma (mg/ml) vs time (hrs)

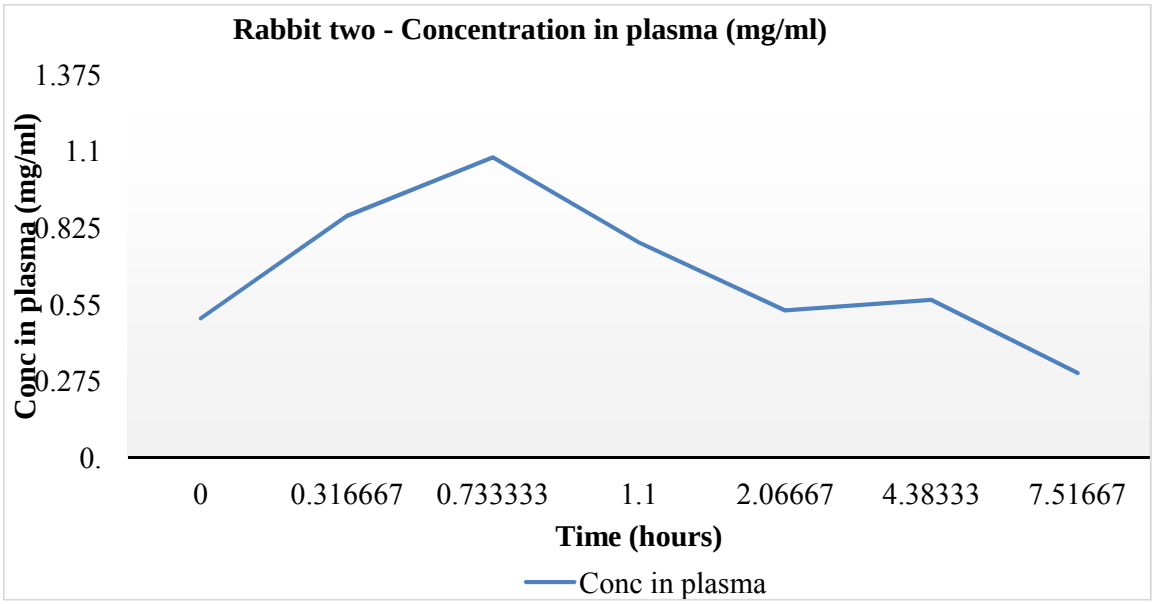
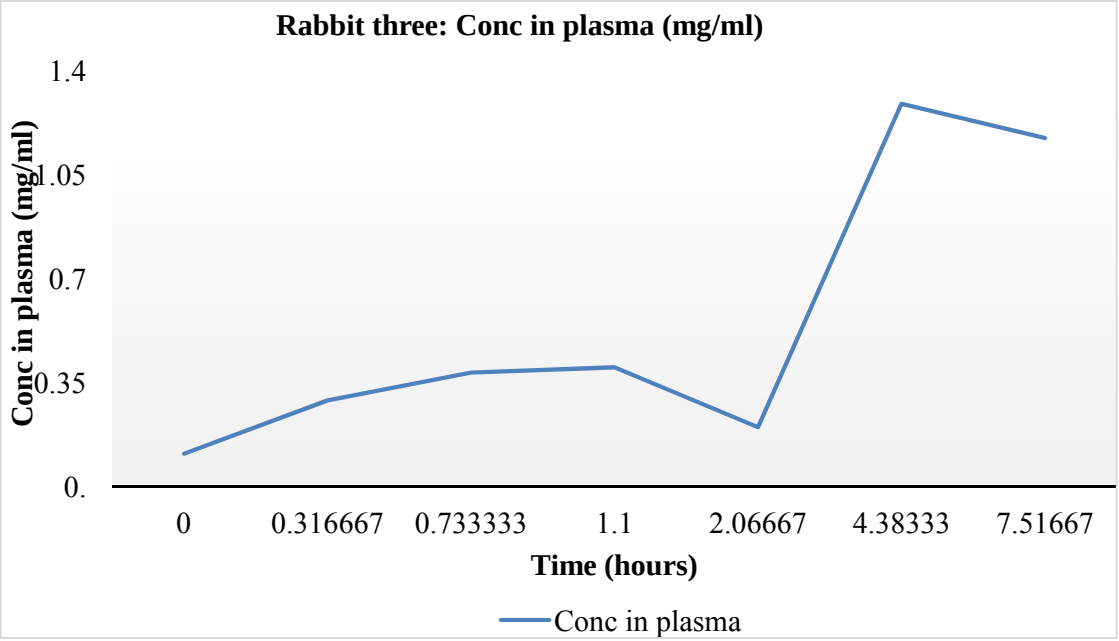


Figure 4: Rabbit 3 - Concentration in plasma (mg/ml) vs time (hrs)



The results tabulated in table 11, table 12 and table 13 were also used to plot log concentration in plasma against the time on a semi log paper as shown in Figures 5, 6 and 7 respectively below:

The results tabulated in table 12, table 13 and table 14 above were used to plot the log concentration in plasma against the time as shown in Figures 2, 3 and 4 respectively below:

Figure 2: Rabbit one - Log Concentration in plasma (mg/ml) vs time (hrs)

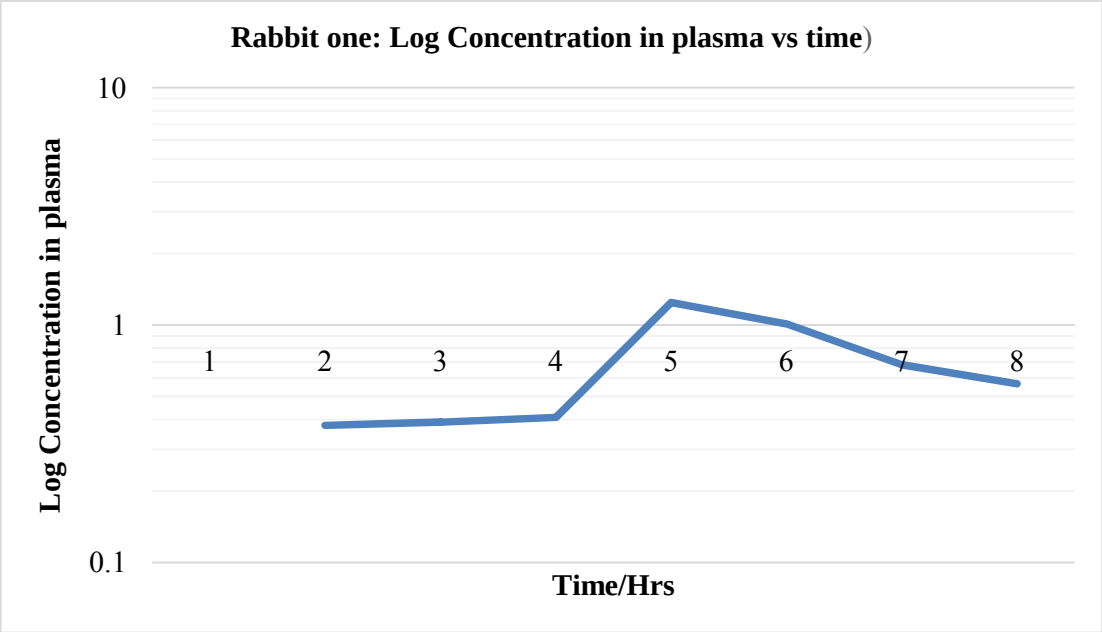


Figure 3: Rabbit two - Log Concentration in plasma (mg/ml) vs time (hrs)

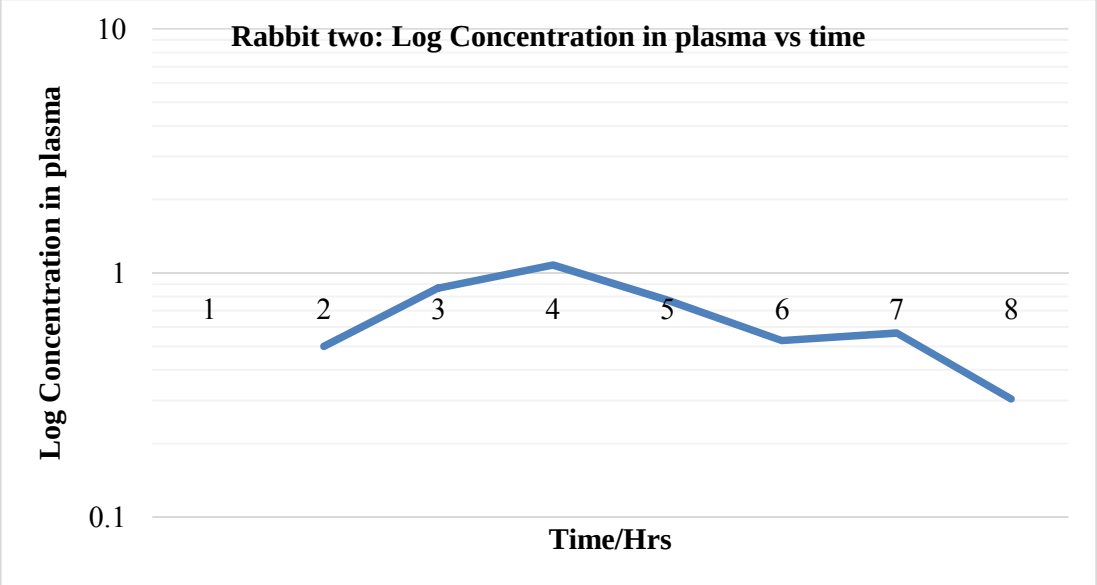
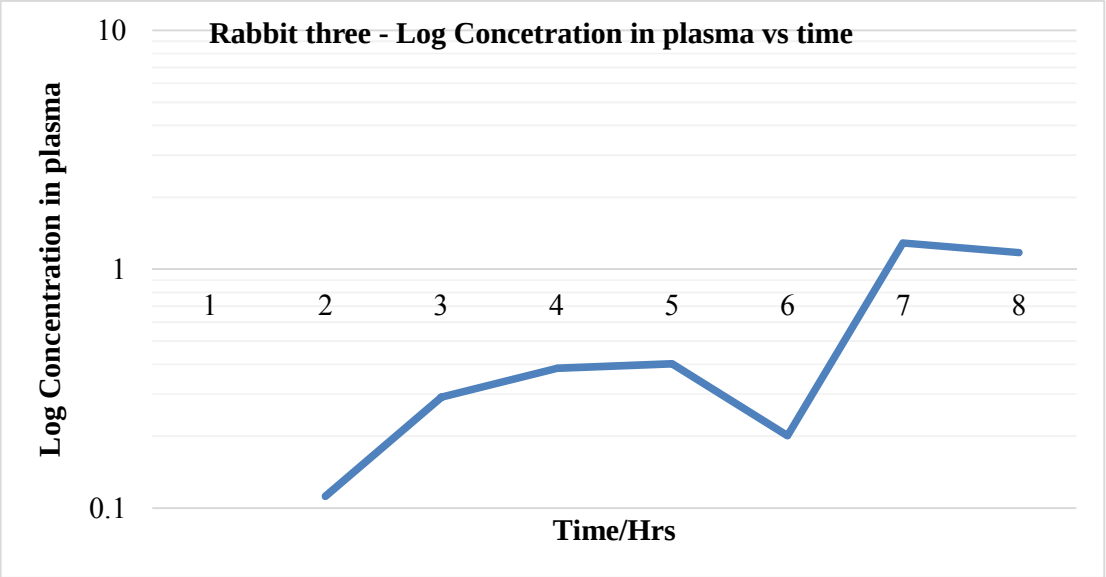


Figure 4: Rabbit three - Log Concentration in plasma mg/ml) vs time (hrs)



The trapeizodal rule was then employed to calculate the area under the curve for rabbits 1, 2 and 3. Table 15, 16 and 17 below gives the area under the curve for respective rabbits.

Table 15: Area under the curve for rabbit one

Time	Conc in plasma	Calculating Points	Calculating Residuals	Area
0.00	0.38		(0.38)	0.11
0.28	0.39	0.39	0.00	0.15
0.65	0.41	0.41	0.00	0.55
1.32	1.25	1.25	0.00	2.20
3.27	1.01	1.01	(0.00)	1.54
5.08	0.68	0.68	(0.00)	1.07
6.80	0.57	0.57	(0.00)	
AREA UNDER THE CURVE				**5.62**

Table 16: Area under the curve for rabbit two

Time	Conc in plasma	Calculating Points	Calculating Residuals	Area
0.00	0.50		(0.50)	0.22
0.32	0.87	0.87	(0.00)	0.41
0.73	1.08	1.08	0.00	0.34
1.10	0.77	0.77	0.00	0.63
2.07	0.53	0.53	0.00	1.27
4.38	0.57	0.57	0.00	1.36
7.52	0.30	0.30	0.00	
AREA UNDER THE CURVE				**4.22**

Table 17: Area under the curve for rabbit three

Time	Conc in plasma	Calculating Points	Calculating Residuals	Area
0.00	0.11		(0.11)	0.06
0.32	0.29	0.29	0.00	0.14
0.73	0.38	0.38	0.00	0.14
1.10	0.40	0.40	0.00	0.29
2.07	0.20	0.20	0.00	1.73
4.38	1.29	1.29	0.00	3.86
7.52	1.17	1.17	(0.00)	
AREA UNDER THE CURVE				**6.22**

4.5.2 Control Group (Rabbit 4, 5 and6)

The results of the concentration in plasma for rabbit 4, 5 and 6 were calculated using tables 18, 19 and 20 respectively:

Table 18: Rabbit four Concentration of vitamin c in plasma sample against time

Sample (Wt = 1.85kg, do;se = 0.000mg)	Absorbance	Conc (mg/ml)	Vol Dissolved (ml)	Amt in plasma (mg)	Plasma vol used (ml)	Conc in plasma (mg/ml)	Time (Hrs)
a	0.068	0.0167	3	0.0500	0.1	0.4995	0.00
b	0.068	0.0167	3	0.0500	0.1	0.4995	0.22
c	0.062	0.0153	3	0.0459	0.1	0.4591	0.62
d	0.066	0.0162	3	0.0486	0.1	0.4861	1.20
e	0.068	0.0167	3	0.0500	0.1	0.4995	2.25
f	0.068	0.0167	3	0.0500	0.1	0.4995	4.50
g	0.057	0.0142	3	0.0425	0.1	0.4255	7.65

Table 19: Rabbit five Concentration of vitamin c in plasma sample against time

Sample (Wt = 1.85kg, dose = 0.000mg)	Absorbance	Conc (mg/ml)	Vol Dissolved (ml)	Amt in plasma (mg)	Plasma vol used (ml)	Conc in plasma (mg/ml)	Time (Hrs)
a	0.066	0.0162	3	0.0486	0.1	0.4861	0.00
b	0.067	0.0164	3	0.0493	0.1	0.4928	0.20
c	0.064	0.0158	3	0.0473	0.1	0.4726	0.58
d	0.063	0.0155	3	0.0466	0.1	0.4659	1.22
e	0.066	0.0162	3	0.0486	0.1	0.4861	2.28
f	0.065	0.0160	3	0.0479	0.1	0.4793	4.55
g	0.056	0.0140	3	0.0419	0.1	0.4187	7.68

Table 20: Rabbit six Concentration of vitamin c in plasma sample against time

Sample (Wt = 1.85kg, dose = 0.000mg)	Absorbance	Conc (mg/ml)	Vol Dissolved (ml)	Amt in plasma (mg)	Plasma vol used (ml)	Conc in plasma (mg/ml)	Time (Hrs)
a	0.065	0.0160	3	0.0479	0.1	0.4793	0.00
b	0.068	0.0167	3	0.0500	0.1	0.4995	0.23
c	0.062	0.0153	3	0.0459	0.1	0.4591	0.67
d	0.066	0.0162	3	0.0486	0.1	0.4861	1.22
e	0.068	0.0167	3	0.0500	0.1	0.4995	2.28
f	0.0685	0.0168	3	0.0503	0.1	0.5029	4.53
g	0.058	0.0144	3	0.0432	0.1	0.4322	7.73

The results tabulated in tables 18, 19 and 20 were also used to plot log concentration in plasma against the time on a semi log paper as shown in Figures 5, 6 and 7 below:

Figure 5: Rabbit four - Log Concentration in plasma (mg/ml) vs Time

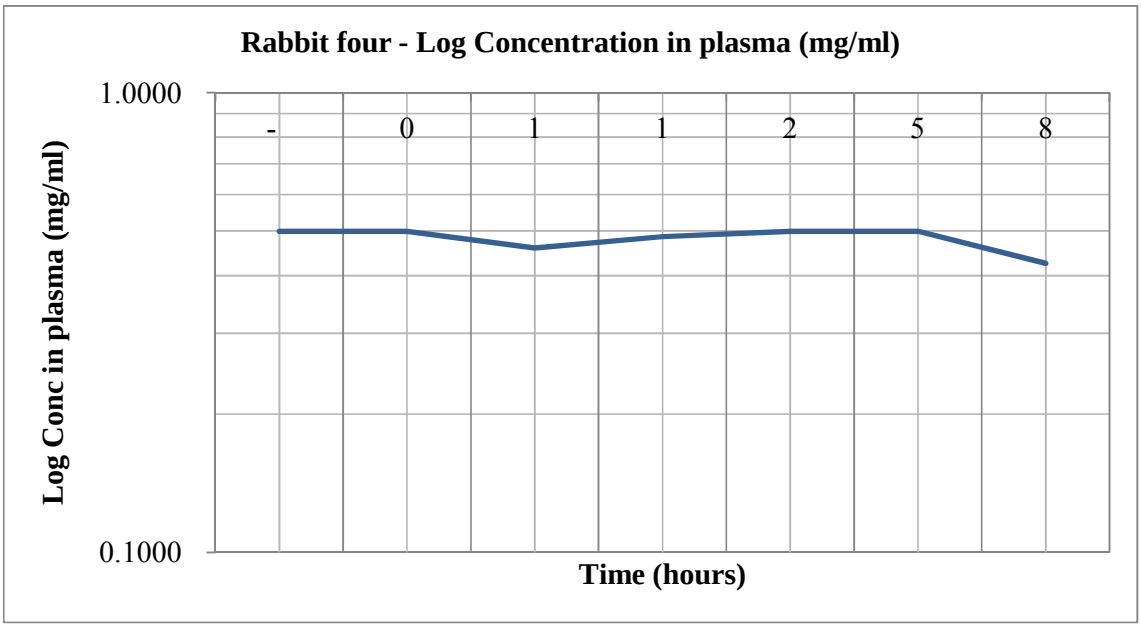


Figure 6: Rabbit five - Log Concentration in plasma (mg/ml) vs Time

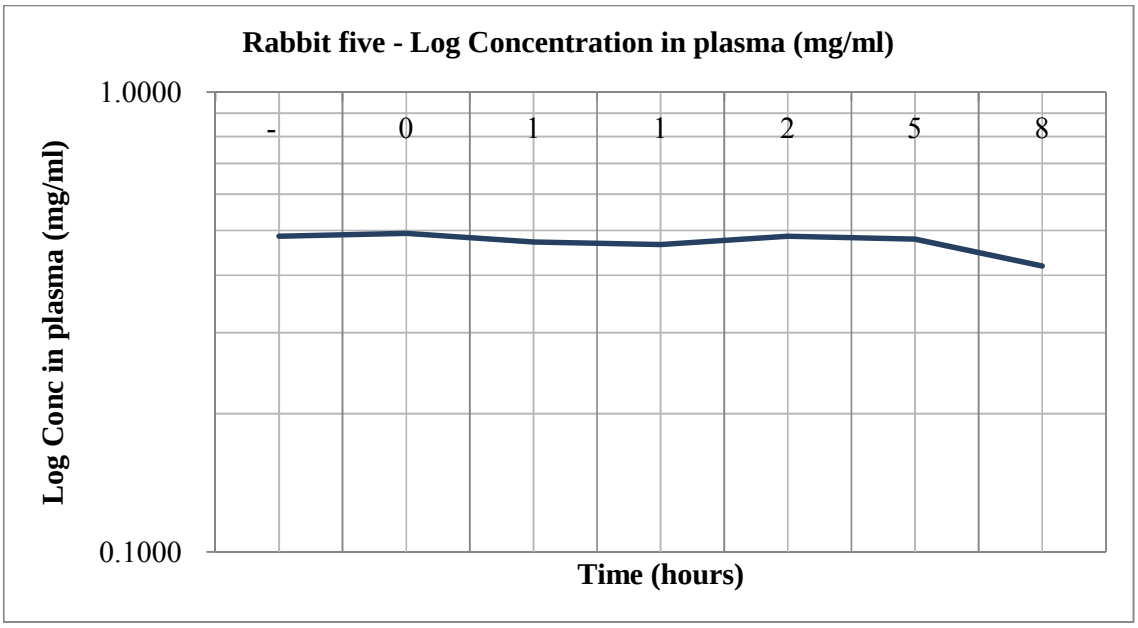
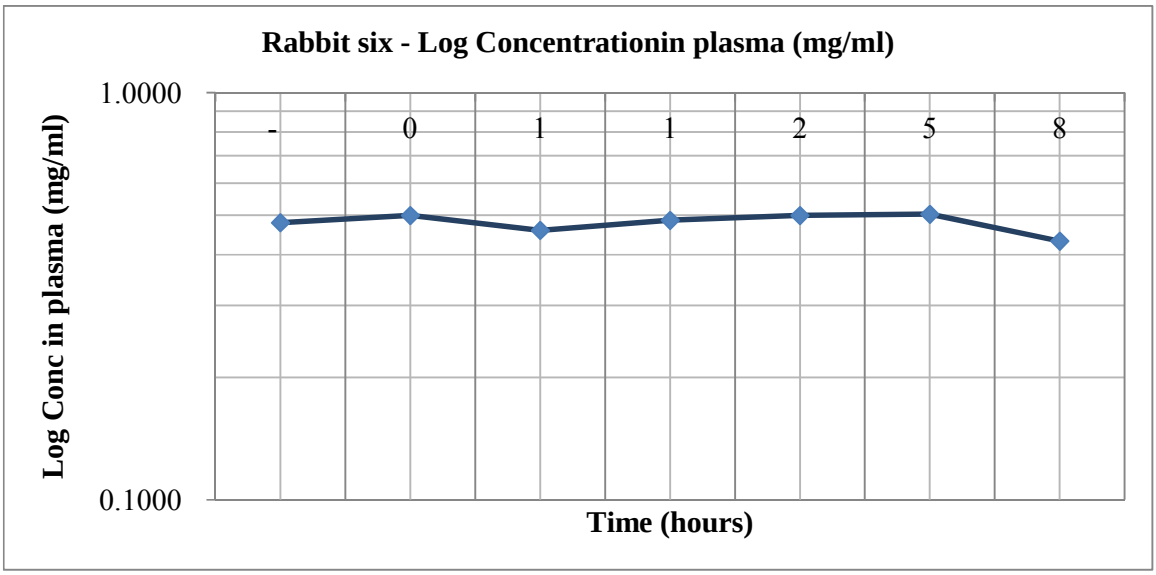


Figure 7: Rabbit six - Log Concentration in plasma (mg/ml) vs Time



The trapezoid rule was then employed to calculate the area under the curve for rabbit 4, 5 and 6. Table 21, 22 and 23 below gives the area under the curve for the respective rabbits

Table 21: Area under the curve for rabbit four

Time	Plasma Concentration	Calculating Points	Calculating Residuals	Area
0.00	0.50		(0.50)	0.11
0.22	0.50	0.50	0.00	0.19
0.62	0.46	0.46	0.00	0.28
1.20	0.49	0.49	0.00	0.52
2.25	0.50	0.50	0.00	1.12
4.50	0.50	0.50	0.00	1.46
7.65	0.43	0.43	(0.00)	
AREA UNDER THE CURVE				**3.67**

Table 22: Area under the curve for rabbit five

Time	Plasma Concentration	Calculating Points	Calculating Residuals	Area
0.00	0.49		(0.49)	0.10
0.20	0.49	0.49	0.00	0.19
0.58	0.47	0.47	0.00	0.30
1.22	0.47	0.47	0.00	0.51
2.28	0.49	0.49	0.00	1.09
4.55	0.48	0.48	0.00	1.41
7.68	0.42	0.42	(0.00)	
AREA UNDER THE CURVE				**3.59**

Table 22: Area under the curve for rabbit six

Time	Plasma Concentration	Calculating Points	Calculating Residuals	Area
0.00	0.4793		(0.48)	0.11
0.23	0.4995	0.50	(0.00)	0.21
0.67	0.4591	0.46	(0.00)	0.26
1.22	0.4861	0.49	(0.00)	0.53
2.28	0.4995	0.50	(0.00)	1.13
4.53	0.5029	0.50	(0.00)	1.50
7.73	0.4322	0.43	0.00	
AREA UNDER THE CURVE				**3.73**

4.5.3 Summary of results

Table 24 below gives the global summary of the research findings

Table 23: Summary of results

Rabbit ID	Dosage of Vitamin C (mg)	Amount which reached the circulation (mg)	Bioavailability (F)	C_{MAX} mg/ml	T_{MAX} (Hrs)	Time to plasma (Hrs)
1	5.964	1.95	0.33	1.0	1.13	0.7
2	7.609	0.56	0.07	0.9	0.73	0.1
3	7.609	2.56	0.34	1.10	1.10	0.32

From table 24 above rabbits 1, 2 and 3 were each dosed with 5.964, 7.609 and 7.609 mg of vitamin C, respectively. The amount that reached the systemic circulation in rabbits 1, 2 and 3 was 1.95, 0.56 and 2.56 mgs respectively. The bioavailability was found to be 0.33, 0.07 and 0.34. The average bioavailability for rabbit 1 and 3 was found to be 0.3; rabbit 2 was not included in finding the average because it is an outlier.

The C_{max} for the rabbits were found to be 1.1mg/ml, 0.9mg/ml and 1.1mg/ml for rabbits 1, 2 and 3 respectively, giving an average of 1.0mg/ml.

The T_{max} for the rabbits were found to be 1.3 hours, 0.73 hours and 1.1 hours for rabbits 1, 2 and 3 respectively, giving an average of 1.04 hour.

Time to reach plasma was found to be 0.7 hours, 0.1 hours and 0.32 hours for rabbits 1, 2 and 3 respectively, giving an average of 0.373

CHAPTER 5:DISCUSSION AND CONCLUSION

5.1 Moringa extract yield

The Moringa extract percentage yield was found to be 16%. This implies that 100g of freshly dried Moringa oleifera Lam leaf powder produces16g of dried extract. This falls within the range of 9-25% yield obtained elsewhere. The variation in percentage yield depends on the type of solvent and extraction method used; and also the season and geographical location of the plants used (Ugwu et al, 2013; Akinyeye et al, 2014).

5.2 Assay of Vitamin C in Moringa extract

The amount of Vitamin C in the Moringa extract was found to be 1.371%. This implies that 100g of the Moringa oleifera Lam extract contained 1371mg of vitamin C. This translates to about 8.5mg of vitamin C per 100g of dried leaf powder. This figure is below the average documented content of about 17.3mg per 100g of leaf powder (Armelle, 2010). This may be due to the degradation of vitamin C caused by heating during rotavapouration.

5.3 Standard curve determination

The vitamin C concentrations used to produce the standard curve were multiplied by a factor of 0.9691 in order to cater for the reduced potency. The plot obtained was a straight line meaning that absorbance is directly proportional to the concentration of vitamin C. The plot has an R^2 value of 0.981, implying that about 98% of the variability in absorbance is explained by the variability in vitamin C concentration. The plot was then used for calculating the vitamin C concentrations in the plasma samples using the equation of the straight line obtained.

5.4 Bioavailability of vitamin C in Moringa oleifera Lam extract

The bioavailability for rabbits 1 and 3 was found to be 0.33, and 0.34 giving in average bioavailability of 0.33. Rabbits 1 and 3 had similar bioavailability whilst there was a very large difference between them and rabbit 2 (variance = 0.29645). This implies that on average only 33% of the vitamin C in Moringa extract reaches the systemic circulation. This may be due to incomplete absorption and first-pass metabolism. For dietary supplements, herbs and other nutrients taken orally, bioavailability is defined as the fraction of the ingested dose that is absorbed (Heaney, 2001). The average bioavailability of vitaimin C from Moringa extract in the rabbits is lower than that of vitamin C 100mg tablets which averages 50% (Srinivasan, 2001).

The bioavailability of a nutrient is governed by external and internal factors (Aggett, 2010; Hurrell, 2010). In this case external factors include the extract matrix, as the vitamin C is part of a mixture of many substances, and the chemical form of the nutrient in question. When in solution vitamin C is unstable and will undergo spontaneous degradation (Srinivasan, 2001)

5.5 C_{max}

Drug bioavailability relies on pharmacokinetic measures, AUC and C_{max} that are reflective of systemic exposure of the drug (FDA, 2013). **C_{max}** is a term used in pharmacokinetics to refer to the peak serum concentration that a drug achieves in a specified compartment of the body after the drug has been administrated (Tracy, 2004). In this case the compartment of interest is plasma. The C_{max} for the rabbits were found to be 1.0mg/ml, 0.9mg/ml and 1.10mg/ml for rabbits 1, 2 and 3 respectively, giving an average of 1.0mg/ml. The variation in C_{max} between the rabbits is small (standard deviation of 0.1). Short term drug side effects are most likely to occur at or near the C_{max} whereas the therapeutic effect of drug with sustained duration of action usually occurs at concentrations slightly above the C_{min}. Therefore Moringa should be

taken in doses that allow the C_{max} of the most toxic compound in Moringa to be below or equal to its maximum tolerated concentration in plasma.

5.6 T_{max} and Time to reach circulation

T_{max} describes the time at which the C_{max} is observed (Midha, 2005). The T_{max} for the rabbits were found to be 1.3 hours, 0.73 hours and 1.10 hours for rabbits 1, 2 and 3 respectively, giving an average of 1.043 hours (standard deviation of 0.29). Thus on average vitamin C from Moringa extract reaches its maximum concentration in plasma just above one hour after oral ingestion. This means that vitamin C from Moringa is more accessible than that from tablets, which have an average T_{max} of two hours (Aggett, 2010; Hurrell, 2010).

When Moringa is consumed, the nutrients contained are released from the matrix, absorbed into the bloodstream and transported to their respective target tissues. Time to reach plasma was found to be 0.7 hours, 0.1 hours and 0.32 hours for rabbits 1,2 and 3 respectively, giving an average of 0.373 (standard deviation of 0.30). Thus on average it takes about twenty two minutes for the vitamin C in Moringa extract to reach the systemic circulation after a bolus oral dose.

5.7 Moringa dose and dosing schedule

There is no enough human evidence concerning the most appropriate dosage Moringa oleifera Lam leaf extract at this point in time, but the majority of animal evidence suggest that 150-200mg/kg oral intake is the optimal dosage. It may be more appropriate to take the ranges above as maximal levels of intake because higher doses have been noted to be genotoxic (Begriche, 2006). It is also documented that Moringa is a food, not a medicine, and it works best preventatively. The recommendations on Moringa dosage are based on an individual's past experiences. Current dosage recommendations include: one teaspoonful once to four times a day in combination with a meal, whereas some people use a lot more

than that. Some professionals recommend splitting this up and taking half the Moringa extra dosage in the morning and the other half in the evening.

It is clear from the above paragraph that there is no agreed dosage for Moringa, whether as dried powder or extract form. This is surprising since Moringa is claimed to heal many ailments, and Moringa is comprised of many nutrients and compounds which are responsible for its medicinal properties (many of which their percentage composition are known). It is critical to know the bioavailability of these different compounds. This will help in determining the most appropriate dosage of Moringa according to the disease of interest. This is the only way to make sure one is getting the right amount of nutrients from the extract. With the correct dosing of Moringa, one will get all of the vitamins, antioxidants, minerals and proteins to help the body function better. Just like any other medicine, several factors need to be considered with dosage. The condition being treated is one of them and some conditions may require a higher dosage than others. The age of the user also matters, generally children take a lower dosage than adults. It is also important to bear in mind that such natural products are not necessarily safe and it's important to have the correct dosage (Garima et al, 2011). For several nutrients, primarily calcium, magnesium, iron, zinc, folate and vitamin A, knowledge of their bioavailability is needed to translate physiological requirements into actual dietary requirements (Gibson, 2007)

5.8 Moringa in relation to other drugs

The widespread use of herbal compounds by people living with HIV/AIDS should be of concern to healthcare practitioners and policy makers. Patients will continue to use herbal medicines as it is important to their local cultural values and beliefs. Therefore, efforts should be made by health professionals to provide validated information to herbalists and patients on the rational use of herbal medicines and supplements. This will likely reduce harm through

failed expectations, pharmacologic adverse effects and unnecessary costs to therapy (Ashwell et al, 2008).

5.9 Conclusion

Knowledge of the pharmacokinetics of profiles of the nutrients and compounds in Moringa oleifera Lam can be used in coming up with a more appropriate dosing regimen for the herbal preparation. Also appropriate dosing schedules in relation to concomitant conventional medicines will be determined to avoid possible interactions. This is important because herbal medicines may interact with modern medicine to increase or decrease the amount of medication in the body.

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