NUTRIENT AND PHYTOCHEMICAL COMPOSITION OF DECOCTION FROM SOME LEAFY VEGETABLES AND THEIR EFFECTS ON BLOOD GLUCOSE, LIPIDS AND LIVER ENZYMES OF DIABETIC MALE WISTAR RATS.

BY

EZEOKEAGU, HELEN NGOZIKA

PG/Ph.D/2011/58387

DEPARTMENT OF NUTRITION AND DIETETICS

FACULTY OF AGRICULTURE

UNIVERSITY OF NIGERIA NSUKKA

OCTOBER, 2021

TITLE PAGE

NUTRIENT AND PHYTOCHEMICAL COMPOSITION OF DECOCTION FROM SOME LEAFY VEGETABLES AND THEIR EFFECTS ON BLOOD GLUCOSE, LIPID PROFILE AND LIVER ENZYMES OF ALLOXAN INDUCED DIABETIC MALE WISTAR RATS

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF NUTRITION AND DIETETICS UNIVERSITY OF NIGERIA NSUKKA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE (PH.D) IN HUMAN NUTRITION DIETETICS

BY

EZEOKEAGU, HELEN NGOZIKA

PG/Ph.D/2011/58387

DEPARTMENT OF NUTRITION AND DIETETICS

FACULTY OF AGRICULTURE

UNIVERSITY OF NIGERIA NSUKKA

PROJECT SUPERVISOR: PROFESSOR N.M NNAM

OCTOBER, 2021

APPROVAL PAGE

This research project has been approved for the award of Doctor of philosophy Degree (Ph.D) in Human Nutrition and Dietetics in the Department of Nutrition and Dietetics, University of Nigeria Nsukka

BY

Prof. N.M. Nnam
(Project Supervisor)

Prof. V.N. Ibeanu
(Head of Department)

Prof. Ignatius .A .Onimawo
External Examiner

OCTOBER, 2021

CERTIFICATION

Ezeokeagu, Helen Ngozi a postgraduate student in the Department of Nutrition and Dietetic University of Nigeria Nsukka with Registration Number PG/Ph.D/2011/58387 has satisfactorily completed the requirement for the award of Doctor of philosophy Degree (Ph.D). The work embodied this research project is original and has not been submitted in part or full for any diploma or degree of this or any other university.

Prof .N M Nnam
Project Supervisor

Date----------

Prof. V.N. Ibeanu
Head of Department

Date----------

DEDICATION

This work is dedicated to God Almighty, deceased lecturers, Ichie and Lolo Ezeanozie Ezeokeagu.

ACKNOWLEDGEMENT

My profound gratitude goes to Almighty and ever living God for his unfailing love, grace, protection, guidance, provision, favour and throughout the period of training. Thank you eternal father. I will forever be grateful to my loving parents late Ichie and lolo M.I. Ezeokeagu and the entire Ezeokeagu family for the light, love support, encouragement during the period. My warm regards to all of you. My sincere thanks go to my dear husband Sir V.O. Onyia and my lovely children Ifeanyi, Somtochukwu, Ekene, Ebubechukwu and the baby of the house Ngozi (Nurse Educator). Thank you for your endurance, prayer and co – operation and for creating conducive environment during the period. Onyia family is not left out, Thank you, I can attest you are really good.

My profound gratitude goes to my great project supervisor Prof. lady N.M Nnam for her understanding, concern, contributions, support encouragement and sacrifices that made it possible for my work to see the light of the day. Thank you so much Prof. for being one of the great women of our days. Once more thank you very much indeed. I remain very grateful to the Head of Department Prof. U.A. Onyechi for piloting the affairs of the Department. God bless you. I am very grateful to the entire lecturers in the Department of Nutrition and Dietetics for their contributions, healthy criticism and concern especially Professor, J.U Nwamarah, Professor. N.K Ngwu, Professor V.N Ibeano, Dr Anyaogu, Dr Chikwendu, Dr Udenta, Dr Bayere , Mr Edeh Mr chukwu and numerous others . You are simply wonderful people. Remain blessed. I cannot forget the non-Academic staff, you too are wonderful and very helpful. God bless you.

My appreciation goes to my classmates Blessing Ugwu, Obiageli Okoli, Ifeoma Ugwu, Chinyere Okpara, Ngozi chukwuka, Ugwu Cosmas and numerous others Thanks for your encouragement, enthusiasm, contribution and concern. Thanks for being there for one another especially when the going became hot. God bless all of you.

All who contributed in one way or the other to see to the success of this work especially Dr Ufele, Silas, Dr Ignatius Maduka,, Dr Emeka Nebo, Dr Okoyeuzu, C and other friends my honest prayer is that God Almighty will bless you in Jesus name Amen.

TABLE OF CONTENTS

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

LIST OF TABLES

LIST OF FIGURES

APPENDIXES

ABSTRACT

The study was conducted to evaluate the nutrient and phytochemical composition of vegetable decoction from *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolua aconitifilius; Morinda lucida* and determine their effects on blood glucose, lipids and liver enzymes of alloxan induced diabetic male Wistar rats. Experimental design was adopted. Matured leaves of each sample were used and the samples were boiled separately in water for 10 to 12 minutes from the boiling time as done traditionally to get the leafy vegetables decoctions. Proximate, mineral, vitamin and phytochemical compositions of the samples as well as the biochemical parameters (Blood glucose, high – density lipoprotein cholesterol, low density lipoprotein cholesterol, triglyceride, total cholesterol and liver enzymes) of the rat treated with the leafy vegetables decoction were done using approved methods. Results of the sample determined in triplicate and rat study in five sets were pooled and analyzed using descriptive statistics and analysis of variance (ANOVA)**,** Proximate analysis revealed moisture (98.64 – 99.61%), protein (0.07 – 0.23 mg/100ml), ash (0.10 – 0.31mg/100), fat and crude fibre (traces) then carbohydrate (0.13 – 0.82 mg/100ml). Mineral analysis revealed iron (0.37 – 0.66mg/100ml), phosphorus (2.08 – 6.69mg/100ml), potassium (54.73 – 72.67mg/100ml), and zinc (0.5 – 0.8 mg/100ml). Result of vitamin composition showed B carotene (54.68 – 63.03mg/100ml), riboflavin (0.04 – 0.14mg/100ml), niacin (0.48 – 4.09mg/100ml), ascorbic acid (6.07 – 7.70mg/100ml). Phytochemicals present were quantified while anthrocynide, anthroquonone, terpernoid and steroid were absent. The quantification showed alkaloids (0.31 – 0.99%), saponin (0.13 – 0.75%) total phenol (5.10 – 12.60mg/100ml), glycosides (1.93 – 6.00 mg/100ml), tannin (1.00 – 2.36mg/100ml), carotenoids (306.67 – 936.67 mg/100ml) and flavonoid (0.42 – 2.44%). For each leafy vegetable decoction, the groups treated with 2ml/kg body weight recorded lowerest effects. There were percentage decreases in blood glucose level across the treated groups (46.03% – 69.64%). Lipid dyslipidemia caused by alloxan administration was also reduced. The increases in high - density lipoprotein cholesterol in some treated groups were higher than the percentage increase of the positive control (33.68%). The highest percentage increase in high density lipoprotein cholesterol was recorded in the group treated with *Luffa aegyptiaca* 3ml/kg body weight (72.15%) consequently decreases were observed thus, low density lipoprotein cholesterol (29.51 – 80.88%), triglyceride (16.10 – 33.56%) and total cholesterol (11.09 – 41.93%). The liver enzyme activity in the treated groups showed an appreciable decrease across board. The photomicrograph of the treated rats showed remarkable improvement compared to the untreated group that showed severe pathology. The present study is suggestive that the nutrient and phytochemical composition of the samples caused synchronized decreases in blood glucose, dyslipidemia and liver enzyme activities with subsequent recovery of the diabetic rat models. Based on these important contributions it is recommended that the entire population especially adults should consume the leafy vegetables decoction to prevent increase blood glucose level and its attendant complications.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Diabetes mellitus a disorder of energy metabolism is becoming increasingly common. Consequently, the basis of abnormality in carbohydrates, protein and fat metabolism is deficient action of insulin on target skeletal muscle, tissue or liver. The disease can be considered a major global health problem. Globally an estimated number of 366 million people have diabetes and it is predicted that more than half a billion people will have diabetes by the year 2030 (International Diabetes Fed. 2011). The World Health Organization (2013) reported that worldwide global population is in the midst of diabetes epidemic. The people in Southeast Asia and sub-Sahara Africa being under greater risk probably due to adoption of western diet and lifestyle within the region. These are facilitated by advertisement for consumption of unhealthy foods and lack of physical exercise (Nnam, Onyechi and Madukwe, 2012).

Despite numerous advances in the field of Medicine and Human Nutrition complications associated with poor glycaemic control is on the increase. Each year the orthodox drugs become increasingly ineffective in 5% to 10% of treated patients and, over ten years, they remain effective in only 20% of those patients initially responsive (Gallengher, 2003). Furthermore, existing orthodox hypoglycemic therapies are generally ineffective in about 20% of Type 2 cases, due to non-compliance with diet and impaired pancreatic function. Also the few western (orthodox) treatments available are not just often very expensive, associated with severe side effects but also require rigorous training to administer them effectively. All these are not readily affordable to the poor.

Following the inefficiency of modern medicine to combat the scourge of diabetes, alternative strategies are urgently needed (Fifa, *et al.*, 2013). Besides, culturally appropriate alternative therapies are desperately needed to treat indigenous populations who are ideologically resistant or have limited access to western medicine. The next option is dietary treatment using foods that are locally available with hypoglycemic effect (Onyechi and Ibeanu, 2010).

In developing countries, its not uncommon for patients to use herbs for conditions such as hypertension, diabetes mellitus and weight loss despite unproved effect on lowering blood sugar.

Many patients use plant remedies as first line treatment for many chronic ailments including diabetes. This paradigm shift is also promoted by the fact that WHO has authenticated the use of food based remedies for the treatment of diabetes (Anthony, Afolayan and Toafik, 2010). Infact, World Health Organization estimated that 80% of the world's population use botanical medicine for their primary health care needs (Fifa *et al*., 2013).

In spite of strong arguments supporting the use of plants with medicinal properties a lot of mist is associated with the use of traditional remedies. Its argued that some plants in use may not be efficacious. Therefore, using traditional remedies that have no proven clinical benefit to patients may lead to delays in seeking appropriate treatment leading to severe diabetes related complications and associated disability and mortality (Elizues, *et al.*, 2013). Most often, interventions are sort by the indigenous people using food plants whose efficacies as consumed have not been tested in management of diabetes. And consuming medicinal food plants whose efficacies as consumed have not been untested may expose consumers to death or developing severe complications of diabetes. Also based on the historical success of natural herbs and ever increasing need for anti-diabetic therapy, a number of Nigerian population divert to traditional medicine in combination with orthodox medicine for the management of diabetes. This may have resulted in dangerous pluralistic approach in diabetic management.

This risk and assumptive behavours call for indebt studies to discover the actual medicinal potentials of the indigenous foods commonly used in Nigeria for treatment. And lack of information on these plants as consumed puts a big question mark on their use as remedy for the management of diabetes.

In Nigeria several edible trees have been identified within the forest and savanna region. Also among them are a wide variety of trees and herbs that are of medicinal value ranging from the popular and highly utilized ones to the less popular and under-utilized ones. Among the underutilized ones are bush buck (*Gongronema latifilium),* a tropical rainforest plant mainly used as vegetable, medicine or spice by the people, Egyptian cucumber *(Luffa aegyptiaca):* an excellent fruit in nature containing many essential constituent required for good health, chaya plant *(Cnidoscolus aconitifolius):* a large, fast-growing leafy vegetable with some medicinal properties that originated from Mexico, and brim- stone tree (*Morinda lucida) :* a tropical ever

green tree found in African rainforest mainly used by the indigenous people as food and medicine.

Obviously, works have been done on extracts of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* to determine the proximate compositions, as well as some vitamins and minerals contents. Also, several works have been done to determine the hypoglycaemic potentials of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifiliu* and *Morinda lucida* extracts. These studies so far on the effect of these plants on diabetes mellitus concentrated on the use of extracts. Based on proven efficacy of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifiliu* and *Morinda lucida* plant extracts on animal model, many people with diabetes resort to these plant remedies for treatment of diabetes. Though the extracts may have shown that they possess some hypoglycaemic potentials but using conventional or traditional method of preparation may have effects on the bioactive contents. Unfortunately, information is seriously lacking on the use of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifiliu* and *Morinda lucida* leafy vegetable decoction in treatment of diabetes.

Therefore, to bridge that gap, the present study was designed to investigate the effects of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction on blood glucose, lipids, liver enzymes, pancreatic and liver cells of alloxan induced diabetic male Wistar rats so as to evaluate their potential roles as alternative and cost effective therapy in management of diabetes and its attendant complications.

1.2 Statement of problem

Chronic illnesses like diabetes mellitus, hypertension, cancer, and renal failures are major problems of many people in the world. Systemic hypertension and diabetes mellitus are common chronic

diseases that frequently co-exist and can significantly affect the health care need and clinical outcome of the affected individual. Most of the chronic diseases are the principal causes of death in the developed and developing countries. Diabetes mellitus is a complex syndrome that causes a lot of physiological changes including alteration in carbohydrate (CHO), protein and fat metabolism. Diabetes, being a chronic disease, unlike the communicable diseases, is often a slow, insidious process. Therefore, uncontrolled diabetes may result in long term damage, dysfunction and failure of various organs especially the heart, kidney and eyes (Bassavanthappa, 2010).

Diabetes mellitus is a chronic medical condition whose prevalence is rising globally. In Nigeria the number of people suffering from type 2 diabetes has been rising steadily over the past two decades**.** The increasing prevalence has also been demonstrated in rural Nigeria probably due to predominance of starch in their foods coupled with the adoption of western lifestyle. As a result, diabetes mellitus affects all ages. Both type I (insulin dependent or juvenile) and type 2 (non-insulin dependent or adult onset) diabetes impose serious complications for economic self- reliance and national development. Type 2 diabetes is a fast growing epidemic affecting people globally. Furthermore, multiple complication and co morbidity are also associated with type 2 diabetes.

In sub-Saharan Africa Nigeria inclusive, it is one of the major medical problems that imperil public health. In Nigeria the number of people suffering from type 2 diabetes has been rising steadily over the past two decades**.** Diabetes has in the last two decades become a household disease and has robbed Nigeria - nation of an increasing number of distinguished leaders and scholars of high repute (Diabetes Association of Nigeria, 2011).

We have left millennium development goals (MDGs) 2015 as the expiration of vision 2020, the threat of diabetes remains even more a serious challenge to achieving the objectives. Consequently, the integrity of Nigeria as the largest black nation and one of the African leading nations is seriously challenged. Nigeria with over 250 tribes and different culture and food value has a diabetic prevalence rate of 3.9 % as recorded by the International Diabetes Federation

(2011), in its current atlas. However, Federal Ministry of Health (2011) recorded 2.2% prevalence rate in Nigeria.

Finding solution to the problem has continued to be a major public health concern. Currently, there is no orthodox therapy that can cure the disease. Modern medicine though very costly only tries to

control hyperglycemia which is the hallmark of the disease. Orthodox drugs only provide relief from symptoms and prevent or delay complication.

Another strong argument supporting the paradigm shift to use of the traditional food plants as source of cure for diabetes is the fact that decades ago life span was longer. The indigenous people based their food and cure for ailments on what they pick from within which were planted there for such purposes. And a shift from that may be solely responsible for increased prevalence of what were initially considered as "western diseases".

The cost of diabetes is a high one and its only within the past few years that it has begun to be fully appreciated. Currently there is national economic crunch and people affected with diabetes are trying to manage the merger resources for both treatment and other needs therefore, managing diabetes effectively with orthodox medicine in the present dispensation is very difficult especially for the average Nigerians creating urgent need for cheaper alternatives remedies

As a result, Health care in Nigeria is a complex business. In developed countries, diabetic care is largely sort in the hospitals, but in Nigeria, a rather different pluralistic approach prevails. Many people often supplement the care they receive from hospitals with treatment from traditional healers. In fact, it is estimated that traditional medicine provides 80% - 90% of health care in Africa, Nigeria, inclusive (Anthony, Afolayan and Toafik, 2010). Another major problem emanates from the traditional belief that every disease has a cure. Therefore, scientific description of diabetes mellitus as chronic non - communicable disease exposes limitations of orthodox medicine and motivates people who subscribe to this wild belief turn to any or unproven plant remedy as the alternative pathway. In view of the serious problems emerging with old and new diseases, a broad screen of natural product is urgently needed, and plants are the natural and under- exploited source of novel compounds.

The continuous spread of diabetes mellitus despite the use of orthodox remedies calls for immediate alternative remedies for the indigenous people. The remedies are sort by the indigenous people using herbs whose efficacies as consumed have not been tested in management of diabetes. All previous studies concentrated on extracts which showed positive results. And lack of information on these herbs as consumed put a big question mark on their use as remedy for the management of diabetes. Perhaps, preparing the leafy vegetables as consumed may have increased or decreased the

contents making it impossible for consumers to take exactly the quantity contained as shown by the extracts. It is very pertinent to point out that the amount given as extracts cannot be easily measured out for human studies since humans do not consume the leafy vegetables as extracts rather as tea or decoction, therefore, making it difficult to extrapolate the studies on use of extracts on animal models to human situations. The quantity taken as leafy vegetable decoction may be either too much on inadequate to effect a change in an individual's blood glucose.

Based on the complication that arise from poorly managed diabetes as well as the claim that traditional remedies can cure diabetes, there is an urgent need to carry out a detailed nutritional and bioactive evaluation of *Luffa aegyptiaca, Cnidoscolus aconitifolius, Morinda lucida and Gongronema latifolium* leafy vegetables decoction as an alternative cost effective therapy in management of diabetes in animal model.

1.3 Objective of the study

General objective

The aim of the study was to evaluate nutrient and phytochemical composition of leafy vegetables decoction from *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida*, and determine their effects on blood glucose, lipids and liver enymes of alloxan induced diabetic male wistar rats.

The specific objectives of the study were to;

1. evaluate: the proximate (moisture, protein, carbohydrate, crude fibre, ash, and lipids) composition of leafy vegetables decoction from *Gongronema latifilium, Luffaa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida*, respectively.

2. evaluate some vitamins (pro vitamin A, thiamin, riboflavin niacin and ascorbic acid) composition of leafy vegetables decoction from *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida*, respectively.

3. evaluate some minerals (iron, potassium, selenium, phosphosrous and zinc) composition of leafy vegetables decoction from *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida,* respectively.

4. determine the presence of some phytochemicals (phenol, anthocyanide alkaloids, saponin, flavonoids tannin, glycoside, terpenoid, carotenoid, anthraquinone and steroids) in leafy vegetables decoction from *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida,* respectively.

5. determine the quantity of some phytochemicals (phenol, alkaloids, saponin, flavonoids tannin, glycoside, terpenoid and carotenoid) present in leafy vegetables decoction from *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida*, respectively.

6. determine the effect of leafy vegetables decoction from *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* respectively, on blood glucose of alloxan induced diabetic adult male Wistar rats.

7. determine the effect of leafy vegetables decoction from *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* respectively, on lipid (high density lipoprotein, low density lipoprotein, triglycerides and total cholesterol) profile of alloxan induced diabetic adult male Wistar rats.

8. determine the effect of leafy vegetables decoction from *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* respectively, on liver enzymes (aspartate amino transeferase (AST), alanine transeferase (ALT) and alkaline phosphate (ALP) of alloxan induced diabetic adult male Wistar rats.

9. determine the effect of leafy vegetables decoction from *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* respectively on the liver and pancreatic cells of alloxan induced diabetic adult male Wistar rats.

1.4 Significant of the study

This study when published would provide information to the general public on the possible roles of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoctions in management of diabetes and its associated complications and this would be more applicable than works on extract since humans do not consume the leafy vegetables as extracts.

From the study, it was predicted that a dose higher than 4ml/kg body weight may result in hypoglycaemia. This information when published would serve as eye opner to the general public and health workers, the knowledge gained would be taken down to the grassroot. This may sensitize the society on uncontrolled intake of herbal remedies which has become a major public health problem as majority of the population especially people with diabetes tend to concentrate on their use for cure and exceed their modest requirement

The result of the work may also be useful to diabetics particularly those adversely affected by herbal medicine due to misinformation and inability to seek proper intervention. It may help diabetics in self-management of diabetes and to become authorities in their own health management.

There is a decline in interest and consumption of some local foods. Thus, uses of some plants have gone into extinction. This work when published may reinkindle the interest of the indiginous people as regards the uses of vegetables. Hence, this work may generate and promote interest in public enlightenment on use of indigenous plants for optimal health. It is presumed that the result of the study may change the orientation of the indigenous people and make the general public appreciate the roles and contributions of indigenous plants in maintaining optimum health and management of ill health.

The findings of the study would serve as reference material for new researchers who may want to carry out similar works using indigenous foods and plants as consumed. The result of the work when published may progress to further researches and establishment of indigenous industries for the manufacturing of drugs from indigenous food plants for local and international use and creat income for drug manufactures.

As the plants are found to be rich in vitamins and minerals the work may help Dieticians to combat micronutrient deficiency present among the indigenous people by encouraging them to consume the leafy vegetables decoctions.The work would be of great value to health workers, community leaders and NGO's in their quest to contro diabetes.The method of preparation is cheap, conventional and easier for the users (diabetics) than preparing aqueous extracts which require sophisticated equipment and calculation.

The information from this work may present households, more available and accessible prophylactic and therapeutic options for diabetics in African countries where vegetables are popularly used as first line therapy in the management of diabetes.

Most of the plants are easily accessible and found within the tropical rain forest, as the plants are effective in management of diabetes in rats, they could be useful in management of diabetes in human and may help persons with diabetes identify and use cheap and easy ways to achieve normal blood glucose level.

Since the plants are found to have an encouraging effect on lipid profile, the use may be advocated by health workers for the control of lipid dysfunction in diabetics and other disease conditions apart from diabetes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Vegetables

Vegetable usually refers to the fresh edible portion of an herbaceous plant – root stem, leaves, flower or fruit (Encarta, 2009). Vegetable can also be defined as a plant grown for any edible part of it such as the leaves, root, fruits or flowers. Vegetables are the edible parts of plant that are consumed wholly or in parts, raw or cooked as part of main dish or salad. Vegetable include those leafy outgrowths of plants or parts of plants that are used in making soup or eaten with the principal part of the meal (Onimawo and Egbekun, 1998). The use of vegetable is part of Africa's cultural heritage and vegetables play important roles in the food culture of African households (Ene - Obong, 2001).

Leafy vegetables are many ranging from leaves of annuals and shrub to leaves of trees. Green leafy vegetables and fruits occupy an important place among the food crops. Vegetables in diets have many positive effects upon health because of their constituents. Vegetables provide essential vitamins, minerals and other substances that are important to good health. They are cheapest most available rich sources of important proteins, amino acids, vitamins and minerals for humans (Okaka, Akobundu and Okaka, 2000). Leafy vegetables are important items of diet in many Nigerian homes, apart from the variety which they add to the menu (Mepha, Eboh, and Banigo, 2007). They are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, minerals, vitamins, fibers and other nutrients which are usually in short supply in daily diets (Mohammed and Sharif, 2011).

It is worthwhile to note that consumption of numerous types of edible plants as sources of food could be beneficial to nutritionally marginal population especially in developing countries where poverty and climate is causing havoc to the rural populace. Consumption of vegetables is very important because in many developing countries the supply of minerals is inadequate to meet the mineral requirements of farm animals and rapidly growing population. Minerals cannot be synthesized by animals therefore, must be provided from plants or mineral-rich water (Anjorin, Ikokoh, and Okolona, 2010).

Table 2.1 Classification of vegetables

Vegetables can be classified according to their type and taste

Type	Example
Bulb vegetable	onions, garlic and shallots

Fruit vegetables	avocados, cucumber, okra, tomatoes, pepper and egg plants
Leafy vegetables	bitter leave scent leave spinach and cabbage
Inflorescent vegetables	broccolis and artichokes
Root vegetables	carrots, beets, radishes and turnips
Stalk vegetables	asparagus bamboo celery
Tuber vegetables	cassava, sweet potatoes and taro.

Source Iloveindii, 2004

2.1.1 Intake of fruits and vegetables.

Nutrient intake is determined by so many factors and the same applies to fruits and vegetables. These factors include, age, gender health status and physical activity (www.healtyliving lookinglight.com). Pregnant and lactating mothers should aim to eat 4 to 5 serves of fruits and 5 to 7 serves of vegetables to meet extra demands of the body.

Table 2.2 Recommended daily intake of fruits and vegetables for adults

Lightly active	**Woman**	**19-30**	**2**	**$2_{1/2}$**	**$4_{1/2}$**
		31-50	$1_{1/2}$	$2_{1/2}$	4
	Men	19-50	2	3	5
		50+	2	$2_{1/2}$	$4_{1/2}$
Moderately active	Woman	19-30	2	$2_{1/2}$	$4_{1/2}$
		50+	$1_{1/2}$	$2_{1/2}$	4
	Men	19-30	2	$3_{1/2}$	$5^{1}/_{2}$
		31+	2	3	5
Very active	Woman	19-30	2	3	5
		31-50	$2_{1/2}$	$3_{1/2}$	6
		51+	2	$2_{1/2}$	$4^{1}/_{2}$
	Men	19-30	$2^{1}/_{2}$	4	$6^{1}/_{2}$
		31-50	$2_{1/2}$	$3_{1/2}$	6
		50+	2	3	5

Source (www. Healthy livinglookinglight.com)

2.1.2 Some of the major nutrients found in green leafy vegetables

Nutrients are the major chemical substances found within the foods that the body utilizes for optimum wellness. There are six classes of nutrients namely carbohydrate, protein, fats and oil,

(lipids) vitamins, minerals and water. These nutrients can further be grouped according to their functions. The groups are as follows:

Energy yielding nutrients viz carbohydrates, protein, fats and oil (lipids); Nutrients needed for growth and development; protein, minerals lipids and water. For promoting body processes; are protein, vitamins, minerals, lipids and water (Nnam, 2010). The sources of carbohydrates include starchy roots and tuber, cereals and etc. Rich sources of protein are meat, fish, legumes etc. Fats and oil can be gotten from plant and animals fats such butter, margarine, palm oil, soy bean etc. Green leafy vegetables are very rich sources of water, vitamins and minerals.

2. 1. 2.1: Proximate composition of green leafy vegetables

Analysis of some leafy vegetables revealed an appreciable quantities' of different nutrients. The proximate analysis of many vegetables reveals the presence of Moisture, protein, ash, fat crude fibre and carbohydrate. For example, proximate analysis carried out on bitter leaf sample showed the following results: crude protein 22.45 ± 0.01%; crude fat (3.45 ± 0.0%); crude fiber (16.0 ± 0.0%) and Ash (9.95 ± 0.2%), (Nwaoguikpe, 2010*)*.

Moisture: Moisture or water is a universal solvent. It dissolves other substances, carries nutrients and water soluble materials round the body, making it possible for every organ to perform its functions effectively. Consumption of vegetables is one of the major sources of water for the cells of the body (Raven, Johnson and Madison, 1999). The high moisture content of vegetables provides for greater activity of water soluble enzymes and co-enzymes needed for metabolic activities of the leafy vegetables (Iheanacho and Udebuani, 2009). Fresh green leafy vegetables generally have more moisture than dried vegetables. In different studies to determine the moisture contents of vegetables these results were recorded: Wake (2014) documented moisture content of (11.325%) for *Combretum zenkeri***.** Ujowundu *et al. (*2010) recorded **(**67.20%) for *Hymenocardia ulmoides* While according to Andzouana, and Mombouli (2011) *Vitex ferruginea* had (55.41%). However, Ajibade and Fagbohu (2010) recorded 7. 11% for *Tylophora glauca* and Moyo, Masika, Hugo, and Muchenje (2012) recorded (9.533%) for *Morinda Oleifera.*

Protein; It is a complex compound that contains nitrogen in addition to carbon, hydrogen and oxygen. The presence of nitrogen supports growth. Basically protein is either categorized as animal

or plant protein. Animal protein contains proteins with high biological values. When eaten in sufficient amount, animal will provide all the essential amino acids. However, plant proteins do not contain all the essential amino acids in the right proportion. For example, legume does not contain enough sulphur amino acid like cysteine and methionine but have abundant lysine. Proteins also regulate body process example hormones and enzyme activities etc. leafy vegetables are sources of protein and can add to the protein status of the body. According to Caunii, Cuciureanu, Zakar, -Tenea and Guichici. (2010) protein values of (1.21g/100g), (1.62g/100g), (2.97g/100g) (3.46g/100g) were reported for Cabbage, Lettuce, Celery leaves and leaf parsley respectively. Also, (Ujuwundu *et al.*, 2010) reported 53.98% for *C. zenkeri* while Moyo, Masika, Hugo, and Muchenje (2012) documented protein value of 20.5398% for *M. Oleifera.*

Fats and oil; Vegetables generally are not good sources of oil. Vegetables as low sources of oil have been documented by earlier studies. According to Adesuyi *et al. (*2011), *A. barbadensis* recorded 0.27% fat while (Ujuwundu *et al.*, 2010) recorded, that *C. Zenkeri* had 2.27% fat. *A. Vera* 2.91%. Ahmed, and Hussain (2013) and *Moringa olifera* 6.5% fat as noted by Moyo, Masika, Hugo, and Muchenje (2011). Vegetable fats and oil lower blood lipids thereby reducing the occurrence of disease associated with damage of coronary artery (Ononugbu, 2012). Vegetable fat has been recommended for better health since mid-1960's Mc Dougall (2013).

Ash; Ash content of plant based food is the function of the mineral elements present. The total ash content which is a measure of the non-volatile inorganic constituents remaining after ashing is the measure of the mineral composition of the food (Ijeh and Ejike, 2011). The greater the ash contents of a green leafy vegetable the greater the mineral composition of the leaf. In previous studies ash values of.1.386%, 2.36%, 7.64% were reported for leaves of *C. zenkeri* (Ujuwundu *et al.*, 2010), *A. barbandensis* Adesuyi *et al. (*2011) and *Moringa olifera* (Moyo *et al.*, 2011), respectively

Carbohydrates; Carbohydrates are plant products which are synthesized as the by-product of photosynthesis. This is consumed by man and animals as the major source of energy. Carbohydrates are hydrolyzed in the body to yield glucose, which can be utilized immediately or stored as glycogen in the muscles and liver for future use.

Carbohydrate provides energy to cells in the body particularly the brain, the only carbohydrate dependent organ in the body (Obizoba, 2009: Ujuwundu *et al* 2010). Interestingly, leaves of *P. thonningii* was reported to have carbohydrate value of 65.28% (Tijjani, *et al.*, 2014) while *H. ulmoides* and *V. ferruginea* were reported to have carbohydrate values of 8.57% and 4.53%, respectively (Andzouana and Mombouli, 2011). Asaolu, *et al*., 2012 reported lower carbohydrate values of 1.22 and 1.16mg respectively for *Gongronema latifilium and Ocimum gratissimum* leaves respectively.

2.1. 2. .2: Vitamins

Vitamins are a diverse group of organic molecules required in very small quantities in the diet for optimum health, growth and survival. These minor nutrients play a role in the metabolism of macronutrients (carbohydrate, protein and fats). The vitamin content of food can vary quite considerable especially in fruits and vegetables. Vitamin level depends among other things on freshness, variety, soil, climate condition during growth and storage.

The different vitamins are either chemically or functionally related. Two classical groups exist viz water and fat soluble vitamins. Water soluble vitamins are C and B group of vitamins. Fat soluble. Vitamins are A, D, E and K. Fat soluble vitamins can be toxic at very high dose because they can be stored in the body. Generally, the water soluble vitamins are harmless to the organism even in high doses since the excess is eliminated in urine.

The absence of vitamin from the diet or an inadequate intake results in characteristics deficiency signs or death (Gordom and kessel, 2002; Bakere *et al.,* 2010). Green leafy vegetables possess significant quantities of fat soluble and water soluble vitamins and the precursors. The study of vitamin contents of green leafy vegetables showed some of the vitamins present to include: Pro vitamin A (345.50 ± 0.0 IU), Vitamin C (228.40 ± 0.0 mg), Vitamin E (37.30 ± 0.01 mg), Vitamin. B_1 (1.0 ± 0.00 mg), Vitamin, B_2 (3.10 ± 0.00 mg) and Niacin, B_3 (0.41 ± 0.0 mg), *(*Nwaoguikpe, 2010*)*. However, sida acuta was shown to contain higher values of the vitamins 925+0.02(µg/100 g), 0.10+0.00(mg/100g), 0.02+0.01(mg/100g), 0.33+0.06(mg/100g) and 22.43+0.21(mg/100g) for β-Carotene, Thiamin, Riboflavin, Niacin, Ascorbic acid respectively (www.iosrjournals.org)

The B group of vitamins participates a lot in metabolism of the macro nutrients (carbohydrate, protein and fat).

Vitamin A; Vitamin A is a versatile vitamin and an important component of the visual pigments in the retina. Vitamin A regulates version, gene expression and cell differentiation thereby maintaining the health of the body lining and skin, immunity reproduction and growth. Retinol supports reproduction and is the major transport form of the vitamin.

Retinol is picked up by retinol binding protein from the liver to the blood. Vitamin A is stored in the liver and zinc is required for the release of vitamin A form where it is stored to the part of the body where it is needed. Half-life of vitamin A in the body is 200 – 300 days (Gordom and kessel, 2002: Vitamin A balance is not affected by minor disturbances in intake. It can only be affected by prolonged deprivation of vitamin A for about six months. Vitamin A in plants occurs as pro vitamin A.

Vegetable sources contain carotenoids such as beta carotene which is converted to retinol by the body. The deficiency may lead to night blindness, xeropthlamia, keratinization of skin (Gordom and kessel, 2002; Basavathappa, 2010).

Functions of vitamin A

Apart from being very active in maintaining a normal sight, it is also an anti-oxidant. It protects the cells of the body against damage caused by free radicals. Plant foods contain vitamin A only as beta carotene and are not as readily available as the profound form of vitamin A.

Thiamin (vitamin B_1); Thiamin is water soluble vitamin. The name comes from thia meaning sulphur and amine referring to nitrogen, Thiamin is destroyed when the chemical bond between each ring and central carbon is broken by prolonged heating. Thiamin is responsible for energy production in the cells. It participates actively in the metabolism of carbohydrates. It is very essential for mental health, normal functioning of the brain, muscle and nerves. Thiamin acts as antioxidant, together with other antioxidants; it protects the cells against acetyldehyde the substance that can cause cell mutation and cause cancer. A healthy adult needs 1mg/ day; pregnant women

and elderly need more of this vitamin. Recommended daily allowance is also increased by alcoholism, smoking, operation stress and high proportion of carbohydrate intake (Nnam, 2010).

Riboflavin (Vitamin B_2); Riboflavin is a component of two coenzymes: flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). It is synthesized by all green plants, most bacteria, yeast and molds. Animals have so far not been shown to synthesize riboflavin. It is very essential in production of niacin from tryptophan.

Riboflavin regulates the metabolism of carbohydrate, protein and fat. It ensures that oxygen is used for the production of energy. It participates as part of enzyme acting as oxidant factor. Persons deficient in vitamin B_2 show cheilosis, glossitis, keratitis, corneal vascularization and seborrheic dermatitis

Niacin (Vitamin B_3); Niacin is unique among the B vitamins in the sense that the body can synthesis it from protein. The amino acid tryptophan is partially converted to niacin in the body in the presence of vitamin B_2. Niacin plays a vital role in the release of energy from carbohydrate protein and fat during metabolism, niacin reduces the low density lipoprotein (LDC) cholesterol which increases the risk of hardening of the coronary artery suggesting that niacin may contribute in preventing heart diseases.

Niacin is found in dairy products, poultry, fish, lean meats, nuts, eggs and to a lesser extent in peas, beans, lentils and enriched breads and cereals. It can also be taken as a supplement. In one recent study, niacin increased HDL 'good' cholesterol by 29%, reduced triglyceride levels by 23% and reduced LDL 'bad' cholesterol by 8% in people with diabetes (www.healio.com/endocri minology). For people with diabetes, constant use of niacin will raise blood glucose levels, but at niacin levels between 750-2,000 mg per day, the rise in blood sugar is modest **(**www.healio.com/endocriminology).

Niacin mono therapy or in combination with other agents was in clinical use during the late 1970s and 1980s, but lipid-lowering treatments shifted largely to statins (American Diabetes care, 2009). Recent failures in developing HDL cholesterol–raising drugs have rekindled interest in this drug.

High-dose niacin (1–3 g) decreases VLDL, increases HDL, and has a modest effect on LDL. Among lipid-modifying agents, niacin is the most potent agent currently available to increase HDL cholesterol and the only one that reduces lipoprotein concentrations (American Diabetes care, 2009).

Much of VLDL production is controlled by the provision of fatty acids to the liver. Niacin is thought to decrease circulating fatty acids by inhibiting the release of fatty acids in adipose tissue mediated by hormone - sensitive lipase. The underlying mechanism is unknown, but recent information suggests that an orphan G protein–coupled receptor may be the nicotinic acid receptor and mediate the anti lipolytic effects of this vitamin (American Diabetes care, 2009). Niacin has the best effect on the cholesterol profile of people with diabetes, but if care is not taken, it may also increase blood sugar levels and can be difficult to tolerate.

Deficiencies of the energy releasing vitamins (thiamin, riboflavin, niacin) produce a number of overlapping symptoms. A deficiency of niacin causes pellagra in man and is characterized by 3D's namely dermatitis of the exposed parts, diarrhea and dementia (Jain, Jain, and Jain, 2005) Symptoms of thiamin deficiencies depends on the severity; mild deficiency; loss of appetite, fatigue, constipation, moderately severe deficiency; mental confusion, opthalmoplegia, and severe thiamin deficiency; dry and wet beriber (Bassavanthappa, 2010).

Ascorbic Acid (Vitamin C); Vitamin C unlike others is generally distributed throughout the body tissues. However, it is very unstable as it is destroyed by oxidation, heat, light or alkalis but fairly stable in acids. Humans and guinea pigs are not able to synthesize vitamin C and must get the vitamin from diet. Vitamin C maintains blood vessel flexibility and improves blood circulation in the arteries especially that of smokers. The most important benefit claimed for vitamins A and C is their role as antioxidants in which they scavenge oxygen free radicals reactive Nitrogen species (RNS) and reactive oxygen species (ROS). These chemically active particles are byproducts of many body's normal chemical processes. Their numbers are increased by environmental assaults such as smoking, chemicals, toxins and stress.

Vitamins C helps the body to absorb iron and to breakdown histamine, the inflammatory components of many allergic reactions (Omale, Nnacheta and Ijeh, 2009). Physiologically, the

major function appears to be the maintenance of normal intercellular substance throughout the body, Vitamin C is a ready scavenger. Vitamin C as an antioxidant is oxidized to regenerate already oxidized substance such as iron and copper to their original forms. Also in the process it removes the damaging oxidizing agents. Deficiency of vitamin C causes scurvy.

2.1.2.3: Minerals

Minerals are micro nutrients. They are inorganic substances essential for the correct functioning of the body. Minerals exist as inorganic element and in that state they can combine with any other element in the body to form salt. However, in the body it dissociates and is used separately. Because they are water soluble they associate with water to form acids or base as the case may be. Mineral bioavailability can be affected by none mineral substances in the diet. Minerals are important constituents of important compounds in the body for example thyroxin contains iodine, haemoglobin contains iron and insulin contains zinc.

Minerals play catalytic roles in the enzyme system especially during metabolism. The mineral composition of green leafy vegetable showed appreciable quantities of mineral for e.g Fe (11.0 ± 0.0 mg), This may explain why leafy vegetable are widely used in virtually all the homes for so many things *(*Nwaoguikpe, 2010*)*. Green leafy vegetables contain many minerals such as;

Iron (Fe); Iron is a necessary component of hemoglobin and myoglobin for oxygen transport and cellular processes of growth and division. Iron is also an essential trace element for normal functioning of the central nervous system and in oxidation of carbohydrates, proteins and fats (Moyo, Masika, Hugo, and Muchenje, 2011). The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide (Trowbridge and Martorell, 2002). The consequences of iron deficiency include reduced work capacity, impairment in behaviour and intellectual performance and decrease resistance to infection (Dioxin and Haris, 2004). Recent studies have shown that vegetables contain reasonable amount of iron. Asaolu et al. (2012) recorded Iron contents of 16.43mg/100g, 34.47 mg/100g, 18.41 mg/100g, 23.36 mg/100g, 39.04 mg/100g, 21.84 mg/100g and 34.92 mg/100g for Bitter leaf, Indian, Bush bulk, Scent leaf, Amaranthus, Hibiscus and Telfaria respectively. (Asaolu *et al.*, 2012).

Phosphorus (Ph); Phosphorus is essential component of bone mineral. Phosphorus has been reported to be good for bones and teeth formation. A balance proportion of calcium and phosphorus is needed in the body. It contributes to energy production by participating in the breakdown of carbohydrates, protein and fats. It is needed for growth, maintenance and repair of tissues and cells and for the production of DNA and RNA (Ajibade *et al.,* 2010). Deficiency of phosphorus- calcium balance results in osteoporosis, arthritis, rickets and tooth decay. The level of phosphorus in leafy vegetables showed moderate concentration (Ajibade *et al.,* 2010). Asaolu *et al* documented Phosphorus values of 12.52mg/100g, 15.38mg/100g, 29.42 mg/100, 21.65 mg/100g, 32.63 mg/100g, 36.91 mg/100g, 26.19 mg/100g for Bitter leaf, Indian, Bush bulk, Scent leaf, Amaranthus, Hibiscus, and Telfaria respectively. However, (Ajibade *et al.,* 2010) documented phosphorus ranged between 26.19mg/100g and 36.91mg/100g in their studied vegetable samples.

Selenium; Selenium one of the phytochemicals with physiological properties may be elements rather than complex organic molecules. Selenium, abundant in many fruits and vegetables, is a dietary mineral involved in major metabolic pathways, including thyroid hormone metabolism and immune function Papp, Lu, Holmgren, and Khanna (2007). Particularly, it is an essential nutrient and cofactor for the enzymatic synthesis of glutathione, an endogenous antioxidant. Selenium is a naturally occurring trace mineral required to maintain good health (Marcason, 2008; Rayman, 2008). Selenium is a key component of a number of selenium proteins involved in essential enzymatic functions such as redox homeostasis, thyroid hormone meta-bolism, immunity and reproduction (Burk, 1998). The redox homeostatic function helps to maintain membrane integrity, protect prostacyclin production and reduce the likelihood of propagation of further oxidative damage to biomolecules which is usually associated with increased risk of diseases**.**

Potassium (K);

Potassium is an important nutrient in plants, essential in many processes needed to sustain plant growth and reproduction. It helps plants to resist drought and the effect of excessive temperature. It also increases plant resistant to diseases. Pottasium aids plants in the production os starches, controls root growth and regulates the opening and closing of stomatal pores which is important for efficient water use. In human, the main function of potassium in the body is the maintenance of the osmotic pressure and the acid-base equilibrium of the body. Potassium is also important in

the formation of proteins and glycogen. Potassium and sodium ions are known activators of energy potentials across nerve membrane. Together with calcium ions, potassium may serve as replenishment in diarrhea, maintenance of normal nervous function and gut peristalsis. Insulin causes K^+ to enter cells with a resultant lowering of the extracellular K^+ concentration. Infusion of insulin and glucose significantly lower the plasma K^+ level in normal individuals and are very effective for the temporary relief of hyperkalemia in patients with renal failure. Hypokalemia often develops when patients with diabetic acidosis are treated with insulin. The reason for the intracellular migration of K^+ is still uncertain. However, insulin increase the activity of N^+ - K^+ ATPase in cell membranes, so that more K^+ is pumped into cells (Kim, 2010).

In a study of seven leafy vegetables namely Bitter leaf, Indian leaf, Bush bulk Scent leaf Amaranthus, Hibiscus and Telfaria by Asaolu *et al (*2012), they recorded potassium values of 73.25mg/100g, 16.85 mg/100g, 99.01 mg/100g, 86.24 mg/100g, 168.96 mg/100g, 84.11 mg/100g and 130.24 mg/100g respectively.

Zinc (Zn); Zinc is a trace element and it is found in all human tissues. Zinc content of the human body has been estimated to be 2-3g. About 6 and 30% are present in the skin. The zinc in these tissues is not accessible in times of deprivation. It is assumed that the body has specific reserve and it is dependent on regular supply of the element. Zinc is transported in the plasma bound to albumen and α_2 – macroglobulin but only 0.1% of the body. Absorption is hampered by phytate and calcium. Studies have shown up to 50% decrease in zinc absorption when calcium supplements are taken Also, Asaolu and Asaolu (2010) reported moderately high level of Zn between 13.73 and 24.64mg in their leafy vegetables samples. Zinc is an essential micronutrient for human growth and immune functions. An estimated 20% of the world population is reported to be at risk of inadequate Zinc intake (Hotz and Brown, 2009). Studies on Nigerians show that zinc deficiency affects 20% of children less than five years; 28.1% of mothers and 43.9% of pregnant women (Dioxin and Haris, 2004). Zinc is also assumed to play a significant role in insulin production and function.

Zinc has been shown to be associated with three main functions: catalytic, regulatory and structural roles.

1. Zinc plays a significant role in large number of zinc requiring enzyme, the zinc complex enzymes and the metalo enzymes.
2. It plays a role in activating the enzymes needed in protein synthesis
3. It is loosely associated with insulin (An important hormone regulating carbohydrate metabolism).

2 .1.2.4: Phytochemicals

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Phytochemicals are important biochemical used for body proceses. These important biochemicals help to protect health. They are found only in plant based foods in very small quantity (Nnam, 2011). Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients (USA Guidance for Industry, 2014). There may be as many as 4,000 different phytochemicals (USA Guidance for Industry, 2014). The phytochemicals, tannins, saponins, alkaloids, flavonoids, terpenes and phenols were found to be present in leaves and are in amounts to be of medicinal value. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals.

Without specific knowledge of their cellular actions or mechanisms, phytochemicals have been considered possible drugs for millennia. For example, Hippocrates may have prescribed willow tree leaves to abate fever. Salicin, having anti-inflammatory and pain-relieving properties, was originally extracted from the bark of the white willow tree and later synthetically produced to become the staple, over-the-counter drug aspirin (Brown and Authur, 2001; Landau, 2014).

Plants used in the treatment of diseases are said to contain bioactive principles with biological activities. Some of which are responsible for the characteristic odour, pungencies and colour of plant, while others give the particular plant its culinary, medicinal or poisonous virtue (Evans, Trease and Evans, 2002). Numerous studies have shown that phytochemicals found in large quantities in fruits and vegetables are responsible for their protective effect (Sundarrayanan, Kumia and Sekar, 2011).

Many plants containing alkaloids and flavonoids have diuretic, antispasmodic, anti-inflammatory and analgesic effects (Ujowundu *et al.,* 2010). The quantitative phytochemical analysis of green leafy vegetable revealed the presence of flavonoids (0.85 ± 0.11 mg), tannins (0.37 ± 0.2 mg), saponins (2.2 ± 0.0 mg), polyphenol (0.35 ± 0.11 mg), alkaloids (2.13 ± 0.10 mg) and HCN (12.25 ± 0.10 mg *(*Nwaoguikpe, 2010*).*

Furthermore, phytochemical composition of 125.00+0.00mg/100g, 406.67+2.89mg/100g, 1751.67+2.89mg/100g,1255.00+0.00mg/100g,85.00+0.00mg/100 and 90.00+0.00mg/100g were recorded for Tannins, Saponins, Alkaloids, Flavonoids, Terpenoids and Phenolics resprctively (www.iosrjournals.org)

Phytochemicals can be candidate nutrients for example, specific phytochemicals, such as fermentable dietary fibers, are allowed limited health claims (United States Food and Drug Administration (US FD, 2014; American Cancer Society, 2013).

Clinical trials and health claim status of phytochemicals

Phytochemical-based dietary supplements can also be purchased (Bongoni *et al.,* 2014). Available scientific evidence does not support claims that taking phytochemical supplements is as good for long-term health as consuming the fruits, vegetables, beans, and grains from which they are taken American Cancer Society (2014) as cited by (Bongoni *et al.,* 2014)

Food processing and phytochemicals

Phytochemicals in freshly harvested plant foods may be degraded by processing techniques, including cooking (Liu, 2004; Rao and Rao, 2007; Dekker, 2013; Bongoni *et al.,* 2014*;* Palermo, Pellegrini and Fogliano, 2014). The main cause of phytochemical loss from cooking is thermal decomposition Palermo *et al.* (2014). A converse exists in the case of carotenoids, such as lycopene present in tomatoes, which may remain stable or increase in content from cooking due to liberation from cellular membranes in the cooked food (Agarwal, Shen, Agarwal and Rao, 2001; Dewanto, Wu, Adom, Liu, 2002). Food processing techniques like mechanical processing can also free carotenoids and other phytochemicals from the food matrix, increasing dietary intake (Hotz and Gibson, 2007; Palermo *et al.,* 2014). The phytochemicals are numerous and includes alkaloids, flavonoids, saponins tannins etc.

Alkaloids; Alkaloids are capable of reducing headache associated with hypertension. It has been reported that alkaloids can be used in the management of cold, fever and chronic catarrh. Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity. However, when ingested by animals, they affect glucagon, thyroid stimulating hormones and inhibit certain enzymatic activities (Okaka, Enoch, and Okaka, 1992)) as cited by (Eyong, Agiang, Atangwho, Iwara and Ebong, 2011).

Carotenoids; Carotenoids, the basic source of yellow, orange, and red plant pigments, are widely distributed in nature (Aoki *et al.,* 2002). They are present in all living organisms, from bacteria, yeast, algae to higher plants and animals (Basu, Vecchio, Flider and Orthoefer, 2001). Carotenoids are compounds constituted by 5 carbons, eight isoprene units joined in a head to tail pattern; most of them have 40 carbon atoms (Bonnie and Choo, 1999). Carotenoids are lycopene, which are exclusively hydrocarbons (those without any oxygen molecule), Aizawa and Inakuma (2007) and secondly, xanthophylls (for example, lutein, zeaxanthin, fucoxanthin and astaxanthi) which are oxygenated containing hydroxyl, methoxy, carboxyl, keto, or epoxy groups (Britton, 1995; Basu *et al.*, 2001). Zeaxanthin content in wolfberry is very high (Chang, Ho, Yu and So 2010). This compound has been used in the cosmetic industry and the feed industry for birds, swine and fish.

These compounds are a group of more than 700 naturally occurring pigments. Most of them can be found in higher plants, especially in their leaves, flower and fruits. Humans do not produce carotenoids however; they must ingest carotenoids from food and metabolize them for use in physiological functions (Chang, Ho, Yu, and So 2010). Carotenoids play important biological roles as accessory light-harvesting components of photosynthetic systems, photo protecting, antioxidants, and regulators of membrane fluidity (Aoki *et al*., 2002).

These compounds responsible for color ranging from light yellow through orange to deep red are biosynthesized in all of the photosynthetic organisms containing cyanobacteria, algae and higher plants, and also in some of non-photosynthetic bacteria, yeasts and fungi (Misra, 2009).

Carotenoids as accessory light-harvesting pigments play an essential role in the protection of plants against excess light and photo oxidative stress (Demmig-Adams and Adams, 2002) as cited by (Irwandi, 2011). Furthermore, Carotenoids are intracellular products and are usually located in

membranes of mitochondria, chloroplasts or endoplasmic reticulum Carotenoids are also widely used in food applications (Bonnie and Choo, 1999). Carotenoids such as β -carotene and lycopene have plenty of scientific and commercial values. Traditionally, carotenoids have been used in the feed, food and nutraceutical industries.

The recent discoveries of health-related beneficial properties attributed to carotenoids have spurred great interest in the production of structurally diverse carotenoids for pharmaceutical applications. Currently, carotenoids are used commercially as natural food colorants, nutrient supplement Bonnie and Choo (1999), feed additives (Bhosale and Bernstein, 2005)**,** animal feed supplements and, more recently, as nutraceuticals for cosmetic and pharmaceutical purposes Carotenoids have been known to have some medicinal properties. Presently, only a few

carotenoids can be produced commercially by chemical synthesis. Currently, commercial production of carotenoids from microorganisms competes mainly with synthetic manufacture by chemical synthesis. However, most of the commercially used carotenoids (for example, β-carotene, astaxanthin and cantaxanthin) are produced by chemical synthesis. Furthermore, in most countries, such as Europe and United Statea of America, the application of carotenoid, namely as food additives (including colorants) is governed by strict regulation.

Fruits and vegetables constitute the major sources of carotenoid in human diet. About 40 are present in atypical human diet (Rao and Rao, 2007). Although, carotenoids are present in many common human foods, deeply pigmented fruits, juices and vegetables constitute the major dietary sources with yellow-orange vegetables and fruits providing most of the β-carotene and α-carotene, orange fruits providing α-cryptoxanthin, dark green vegetables providing lutein and tomatoes products lycopene

Sources

Carotenoids are found in colored fruits and vegetables. Apricots, cantaloupe, carrots, pumpkin and sweet potato are sources of α-carotene and β-carotene. Pink grapefruit, tomatoes and watermelon are sources of lycopene, α- carotene, β -carotene, phytofluene and phytoene. Spinach is a source of lutein, zeaxanthin, α- and β -carotene; mango, papaya, peaches, prunes, squash and oranges are sources of lutein, zeaxanthin, β -cryptoxanthin, α -, β-carotene, phytofluene and phytoene. The

content of carotenoids (β-carotene, lycopene, lutein, zeaxanthin and β -cryptoxanthin) have been reported by Britton and Khachik (2009). However, the precise values are not given but the content is indicated as a range, as follows; low: 0 to 0.1 mg/100 g, medium: 0.1 to 0.5 mg/100 g, high: 0.5 to 2 mg/100 g, very high: >2 mg/100.

Flavonoids; Flavonoids are known for their antioxidant activity and hence they help to protect the body against cancer and other degenerative diseases (Mensah, Ihenyen and Okhiure, 2013). Flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-viral, anti-neoplastic, anti-thrombotic and vasodilatory activity (Iniaghe, Malomo, and Adebayo, 2009). Flavonoids in general serve as flavouring ingredients in plants. Besides their role as flavouring agents they are also expressed in plants in response to microbial infection suggesting their antimicrobial activity (Eyong *et al.*, 2011). Flavonoids have also been implicated as antioxidants both in physiological and diseased states. For instance, tea flavonoids have been reported to reduce the oxidation of low-density lipoprotein, lower the blood level of cholesterol and triglycerides (Erdman, 2007).

Tannins; This bioactive compound is known to have potentials anti-viral activity (Cheng, Lin, and Lin, 2002) as well as potential prophylactic and therapeutic effect against cancer cells, but via different mechanisms. Tannins are known to exhibit antiviral, antibacterial and antitumor activities http;//scollar.goggle.com. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as diuretic. Tannins are well known for their antioxidant and antimicrobial properties as well as for soothing relief, skin regeneration, as anti-inflammatory and diuresis. Tannin is also an anti nutrient that can affect the absorption of food. Its effect is felt at a range between 3 – 8mg/100g (Chung, 1998).

Saponnins; Saponnins are expectorants, cough depressants and are administered for haemolytic activities (Mensah *et al.*, 2012). Saponins are known for their multi biological function including their anti-diabetic effects (Kenjiro et al., 2006; Alyemeni et al., 2011). Saponin as a class of natural products is also involved in complex with cholesterol to form pores in cell membrane bilayers (Francis et *al.*, 2002**;** Eyong *et al.*, 2011). Saponins are known bioactive substances that can reduce the uptake of cholesterol and glucose at the gut through intra-luminal physiochemical interaction (Price, johnson and Feriwick, 1987). Therefore, in medicine, saponnin is used for

hypercholesterolemia, hyperglycaemia, and weight loss and also as antioxidant, anticancer and anti-inflammatory. It has also been reported to have antifungal properties (Tijjani *et al.*, 2012). Saponins exhibit cytotoxic effect and growth inhibition against a variety of cells making them have anti-inflammatory and anticancer properties (Iniaghe, *et al.*, 2009).

Terpenoids, Traditional medicine has been a fertile source for revealing novel lead molecules for modern drug discovery. In plants, terpenoids represent a chemical defense against environmental stress and provide a repair mechanism for wounds and injuries. Interestingly, effective ingredients in several plant-derived medicinal extracts are also terpenoid compounds of monoterpenoid, sesquiterpenoid, diterpenoid, triterpenoid and carotenoid groups (Salminen, Lehtonen and Suuronen, 2008).

Inflammatory diseases and cancer are typical therapeutic indications of traditional medicines. It seems that during evolution, plants have established a terpene-based host defense which also

represents a cornucopia of effective therapeutic compounds for common human diseases (Salminen, *et al.*, 2008). Terpenes are very important group of organic compounds that have been reported as potent drugs used in treatment of wide range of ailments. The most rapidly acting anti - malarial Artemisin and its derivate are terpenes (Tijjani *et al.*, 2012)

Phenols; Phenols are also present in plant. It is one of the major groups of compounds acting as primary antioxidant or free radical scavenger (Adesina 2011). The efficacy of the utilization of leaves in herbal/ traditional/ folklore medicine may be attributed to its phytochemical profile.

2.1.3: Vegetables and chronic diseases

Vegetables provide essential vitamins, minerals, fibre, phytochemicals and other substances that are very important to good health. Some leafy vegetables have herbal/folklore preparation with anti-diabetic activity (Nnam, Onyechi and Madukwe, 2012).

Sathanarayan and Fasik (2009) also, identified that turning to green leafy vegetables is a great advantage in the diabetes management as it provides safety and freedom from the hazardous effects of diabetes on other systems. Many vegetables apart from serving as foods also possess some medicinal properties and as such are used in folk medicine for the treatment of various diseases including chronic non-communicable diseases such as diabetes, cardiovascular diseases etc.

Numerous epidemiological studies have correlated human consumption of foods rich in fruits and vegetables containing high levels of phytochemicals to lower the risk for chronic diseases such as diabetes, cancer, cardiovascular diseases (Shitu, 2009). Many leafy vegetables and their extracts are effective in the treatment of many non - communicable diseases (NCDs). Fifa *et al*. (2013) also affirmed that herbs play important role in diabetic therapy particularly in developing countries where most people have limited resources and do not have access to modern medical treatment for chronic diseases such as hypertension, cardiovascular diseases, diabetes mellitus etc.

Vegetables are low in calories and fat, high in fiber and are filling thus they can help to control weight and maintain good health. Eating plenty of vegetables everyday can help reduce risk of heart disease, high blood pressure, type 2 diabetes and certain cancers. Since vegetables are usually high in fiber and researches have shown that eating foods high in fiber lower blood sugar

levels, as such, they are important, protective and highly beneficial foods for the maintenance of good health and prevention of diseases Furthermore, diets containing vegetables are usually high in fibre and researches have shown that eating foods high in fibre lower blood sugar levels and such food is very beneficial in the management of diabetes

2.2 Diabetes mellitus

The constellation of abnormalities caused by insulin deficiency is called diabetes mellitus. The word *diabetes* is borrowed from the *Greek* word sipho meaning a *siphon* because affected individuals experience polyuria and pass water like a siphon. Diabetes mellitus, one of the most common metabolic disorders affecting human kind is known since ancient times. Ever since then man continued to search for its cure. Discovery of insulin since 1921 and some other breakthroughs in medicine and human nutrition have neither contributed to clear understanding of its aetiology nor to find its cure. Diabetes mellitus is as old as man.

Diabetes could either be mellitus or less often, insipidus. Greek and Roman Physicians used the term “diabetes” to refer to the condition in which the cardinal finding was the large urine volume and two types were distinguished:” diabetes mellitus” in which the urine tasted sweet; and “diabetes insipidus,” in which the urine had little taste. Today, the term diabetes insipidus” is reserved for

conditions in which there is a deficiency of the production or actions of vasopressin and the unmodified word “diabetes” is generally used as a synonym for diabetes mellitus.

Therefore, diabetes mellitus (DM) describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Dhanush, Krishna, Suguna, and Sathanarayan, 2014).

There are two main types of diabetes mellitus viz juvenile (type1) and adult onset diabetes (type 2) diabetes. Though there is juvenile (type 1) diabetes but the most common type of diabetes is type 2 diabetes. This was formally called adult onset diabetes since the disease usually develops during adulthood. In type2 diabetes the pancreas usually produces insulin but the body cell cannot respond to it. Hyperglycemia therefore results from impairment in insulin secretion combined with or without impairment of insulin action. In diabetes there is defect in insulin secretions, action or both. The two forms of diabetes (Types 1 and 2) differ in their pathogenesis but both have hyperglycemia as a common hallmark. Chronic hyperglycaemia is known to damage almost all cell types in the body.

The United Nations (UN) defines diabetes as a chronic, debilitating, and costly disease associated with severe complications which pose severe risks for families, member states, and the entire world; and serious challenges to the achievement of the internationally agreed development goals, including the Millennium Development Goals (MDGs) (Oputa and Chinenye, 2012).

Often, the predisposing factors of most types of diabetes are multi- factorial: environmental, lifestyle and/or genetic factors are at play (Gerald and Sandra, 2010). Pre-diabetes, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) may progress to overt type 2 diabetes (Walsh and Crumbie, 2007; Oputa, and Chinenye, 2012).

The cause of clinical diabetes is always a deficiency of the effects of insulin at the tissue level (Guyton, 1984; Bassavanthappa, 2010; Gerald and Sandra, 2010). Insulin is recognized for its important role in proper glucose transport, vasodilatation, and anti-oxidant, anti-inflammatory, as well as profibrolytic properties (Vink, 2009).

Insulin resistance is also accompanied by compensatory loss of hormonal homeostasis and activation of local tissue is responsible for most of the complications associated with diabetes mellitus. Types 1 diabetes, or insulin-dependent diabetes mellitus (IDDM), is due to insulin deficiency caused by autoimmune destruction of the Beta cells in the pancreatic islets, and it accounts for 3-5% of cases and usually present in children. Types 2 diabetes, or non-insulin-dependent diabetes mellitus (NIDDM), is characterized by the deregulations of insulin release from the Beta cells, along with insulin resistance in peripheral tissues such as skeletal muscles, brain, and liver. Type 2 diabetes is usually present in overweight or obese adults (Bassavanthappa. 2010; Gerald and Sandra, 2010). The number of person diagnosed with type 2 diabetes has continued to rise globally due to adoption of unhealthy lifestyle.

Diabetes is characterized by polyuria (passage of large volumes of urine), polydipsia (excessive drinking), and weight loss in spite of polyphagia (increased appetite), hyperglycemia, glycosuria, ketosis, acidosis, and coma (Gerald and Sandra, 2010). Widespread biochemical abnormalities are present but the fundamental defects to which most of the abnormalities can be traced are reduced entry of glucose unto various "peripheral" tissues and increased liberation of glucose into circulation from the liver (Gerald and Sandra, 2010) Therefore, there is an extracellular glucose excess and, in many cells, an intracellular glucose deficient-a situation that has been called "starvation in the midst of plenty". Also the entry of amino acids into muscle is decreased and lipolysis is increased. Chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually failure of organs, especially the kidney, nerves, heart, eyes and blood vessels (Rajeswaria and Sridevi, 2014).

Therefore, Diabetes mellitus as a metabolic disorder affects the way the body handles basic food components like carbohydrates, protein and fats. This is mostly due to lack or abnormal action/ effect of the hormone insulin. In diabetic patients, the body loses insulin producing capacity as a result of pancreatic B- cell apoptosis or insulin insensitivity (Kim, Lee, Park and Cho 2010). The cytokines, lipo-toxicity and gluco-toxicity are three major stimuli for B-cell apoptosis. Evidence of increasing control of hyperglycaemia, hypertension and dyslipidemia may postpone the development of diabetic complications in type 2 diabetes mellitus.

Diabetes being a chronic disease can only be managed. A diagnosis of diabetes mellitus brings about a total change in the patient's lifestyle, including employment, self-esteem, diet, physical activity and relationship with people. Diabetes places individuals in a state of depression as they may have to refrain from some childhood ingrained family feeding habit due to the need to monitor or regulate the blood sugar. Therefore, diabetes is one of the most challenging chronic diseases which pose life threatening complications. It requires lifelong compliance to treatment and diet on the part of the patient to prevent complications and attain better glycaemic control. Unfortunately, only a small portion of patient with type 2 diabetes attains the recommended dietary regimen (Vink, 2009).

As a result of that, diabetes is often complicated by recurrent infections, varied eye problems, erectile dysfunction, poor obstetric outcome, skin ulcers and gangrene, renal disease, and acute hyperglycaemic emergencies (American Diabetic Association, 2011; Oputa, and Chinenye, 2012). All the above complications ensure a high morbidity and mortality. The prevention and the delay in time of onset of type 2 diabetes, will curtail the current global diabetic epidemic.

2.2.1: Prevalence of diabetes mellitus

Globally, there are different prevalent levels varying from country to country, race and ethnic groups. The International Diabetes Federation (IDF) in its publication in 2011 came up with prevalence and incidence values around different regions and countries of the World. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is expected to rise from 171 million in 2000 to 366 million in 2030. Nigeria with over 250 tribes and different culture and food values, the prevalence values has not been uniform though the International Diabetes Federation recorded 3.9% in its Diabetes Atlas (Akaogu, 2010).

In Nigeria, with over 140 million people (2006 census), an estimated six million people have full blown diabetes mellitus. The 1992 National prevalence study on Non-communicable diseases

conducted by the Federal Ministry of Health in 13 states of the Federation indicated a prevalence rate of 2.7% with a prevalence of 2.6% in adult males and 2.8% in adult females. Reports from subsequent studies such as hospital records indicated an alarming increase in both prevalence and incidence of Diabetes among all ethnic groups and social classes in Nigeria (Diabetes Association of Nigeria, 2011). Indeed**,** Diabetes has in the last two decades become a household disease and has robbed Nigeria - nation of an increasing number of distinguished leaders and scholars of high repute (Diabetes Association of Nigeria, 2011).

The International Diabetes Federation (IDF) estimated in 2011 that 366 million adults, aged 20–79 years of the world's 7 billion people have diabetes. This gives a comparative prevalence of 8.5%. Since more than 90% of the global cases of diabetes are type 2, it is evident that the epidemic is mainly due to the escalation of the causes of type 2 diabetes. A recent revelation by (WHO,2013) indicates that Diabetes has tripled in the last two decades globally with the highest prevalence rates found in developing countries. The WHO report indicates that in extreme cases, up to 30 - 50% of the adult population in some developing countries have been afflicted with diabetes. The (IDF) report of 2011 further alerted that diabetes will continue to be a major threat to public health beyond the year 2030 and is set to increase worldwide if appropriate preventive strategies are not instituted.

The prevalence of asymptomatic or undiagnosed diabetes is high (50–85%) in the general population, depending on the country in question. Therefore, only a small proportion of cases present in health facilities with classical symptoms of polyuria, polydipsia and weight loss. The gradual progression of pre- diabetes to diabetes may account for the high prevalence of most asymptomatic and undiagnosed diabetes. The progressive increase in the prevalence rates of diabetes is associated with lifestyle changes; overweight and obesity, physical inactivity, alcohol consumption, dietary changes, cigarette smoking and a lot of other factors that are potentially modifiable. Therefore, Diabetes remains the 7th leading cause of death in the United States in 2010 with 69 07% death certificate and a total of 234051 deaths listing it as the underlying or contributing cause of death (American Diabetes Association, 2011). Diabetes causes more death a year than breast cancer and HIV /AIDs combined together (Diabetic society, 2014).

The disease has reached an epidemic proportion in Nigeria according to recent publications and has resulted to premature death of thousands of Nigerians in addition to permanent disabilities like

blindness, amputation of limbs, impotence, kidney failures, still births, pregnancy wastages etc. Apart from HIV-AIDS which is an infection, Diabetes is now the major disease of this century, the next century in Nigeria and the world at large (Diabetes Association of Nigeria, 2011).

Unfortunately, so much attention is being given even recently to communicable diseases like HIV, tuberculosis and malaria at the detriment of the emerging epidemic of non-communicable disease like diabetes, hypertension and heart disease. Following the trend of diabetes, it is not surprising that over 30% of our elite population including decision-makers may be diabetic. More painfully so, majority of Nigerian diabetic population cannot afford meaningful treatment; and about over 80% of the healthy population is ignorant about the treatments and management of diabetes.

2.2.2 Pathophysiology of diabetes

Hyperglycemia by itself can cause symptoms resulting from the hyperosmolality of the blood. The plethora of glucose outside the cells in diabetes contrasts with the intracellular deficit. Deficient glucose utilization and deficient hormone sensing (insulin, leptin, CCK) in the cells of the hypothalamus that regulate satiety are the probable causes of hyperplagia in diabetes. The feeding area of the hypothalamus is not inhibited and thus satiety is not sensed so food intake is increased.

In addition, there is glycosuria because the renal capacity for glucose reabsorption is exceeded. Excretion of the osmotically active glucose molecules entails the loss of large amounts of water (osmotic dieresis). The resultant dehydration activates the mechanisms regulating water intake, leading to polydipsia. There is an appreciable urinary loss of Na and k as well. For every gramm of glucose excreted, 4.1 kcal is lost from the body. Increasing the oral caloric intake to cover this loss simply raises the plasma glucose further and increases the glycosuria, so mobilization of endogenous protein and fat stores and weight loss are not prevented. Glucose catabolism is normally a major source of energy for cellular processes, and in diabetes energy requirements can be met only by drawing on protein and fat reserves. So mechanisms are activated that greatly increase the catabolism of protein and fat, and one of the consequences of increased fat catabolism is ketosis. Thus, glycogen depletion is a common consequence of intracellular glucose deficit, and the glycogen content of liver and skeletal muscle in diabetic animals is usually reduced.

When plasma glucose is episodically elevated over time, small amounts of hemoglobin A are nonenzymatically glycated to form HbA_{IC} ln long-standing diabetes additional problems occur. These include microvascular, macrovascular, and neuropathic diseases. The microvascular abnormalities are proliferative scarring of the retina leading to blindness and renal disease (diabetes nephropathy) leading to renal failure. The macrovascular abnormalities are due to accelerated atherosclerosis, which is secondary to increased plasma LDL. The neuropathic abnormalities involve the autonomic nervous system and peripheral nerves. The neuropathic plus the atherosclerotic (diabetes neuropathy) involve the autonomic nervous system and peripheral nerves. The neuropathy plus the atherosclerotic circulatory insufficiency in the extremities and reduced resistance to infection can lead to chronic ulceration and gangrene, particularly in the feet (Kim, 2010). In addition, intracellular glucose can be converted to so-called Amadori products, and these in turn can form advanced glycosylation end products (AGEs), which cross-link matrix proteins. This damages blood vessels. The AGEs also interfere with leukocyte response to infection.

In addition, intracellular glucose can be converted to so-called Amadori products, and these in turn can form advanced glycosylation end products (AGEs), which cross-link matrix proteins. This damages blood vessels. The AGEs also interfere with leukocyte response to infection. (Kim, 2010).

2.2.3 Changes in protein metabolism

In diabetes, the rate at which amino acids are catabolized to CO_2 and H_2O is increased. In addition, more amino acids are converted to glucose in the liver. The increased gluconeogenesis and hyperglucagonemia is generally present in diabetes. Adrenal glucocorticoids also contribute to increased gluconeogenesis when they are elevated in severely ill diabetics. The supply of amino acids is increased for gluconeogenesis because, in the absence of insulin, less protein synthesis occurs in muscle and hence blood amino acid levels are rise. Alanine is particularly easily converted to glucose. In addition, the activity of the enzymes that catalyze the conversion of pyruvate and other two carbon metabolic fragments to glucose is increased. These include phosphoenolpyruvate carboxykinase, which facilities the conversion of oxaloacetate to phosphoenolpyruvate. They also include fructose 1, 6 - diphosphatase, which catalyzes the conversion of fructose diphosphate to fructose 6-phosphate, and glucose 6-phosphatase, which controls the entry of glucose into the circulation from the liver. Increased acetyl-CoA increases pyruvate carboxylase activity, and

insulin deficiency increases the supply of acety- CoA because lipogenesis is decreased. Pyruvate carboxylase catalyzes the conversion of pyruvate to oxaloacetate. In diabetes, the net effect of accelerated protein conversion to CO_2, H_2O, and glucose, plus diminished protein synthesis, is protein depletion and wasting.

2.2.4: Fat metabolism in diabetes

The principal abnormalities of fat metabolism in diabetes are acceleration of lipid catabolism, with increased formation of ketone bodies, and decreased synthesis of fatty acids and triglycerides. The manifestations of the disordered lipid metabolism are so prominent that diabetes has been called "more a disease of lipid than of carbohydrate metabolism".

Fifty percent of an ingested glucose load is normally burned to CO_2 and H_2O; 5% is converted to glycogen; and 30-40% is converted to fat in the fat depots.

In diabetes, less than 5% of ingested glucose is converted to fat, despite a decrease in the amount burned to CO_2 and H_2O, and no change in the amount converted to glycogen. Therefore, glucose accumulates in the bloodstream and spills over into the urine. In diabetes, conversion of glucose to fatty acids is deficient.

The rate of ketone utilization in diabetics is also appreciable. It has been calculated that the maximal rate at which fat can be catabolized without significant ketosis is 2.5 g/kg body weight/d in diabetic humans. In untreated diabetes, production is much greater than this, and ketone bodies pile up in the bloodstream

2.2.5: Pancreatic secretions

About four polypeptides with regulatory activity are secreted by the islets of Langerhans in the pancreas. Two of these are hormones; insulin and glucagon and have important functions in the regulation of the intermediary metabolism of carbohydrates, proteins, and fats (John, 1990).

The third polypeptide, somatostain, plays a role in the regulation of islet cell secretion and the fourth, pancreatic polypeptide is probably concerned primarily with the regulation of HCO^-_3 secretion into the intestine. Glucagon, somatostain, and possibly pancreatic polypeptide are also secreted by cells in the mucosa of the gastrointestinal tract.

Insulin is anabolic, increasing the storage of glucose, fatty acids, and amino acids. Glucagon is catabolic, mobilizing glucose, fatty acids, and the amino acids from stores into the bloodstream (Gerald and Sandra, 2003). The two hormones are thus reciprocal in their overall action and are reciprocally secreted in most circumstances. Insulin excess causes hypoglycemia, which leads to convulsions and coma. Insulin deficiency, either absolute or relative, causes diabetes mellitus (chronic elevated blood glucose), a complex and debilitating disease that if untreated is eventually fatal, Glucagon deficiency can cause hypoglycemia, and glucagon excess makes diabetes worse. Excess pancreatic production of somatostatin causes hyperglycemia and other manifestations of diabetes. A variety of other hormones also have important roles in the regulation of carbohydrate metabolism

Insulin; Insulin, a polypeptide hormone has a molecular weight of 5800 Da. The peptide hormone composed of 51 amino acids (6 kDa), and is secreted by the beta cells of pancreatic Islets of Langerhans. Insulin secretion is triggered by elevated glucose concentration in the blood. This condition usually occurs in healthy individuals after having a meal. Insulin possesses a wide spectrum of biological actions. It stimulates cells in the liver, muscle, and fat tissue to take up glucose from the blood, thereby leading to a decrease in blood glucose level. Intracellular effects of insulin includes: stimulation of glycolysis, glycogen synthesis, and fatty acid and triglyceride synthesis.

It stimulates cellular glucose uptake, glucose oxidation, glycogenesis, lipogenesis, proteogenesis and the formation of DNA and RNA. Therefore, insulin enables the cells to absorb glucose from the blood and also helps in the utilization of the glucose in the cells by glycolysis, tricarboxylic acid cycle, hexose monophosphate shunt, and glycogenesis (Kumar, Augustine and Vijayammal 1999).

Insulin is secreted by the islet of Langerhans. The islets of Langerhans are pvoid, 76x175 cm collection of cells. The islets are scattered through the pancreas, although they are more plentiful in the tail than in the body and head. B-islets make up about 2% of the volume of the gland, whereas the exocrine portion of the pancreas make up 80% and ducts and blood vessels make up the remainder. Humans have 1 to 2 million islets. Each has a copious blood supply. Blood from the islets, like that from the gastrointestinal tract (but unlike that from any other endocrine organs) drains into the hepatic portal vein.

In humans, cells the islets can be divided into types on the basis of their staining properties and morphology. Humans have at least four distinct cell types: A, B, D and F cells. The A cells secrete glucagon, the B cells secrete insulin, the D cells secrete somatostatin, and the F cells secrete pancreatic polypeptide. The B cells which are the most common and account for 60-75% of the cells in the islets are generally located in the center of each islet. They tend to be surrounded by the A cells which make up 20% of the total, and the less common D and F cells.

The islet in the tail of the human pancreas have many A cells and few if any F cells in the outer rim, whereas in rats and probably in some humans, the islets in the posterior part head of the pancreas have relatively large number of D and A cells. The A-cells (glucagon-rich) islets arise embryological from the dorsal pancreatic bud, and the F-cell (pancreatic polypeptide-rich) islets arise from the central pancreatic bud. These buds arise separately from the duodenum.

The B cell granules are packets of insulin in the cell cytoplasm. The shape of the packets varies from species to species in humans, some are round whereas others are rectangular. In the B cells the insulin molecule forms polymers and also complexes with zinc. The differences in the shape of the packets are probably due to difference in the size of polymers or zinc aggregates of insulin. The A granule which contain glucagon, are relatively uniform from species to species Gerald and Sandra (2003).

Structure and species specificity of insulin; Insulin is a polypeptide containing two chains of amino acids linked by disulfide bridges. Minor differences occur in the amino acid composition of the molecules from species to species. The differences are generally not sufficient to affect the biological activity of particular insulin in heterologous species but are sufficient to make the insulin antigenic. If insulin of one specie is injected for a prolonged period into another specie, the anti-insulin antibodies formed inhibit the injected insulin (John, 1990).

Almost all the humans who have received commercial bovine insulin for more than 2 mo have antibodies against bovine insulin, but the titer is usually low. Procine insulin differs from human insulin by only one amino acid residue and has low antigenicity. Human insulin produced in bacterial by recombinant DNA technology is now widely used to avoid antibody formation.

Biosynthesis and secretion of insulin; Insulin is synthesized in the rough endoplasmic reticulum of the B cells. It is then transported to the Golgi apparatus, where it is packaged into membrane-bound granules. These granules move to the plasma membrane by a process involving microtubules, and their contents are expelled by exocytosis. The insulin then crosses the basal lamina of the B cell and a neighboring capillary and the fenestrated endothelium of the capillary to reach the blood stream. Like other polypeptide hormones and related proteins that enter the endoplasmic reticulum, insulin is synthesized as part of a larger preprohormone. The gene for insulin is located on the short arm of chromosome 11 in humans. It has two introns and three exons. The peptide segment connecting the A and B chains then the C peptide facilitates the folding and is detached from the granules before secretion. The C peptide can be measured by radioimmuno assay and its level in blood is an index of beta cell function in patient receiving exogenous insulin (John, 1990).

Role of insulin in diabetes; Impairment of insulin activity is a major patho mechanism of hyperglycemic states. Despite the broad spectrum of information concerning insulin-dependent regulation of glucose levels in human tissues, the role of cellular and molecular mechanisms is not fully explained. Although it is widely accepted that insulin resistance is a major factor in the pathogenesis of type 2 diabetes the precise mechanism by which insulin resistance leads to the development of type 2 diabetes is uncertain (Gerald and Sandra, 2003). Furthermore, patients with type 2 diabetes exhibit insulin resistance in multiple organs (i.e skeletal muscle, adipose tissue, and liver) and it is unknown whether a defect in one or all of these organs is required for the development of diabetes.

However, Insulin increased the uptake of deoxy-D-glucose and the expression of GLUT1, GLUT3, and GLUT4 isoforms in the plasma membrane (Paweł, 2010). Skeletal muscle is responsible for the majority of whole body insulin-mediated glucose metabolism and insulin promotes glucose transport in this tissue by stimulating the translocation of the major insulin-responsive glucose transporter, GLUT4, from intracellular storage vesicles to the plasma membrane. Decreased insulin-stimulated glucose transport in skeletal muscle has been shown to be a major contributing factor to insulin resistance in patients with type 2 diabetes and obesity (Piątkiewicz, 2010).

Skeletal muscle glucose uptake increases dramatically in response to physical exercise (Delphin *et al.*, 2012). Moreover, skeletal muscle comprises the vast majority of insulin-sensitive tissue and is

a site of dysregulation in the insulin-resistant state. The biochemical and histological composition of the muscle is well defined in a variety of species. However, the functional consequences of muscle biochemical and histological adaptations to physiological and pathophysiological conditions are not well understood. The physiological regulation of muscle glucose uptake is complex. Sites involved in the regulation of muscle glucose uptake are defined by a three-step process consisting of: delivery of glucose to muscle, transport of glucose into the muscle by GLUT4 and finally phosphorylation of glucose within the muscle by a hexokinase (HK) *(*Delphin *et al.,* 2012).

Plasma and serum insulin level; Insulin plays a key role in the regulation of plasma glucose levels (hepatic output inhibition, stimulation of peripheral glucose utilization). The resulting hypoglycemic effects of insulin are counterbalanced by hormones with hyperglycemic effects (glucagon, growth hormone, cortisol, and epinephrine). Insulin secretion is controlled by the plasma glucose levels: hyperglycemia induces a prompt increase in circulating insulin levels. Neural influences, as well as various metabolic and hormonal factors (amino acids, glucagon, and gastrointestinal hormone) also participate in the control of insulin secretion and insulin profile of the patients. It is also known that the occurrence of haemolysis in sample used for evaluation of insulin levels results in artefactual changes in insulin levels. Red cells contain an insulin-degrading enzyme which may lead to underestimation of the actual insulin concentrations in haemolysed samples (Kumar *et al.*, 1999).

Insulin causes an increase in glucose capture mainly in the translocation stimulation mechanism of GLUT4 transporter from intracellular resources to cellular membrane. Flow cytometry allows determination of the degree of GLUT protein isoforms gathering in the cellular membrane and hence is an important assessment method of insulin effectiveness on the process of translocation. Hence, expression of GLUT isoforms present on the surface of lymphocytes in metabolically healthy persons and diabetic persons is a very important parameter in assessing the patient's insulin profile. Flow cytometry is a valuable method allowing the estimation of insulin influence on GLUT protein translocation in cellular membrane and the increase of the speed of glucose capture by peripheral blood lymphocytes.

On a theoretical basis, it has already been suggested that using more specific insulin assays could lead to increasing difficulties for showing inappropriate insulin levels in patients with endogenous hyperinsulinism (Estrada, Elliot and Zinman, 1994; Piątkiewicz, 2010)

In healthy subjects, insulin molecular concentration in plasma is only fivefold that of proinsulin. It is known that a high proportion of proinsulin is secreted by insulinomas. Molecular concentrations of insulin and proinsulin in plasma are very similar in patients with insulinoma, so that the percentage of cross-reaction with proinsulin strongly affects the results of plasma insulin. A minimum serum insulin threshold of 6mIU/l (with concomitant symptomatic hypoglycaemia below 0.45g/l) had been recommended using a double antibody radioimmunoassay with a detection limit of 5mIU/l (Moller and Flier 1991), as cited by (Piątkiewicz, 2010). Others recommended a 5mIU/l threshold with concomitant blood glucose below 0.54g/l (Estrada *et al.,* 1994; Piątkiewicz, 2010). A lower threshold of 3mIU/l was recommended with a recent immune chemiluminometric assay with a low detection limit **(**Curto, Piccinini and Rabbone, *1997***)**. Some recent insulin assays are totally devoid of any significant cross- reaction with intact proinsulin.

Serum insulin half-life is very short (about 4min) in subjects with normal metabolic clearance of insulin, so one might suspect that even detectable insulin levels at the time of symptomatic fasting hypoglycaemia (usually after several hours of fasting) might be inappropriate. For example, in muscles and adipose tissue an increase of insulin concentration in the range of physiological

norms (0–100 mU/L) increased the speed of glucose transport (measurement conducted with the use of radiolabelled analog of glucose) by about 300% to 400% (Zierath 1995; Dimitriadis, Leighton and Parry -Billings, 1997)]. The range of these effects is close to the effects observed in lymphocytes, which quantitatively suggests that these cells can constitute a good experimental model for studies of insulin influence on glucose metabolism.

As a result of the difficulties of acquisition of human cells for the examination of insulin activity on the cellular level, lymphocytes could prove to be a useful, easily available tool in studies on insulin activity disorders leading to insulin resistance, encountered, for example, in type 2 diabetes mellitus, obesity, and endocrinopathies (Piątkiewicz, 2010).

The data acquired in the studies of lymphocytes can be combined with the results of earlier in vivo examinations with the use of methods assessing the sensitivity of a whole organism to insulin (such as hyperinsulinemic euglycaemic clamp and oral glucose-tolerance test) and can facilitate the study of the patho mechanism leading to insulin resistance. Such a method was employed in the case of binding of insulin receptor in patients with type 2 diabetes mellitus (Piątkiewicz, 2010). It suggests that in these cases insulin resistance was an effect of post receptor defect of insulin activity. For example, the increase of insulin level in the range of physiological values causes a 60% growth of GLUT4 gathering in the cellular membrane of muscles. The influence of insulin on the speed of glucose transport to lymphocytes was examined in experiment with the use of deoxy-D-glucose (Piątkiewicz Czech and Tatoni, 2007), previously used by other authors for the measurement of glucose transport in myoblasts, mononuclear cells of peripheral blood, monocytes and lymphocytes (Szkudelski, 2001; Piątkiewicz, Czech and Tatoni, 2007). However, none of these studies assessed the effects of insulin influence on the transport of deoxy-D-glucose through cellular membrane. Insulin causes increased deoxy- D-glucose capture by lymphocytes (Piątkiewicz, 2010).

The effect of stimulation was dependent on the administered dose, mean effective doses remained in the range of physiological values (20 mU/L), and reached their peak at an insulin level of 50 mU/L. The blocking of insulin activity caused by the antibody of insulin receptor caused the inhibition of insulin-dependent deoxy-D-glucose capture, which additionally confirmed the significant influence of insulin on the cellular glucose transport in lymphocytes.

2.2.6 Glucose transporters

Glucose enters cells by facilitated diffusion or in the intestine and kidneys, by secondary active transport with Na^+. In muscle, adipose tissue and some other tissues, insulin stimulates glucose entry into cells by increasing the number of glucose transporters in the membrane. Glucose transport is an important phase of cellular metabolism because of the control of oxidation and percentage of carbohydrate (Newsholme and Dimitriadis, 2001). Seven different glucose transporters named GLUT 1-7 in order of discovery have been characterized. In skeletal muscles and adipose tissue, three isoforms of transporter are present: GLUT1, GLUT3, and GLUT4 (Wilson, Mitsumoto and Maher, 2004). The glucose transporters (GLUTs) that are responsible for facilitated diffusion of

glucose across cell membranes are a family of closely related proteins that span the cell membranes 12 times and have their amino and carboxyl terminals inside the cell (John,1990). They differ from and have no homology with the sodium-dependent glucose transporters SGLT 1 and SGLT 2, responsible for the secondary active transport of glucose in the intestine and renal tubules. Although, the SGLTs also have 12 Trans membrane domains their mode of action differ (Newsholme and Dimitriadis, 2001).

Insulin is the major regulator of blood glucose levels in the fed state when skeletal muscle becomes the primary consumer of glucose (Rajeswaria and Sridevi, 2014). The determinant of glucose utilization by muscle is its uptake mediated by glucose transporters. Insulin stimulates glucose uptake in skeletal muscle cells and fat cells by promoting the rapid translocation of GLUT4 glucose transporters to the plasma membrane. It regulates the release of GLUT4 from sequestered intracellular storage pools, and also has effects on docking and fusion of GLUT4 vesicles with plasma membrane (Rajeswaria and Sridevi, 2014). Regulation of GLUT4 activity by insulin will enhance the muscle cell glucose uptake.

Medicinal plants enhance the glucose uptake by GLUT4 translocation and were proven by in vitro glucose uptake model (Rajeswaria and Sridevi, 2014). The L-6 cell line is the best characterized cellular model origin to study glucose uptake and GLUT4 translocation.

Skeletal muscle is the major site for insulin-stimulated glucose uptake in vivo. Besides myocytes, adipocytes are also highly insulin-responsive cells (Kirsi *et al.*, 2002). Until recently, the major role of adipose tissue has been mostly regarded as lipid storage and mobilization. Accumulation

of fat in the abdominal adipose tissue region is believed to be significant in the pathogenesis of insulin resistance syndrome.

With enlargement of the adipose cells, the glucose transport system is less effective because of a reduced number of glucose transporters in the intracellular of the adipose cells. This is accompanied by high concentration of FFA in plasma and increased rate of fat oxidation leading to inhibition of glucose oxidation (Kirsi *et al.*, 2002). In obese subjects, resistance to the effect of insulin to enhance glucose uptake in the adipose tissue has been demonstrated in vitro. The insulin receptor density is decreased. Moreover, the ability of insulin to stimulate triglyceride synthesis and inhibit lipolysis

in adipose tissue is impaired in obesity (Kirsi *et al.*, 2002). Although, there is clear mechanistic evidence for insulin resistance in adipocytes, it is, however, still not clear to what extent this contributes to whole-body resistance to insulin-stimulated glucose uptake in obese subjects (Kirsi *et al.*, 2002).

2.2.7 Diabetic tests

The American Diabetes Association (ADA) recommends testing for diabetes every three years for people ages 45 and older. Individuals with risk factors such as being overweight, having a diabetic parent or sibling, or having high blood pressure or high cholesterol levels should begin testing earlier and should have repeat tests as often as yearly. Although several diagnostic tests are available, two are most useful.

2.2.7.1: Fasting blood sugar (FBS).

It's the simplest, most widely used test, requiring only a single blood sample that is obtained after at least eight hours without caloric intake.

Diagnosis	**FBS**	**HbA1C**
Normal	below 100 mg/dL	below 5.7%
Pre-diabetes	100–125 mg/dL	5.7%–6.4%
Diabetes	126 mg/dL and above	6.5% and above

2.2.7.2: Glycated haemoglobin (Historical perspective)

In 1955, researchers for the first time described, that adult haemoglobin contains heterogenous molecules. The significance of this finding was not explained till 1969 when Rahbar and colleague described that unusual haemoglobin found in diabetic patients was HbA1c, also noted a two-fold increase of HbA1c in diabetic patients. By the mid-1970s, the nature of the chemical reaction had been explained as glycation. Glycation is a spontaneous non-enzymatic reaction in which glucose binds covalently with haemoglobin at amino terminus of the b-globin chain (Bunn, Haney, Gabbay

and Gallop, 1975). In 1976, HbA1c was described as a useful means of monitoring the glycaemic control in diabetic patients (Koenig *et al*., 1976). By the early 1980s, HbA1c test was widely accepted in clinical practice as a means of monitoring the blood glucose concentration of patients with diabetes.

Glycosylated hemoglobin (HbA1C) test: This test measures the percent of red blood cells' oxygen-carrying hemoglobin molecules that have glucose attached to them. A normal value is below 5.7%, meaning that around 5% of hemoglobin molecules have glucose attached to them. At higher levels, glycosylation impairs function. Unlike the FBS, this test does not require fasting or any other dietary changes. Another advantage of this newer test is that it reflects a person's average blood sugar level over the preceding two to three months, while a blood sugar test provides more of a minute-to-minute snapshot.

Beginning in 2010, the American Diabetic Association (ADA) gave the test equal status for the diagnosis of diabetes. Glycated haemoglobin (HbA1c) is defined as haemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chains. This definition does not exclude haemoglobin that is additionally glycated at other sites on alpha or beta chains. HbA1c has been the most widely used and accepted test for monitoring the glycaemic control in individuals with diabetes (Kobold *et al.*, 1994).

Glycated haemoglobin has been in use to monitor control of blood glucose in diabetic patients for about three decades. It provides an average blood glucose level during preceding 10 - 12 weeks. It is a very convenient blood test, can be done in any clinical setting regardless of prandial state. International Federation of Clinical Chemistry (IFCC) developed a new reference method to measure the glycated haemoglobin, and the method is accepted worldwide as only valid anchor for the measurement of HbA1c. In 2009 International Expert Committee (IEC) recommended the use of HbA1c to diagnose diabetes with a threshold 6.5%. IFCC recommended the use of a new unit, i.e. mmol HbA1c /mol of total haemoglobin in place of hpercentage. Meanwhile a trial was conducted to find out relationship between average blood glucose and glycated haemoglobin, and a linear regression equation was developed to measure average blood glucose from HbA1c.

Using the equation one can calculate average blood glucose from glycated haemoglobin in mmol/mol. This average blood glucose was reported as "eAG" (estimated average glucose) and it

was used to monitor glucose control as estimated Glomerular Filtration Rate (eGFR) is used to monitor renal function in chronic kidney disease patients. Once a haemoglobin molecule is glycated, it remains in the red blood cell for the rest of its life-span (120 days). As such, it provides information about the degree of long-term blood glucose control.

The HbA1c level does not reflect exact mean blood glucose; rather, it is weighted proportionally towards recent levels (Tahara and Shima, 1995). The formation of glycated Hb depends upon ambient glucose concentrations in which erythrocytes circulate as well as the duration of exposure. A whole blood sample for glycated Hb is sufficient regardless of prandial state and clinical setting.

Non-enzymatic Glycation verses Enzymatic Deglycation: Most proteins (including haemoglobin) react with sugars to form covalent compounds without the involvement of enzymes. This chemical process is termed non-enzymatic glycation. The resulting accumulation of advanced glycation end products is associated with the progression of the complications of diabetes whereas enzymatic deglycation reverses the process of non-enzymatic glycation and generates free amino groups (Wu and Monnier, 2003).

Enzymatic deglycation is a formidable defense system against non-enzymatic glycation in mammalian cells. This system operates using fructosamine-3-kinase (FN3K), phosphorylating fructoselysine residue on glycated proteins and thereby destabilizing the compound, ultimately causing the decomposition of the glycated proteins (Szwergold, Howell, and Beisswenger, 2002; Depierre, and Van, 2003). This process of enzymatic deglycation is overwhelmed by episodes of extreme hyperglycaemia in individuals with diabetes as non-enzymatic glycation continues unabated (Szwergold *et al.*, 2002).

In the long run, it alters the stability of the protein structure, ultimately leading to cellular dysfunction (Luthra and Balasubramanian, 1999). These Advanced Glycation End products (AGEs) directly and indirectly (via receptors) promote the development of cardiovascular disease (Jandeleit-Dahm and Cooper 2008). They accumulate in different parts of the body and interact with receptors for advanced glycation end products (RAGE), induce oxidative stress, increase inflammation and enhance extracellular matrix deposition, thereby accelerating the process of endothelial dysfunction.

Consequently, they result in accelerated plaque formation and ultimate atherosclerosis in diabetes. (Yan, Agati, Schmidt, and Ramasamy, 2007; Jandeleit and Cooper, 2008). Glycated haemoglobin, intermediary compound is reversible but after some internal rearrangement of the compound, a stable HbA1c is formed. Several glycation sites of the HbA molecule exist; N-terminal valine residue of the b - chain is the predominant glycation site, accounting for 60% of bound glucose. Of the three types of HbA1 namely, HbA1a, HbA1b, and HbA1c, HbA1c represents the most prevalent glycated species.

Clinical use of HbA1c. The world is facing an escalating epidemic of diabetes. More than 220 million people worldwide have been diagnosed with diabetes, although the actual number of people with diabetes is likely to be higher because of the insidious onset of Type 2 diabetes. (T2DM). Moreover, many people who have impaired glucose tolerance remain outside the diagnosed community of patients. The decreasing life expectancy combined with the emergence of T2DM in children has resulted in phenomenal increase in diabetes related complications, becoming one of the major causes of disability and death worldwide. Type 2 diabetes accounts for 90% to 95% of all cases of diabetes. Furthermore, T2DM significantly increases the risk of heart disease and stroke; indeed, 50% of people with diabetes die of cardiovascular disease (WHO, 2009).

As a result of this, there is an urgent need for a method that can easily diagnose diabetes and the extent of complication early enough for early and prompt treatment. The use of HbA1c as a test went through nearly three decades of detailed scrutiny before being accepted as a diagnostic test for diabetes. Researchers had long been searching for test of glycaemia that could be used to screen and diagnose diabetes as well as monitor the chronic glycaemic control; such test, may also be able to predict the onset of complications.

Glycated haemoglobin acquires importance as a test for glycaemia because it has less intra-individual variation and is a better predictor of cardiovascular complications compared to fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT). In addition, it is used for glucose monitoring of diabetic patients (Peters, Davidson, Schriger and Hasselblad, 1996; Bennett, Guo and

Dharmage 2007). In another study HbA1c and FBG showed continuous relationship with cardiovascular disease (Jesudason, Dunstan, Leonge, and Wittest, 2003).

Glycated haemoglobin has been so extensively investigated by clinical trials. Dunn *et al. (*1979) suggested that HbA1c is highly reproducible and responsive to changes in glucose tolerance; as such, it could be used to monitor the control of glycaemia. However, Mulkerrin *et al.,* 1992) reported very poor sensitivity (36%) and predictive value (44%) in their elderly sample (mean age 76 years old), thereby making HbA1c neither useful in screening nor beneficial for diagnosing diabetes.

However, in acutely ill hospitalized patients HbA1c > 6% could reliably diagnose diabetes and level <5.2 % would reliably exclude diabetes. In other words, the HbA1c cut-off of 6.5%, with very low sensitivity and very high specificity, could be used as a supportive marker for the diagnosis of diabetes (Tanaka *et al.,* 2001). Although HbA1c cut-off 6.5% for diagnosis is too high, it gives acceptable sensitivity and specificity rates at 44.6% and 99.6%, respectively (Rohlfing, Harris, Little, Wiedmeyer, England, and Madsen, 2000).

A study from Australia suggested the use of a 7% cut-off rate for HbA1c when screening high risk populations (Rowley, Daniel, and O'Dea, 2005). Other studies have asserted that simultaneous measurement of fasting blood glucose and HbA1c may be used to identify high risk patients at an early stage (Mannucci *et al.,* 2003, Kim *et al.,* 2010). In a consensus statement in 2008 the Society of Endocrinology recommended;

1. HbA1c of 6.5-6.9% or greater, confirmed by FBG or OGTT should establish the diagnosis of diabetes,
2. HbA1c > 6.0% and impaired fasting glucose (> 100mg/dl) or random plasma glucose of 130-199mg/dl should lead to further diagnostic workup and closer follow-up (Saudek *et al.,* 2008).

By using these recommendations, we may identify a high proportion of individuals with undiagnosed diabetes, who would otherwise only be diagnosed once they developed end organ damage.

2.2.8 Treatment of Diabetes

Diabetes is an ancient disease and ever since then man has been trying to find a cure for the disease. In 1912, English physiologist Sir Edward Albert Sharpey-Schafer's study of the pancreas led him to the discovery of a substance that would normally be produced in non-diabetics; "insulin". The name comes from the Latin word insula, meaning island, referencing the insulin-producing islets of Langerhans in the pancreas. In 1916, Elliott Joslin, publishes the first edition of "The Treatment of Diabetes Mellitus". A clinician and educator, Joslin is renowned throughout the world as one of the most influential voices in diabetes care. Following his discovery in 1921 Frederick Banting, and his then student assistant, Charles Best, extracted insulin from dog's pancreas.

Banting and Best were working in laboratory space at the University of Toronto provided by Professor J.J.R. Macleod. They injected the insulin into dogs' whose pancreases have been removed, and the animals' blood sugar levels went down. James Collip purifies the extract so that it can be used in humans. Banting and Macleod were awarded the 1923 Nobel Prize in Physiology or Medicine, though the contributions of all four men have been recognized as important in the discovery of insulin.

In 1923 Eli Lilly and Company started commercial production of insulin. In the decades that follow, manufacturers develop a variety of slower-acting insulin, the first being protamine insulin introduced by Novo Nordisk in 1936. Since then several attempts have been made by different health professionals in the field of medicine and other health professional to find lasting solution to diabetes but to no avail. Broadly diabetes is treated using two main approaches viz: Orthodox and Alternative medicine.

2.2.8.1 Orthodox management

There are several drugs for the treatment of diabetes. They however have prominent side effects (Gupta, Medratia, Singh and Sharm, 2006) and most often out of reach for most diabetics. Currently, there is no cure for diabetes, so treatment aims at keeping blood glucose levels as normal as possible, control the symptoms and prevent health problems from developing later in life. Therefore, the goal of orthodox diabetes management is to keep blood glucose levels as close to normal as safely possible. Since diabetes may greatly increase risk for heart disease and peripheral artery disease, measures to control blood pressure and cholesterol level are essential part of diabetes orthodox treatment as well.

People with diabetes must take responsibility for their day-to-day care. This includes monitoring blood glucose levels, dietary management, maintaining physical activity, keeping weight and stress under control, monitoring oral medications and, if required, insulin use via injections or pump. To help patients achieve this, diabetic education program is crucial. The program enables patients to make more consistent and appropriate adjustments in their therapy and lifestyle. Lifestyle changes are very necessary for effective management of diabetes (Bassavanthappa, 2010). Modifying eating habits and increasing physical activity are typically the first steps toward reducing blood glucose levels. Three major areas in lifestyle changes that need to look closely at are diet, weight and level of physical activity.

Diet

By eating healthily, losing weight (if overweight) and exercising regularly may keep blood glucose at a safe and healthy level without the need for other types of treatment. Increasing the amount of fibre in diet and reducing fat intake, particularly saturated fat, can help prevent type 2 diabetes, as well as managing the condition if already in existence.

Tips to effective management are:

- Increase consumption of high fibre foods, such as wholegrain bread and cereals, beans and lentils as well as fruit and vegetables
- Choose foods that are low in fat – replace butter, ghee and coconut oil with low fat spreads and vegetable oil
- Choose skimmed and semi-skimmed milk, and low fat yoghurts
- Eat fish and lean meat rather than fatty or processed meat, such as sausages and burgers
- Grill, bake, poach or steam food instead of frying or roasting it
- Avoid high fat foods, such as mayonnaise, chips, crisps, pasties, poppadum and samosas
- Eat unsalted nuts and low fat yoghurts as snacks instead of cakes, biscuits

Weight

If overweight or obese (body mass index (BMI) of 25 or over), reduce weight, gradually by reducing calorie intake and encourage safe exercise. Losing 5-10% of overall body weight over the course of a year is a realistic initial target. Patients should aim to continue to lose weight until

desired weight is reached and maintained at BMI within the healthy range, which is: 18.5-24.9kg/m² for the general population and 18.5-22.9kg/m² for people of South Asian or Chinese origin

Physical activities

Being physically active is very important in preventing or managing type 2 diabetes. For adults who are 19-64 years of age, the government recommends a minimum of 150 minutes (2 hours and 30 minutes) of "moderate-intensity" aerobic activity, such as cycling or fast walking a week, which can be taken in sessions of 10 minutes or more, and muscle-strengthening activities on two or more days a week that work will affect all major muscle groups (legs, hips, back, tummy (abdomen), chest, shoulders and arms). An alternative recommendation is to do a minimum of 75 minutes of "vigorous-intensity" aerobic activity, such as running or a game of tennis every week, and muscle-strengthening activities on two or more days a week that work all major muscle groups (legs, hips, back, abdomen, chest, shoulders and arms).

In cases where the above activity levels are unrealistic, even small increases in physical activity are beneficial to health and act as a basis for future improvements. Reduce the amount of time spent watching television or sitting in front of a computer. Going for a daily walk – for example, during lunch break is a good way of introducing regular physical activity into daily schedule.

Oral Medications

Type 2 diabetes usually gets worse over time. Sometimes blood sugar levels remain high in people with type2 diabetes even when healthy eating and exercises are ensured. Making lifestyle changes, such as adjusting diet and taking more exercise may help control blood glucose levels at first, but they will not be enough in the long term. Thus medications are required for effective control of blood glucose levels. Initially, this will usually be in the form of tablets, and can sometimes be a combination of more than one type of tablet. The medications work in several different ways. These ways include; improve the effectiveness of the body's natural insulin, reduce blood sugar production, increase insulin production and inhibit blood sugar absorption. Oral diabetic medications are sometimes taken in combination with insulin (Bradley and Charold, 1998). The drugs of choice in management of diabetes include;

Metformin; Metformin is usually the first medicine used to treat type 2 diabetes. It works by reducing the amount of glucose that the liver releases into bloodstream. It also makes body cells more responsive to insulin. Metformin is recommended for adults with a high risk of developing type 2 diabetes, whose blood glucose is still progressing towards type 2 diabetes despite lifestyle changes in order to control it.

Sulphonylurea; Sulphonylureas increase the amount of insulin produced by pancreas. Examples of sulphonylureas include: glibenclamide, gliclazide, glimepiride, glipizide, gliquidone etc.

Sulphonylureas can increase the risk of hypoglycaemia (low blood sugar), because they increase the amount of insulin in body. They can sometimes cause side effects including weight gain, nausea and diarrhea.

Glitazones (thiazolidinediones, TZDs); Thiazolidinedione medicines (pioglitazone) make body cells more sensitive to insulin so that more glucose is taken from the blood. They are usually used in combination with metformin or sulphonylureas, or both. They may cause weight gain and ankle swelling (oedema). It is contraindicated in heart failure and high risk of bone fracture. However, thiazolidinedione was withdrawn from use in 2010 due to an increased risk of cardiovascular disorders, including heart attack and heart failure.

Gliptins (DPP-4 inhibitors); Gliptins work by preventing the breakdown of a naturally occurring hormone called GLP-1. GLP-1 helps the body produce insulin in response to high blood glucose levels, but is rapidly broken down. By preventing the breakdown of GLP -1, gliptins (linagliptin, saxagliptin, sitagliptin and vildagliptin) prevent high blood glucose levels, but do not result in episodes of hypoglycaemia.

GLP-1 agonists; Exenatide is a GLP-1 agonist, an injectable treatment that acts in a similar way to the natural hormone GLP-1. It is injected twice a day and boosts insulin production when there is high blood glucose levels, reducing blood glucose without the risk of hypoglycaemia episodes ("hypos"). It also leads to modest weight loss in many people who take it. It is mainly used in people on metformin plus sulphonylurea, who are obese.

Liraglutide; Another GLP-1 agonist but once - daily injection. Like exenatide, liraglutide is mainly used for people on metformin plus sulphonylurea, who are obese, and in clinical trials it is been shown to cause modest weight loss.

Acarbose; Acarbose helps prevent blood glucose level from increasing too much after a meal. It slows down the rate at which digestive system breaks carbohydrates down into glucose. Acarbose is not often used to treat type 2 diabetes because it usually causes side effects, such as bloating and diarrhea. However, it may be prescribed if patient cannot take other types of medicine for type 2 diabetes.

Nateglinide and repaglinide; Nateglinide and repaglinide stimulate the release of insulin by the pancreas. They are not commonly used, but may be an option if patient has meals at irregular times. This is because their effects do not last very long, but they are effective when taken just before meal. Nateglinide and repaglinide can cause side effects, such as weight gain and hypoglycaemia (low blood sugar).

Insulin Therapies

People with type 1 diabetes require multiple insulin injections each day to maintain safe insulin levels. Insulin may also be required to treat type 2 diabetes too. Using an insulin pump is an alternative to injections. The pump is about the size of a pager and is usually worn on the belt. Insulin is delivered through a small tube (catheter) that is placed under the skin (usually in the abdomen). There are four major types of insulin: rapid-acting, short-acting, intermediate-acting and long-acting insulin.

Where glucose-lowering tablets are not effective in controlling blood glucose levels, patient may need to have insulin treatment. This can be taken instead of or alongside tablets; depending on the dose and the way the patient takes it. Insulin comes in several different preparations, and each works slightly differently. For example, some last up to a whole day (long-acting), some last up to eight hours (short-acting) and some work quickly but don't last very long (rapid-acting). Insulin must be injected because if it were taken as a tablet, it would be broken down in the stomach like food and unable to enter the bloodstream. Insulin injections are given using either a syringe or an

injection pen, which is also called an insulin pen (auto-injector). Most people need between two and four injections of insulin a day.

In type 2 diabetes been controlled using insulin or certain types of tablets, patient may experience episodes of hypoglycaemia. Mild hypoglycaemia (a "hypo") can make patient feel shaky, weak and hungry, but it can be controlled by eating or drinking something sugary. This should be followed by a longer-acting carbohydrate, such as a cereal bar, sandwich or piece of fruit. In most cases, these measures were enough to raise patient's blood glucose level to normal, although it may take a few hours. If severe hypoglycaemia develops patient may become drowsy and confused, and may even lose consciousness. If this occurs, patient may need to have an injection of glucagon into the muscle or glucose into a vein.

Measures to prevent complications of diabetes

To reduce the risk of developing other serious health conditions, patient may be advised to take other medicines, including:

- anti-hypertensive medicines to control high blood pressure
- a statin, such as simvastatin or atorvastatin, to reduce high cholesterol
- low-dose aspirin to prevent a stroke
- Angiotensin- converting enzyme (ACE) inhibitor, such as enalapril, lisinopril or ramipril, if there is any early signs of diabetic kidney disease. Diabetic kidney disease is identified by the presence of small amounts of albumin (a protein) in urine.

2.2.8.2: Herbal drugs

Many Plants are used in traditional medicine to treat a lot of pathologies. Plants play important roles in the life of rural people, particularly in remote parts of developing countries which do not possess important health facilities. Tropical rain forest is the most species rich and diverse terrestrial ecosystem. Tropical rain forest accounst for only 7% of the earth dry surface but accommodate 70% of the animal and plant species in the world ecosystem (Onyekwelu, Jonathan, Olufunmilayo and Benard, 2011). Tropical rain forest contains many edible fruits and trees which supply fruits and herbs for decades. These plants are well known for health promotion and maintenance by the indigenous people.

The use of herbs is common among many peoples of the world. For example, South Africa is home to over30,000 species of higher plants and over3,000 of these species are used in traditional medicine across the country Mande (2005) as cited by Anthony (2010). The indigenous people of southern Africa have a long history of traditional plant usage for medicinal purposes; with about 4,000 taxa being so employed Anthony (2010).

There are over 27 million users of indigenous medicine and an estimated 200,000 indigenous traditional healers that treat up to 60% of the population using edible plants found in the region Anthony (2010). During the last century, the practice of herbalism became main-stream throughout the world. In spite of the great advances achieved in modern medicine, plants still make an important contribution to health care. Therefore, World Health Organization (WHO, 2013) describes a medicinal plant as any plant in which at least one of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of the bioactive constituents of plant are alkaloids, tannins, flavonoids, saponins and phenolic compounds (Ekha [ise, Soroh and Falodun, 2010).

Rich storehouses of medicinal plants exist everywhere especially in Africa which offers a vast reservoir of plants that have been categorized. The medicinal properties of various plant materials and extracts have been recognized since the beginning of the 5th century. The use of plant whether herbs, shrubs or tree, in parts or whole in the treatment and management of diseases dated back to pre-historic times. Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity (Okanla Oyewale and Akinyanju, 1990).

The use of medicinal food plant is not a new concept in the management of diabetes. Though, there are biomedical drugs for managing different types of diabetes, patients with diabetes use other complementary traditional herbs. This is due to the recognition of the value of traditional medicinal systems. Herbal medicines have long been used effectivelyall over the world in the treatment of diabetes mellitus and some other chronic diseases. Traditional medicines continue to play a significant role in the treatment of life-threatening diseases such as malaria, tuberculosis, diabetes

and AIDS in developing world, although not adequate scientific evidence has been documented in support of their healing properties.

In South Africa, only 32 plant species have been identified for the treatment of diabetes based on the major ethno botanical surveys (Anthony, 2010). From available data Nigeria seems to have a large number of plants for managing diabetes. Out of 115 medicinal plants collected and used in Nigeria, 100 were found to be useful in management of diabetes (Udoamaka, Ezuruike and Preito, 2014). Therefore, herbs have been reported as one of the remedies used for treatment by patients with diabetes in Zimbabwe, Nigeria, Vietnam, Oman, India and China (Elizues *et al.,* 2013).

Over 200 years ago traditional Chinese medicine (TCM) was used in the treatment of diabetes mellitus and lot of successes were documented (Tong, Dong, Chen, and Zhen, 2012). In India cinnamon and bark of an ever green tree (Morinda lucida) that grows in the forest has an ancient history of use for its anti-diabetic properties. World-wide more than 400 plants have been documented as beneficial in the treatment of diabetes (Gallengher, 2003).

In China, herbal preparations account for 30–50% of the total medicinal consumption, while in Ghana, Mali, Nigeria and Zambia, about 60% of children with high fever resulting from malaria are given herbal medicine as a first line of treatment (WHO, 2003). Herbs which are obtained from the wild and sometimes cultivated exist as hundreds of species in Nigeria (Ibe and Nwafo, 2005; Aiyeloja and Bello, 2016).

Among such herbs of significant include Cinnamon which has a long history as an anti-diabetic spice, but trials involving cinnamon supplementation have produced contrasting results. Cinnamon extracts are reported to have beneficial effects on people with normal and impaired glucoses tolerance, the metabolic syndrome, type 2 diabetes, insulin sensitivity and insulin resistance. All show beneficial effects of whole cinnamon and/or aqueous extracts of cinnamon on glucose. Lipids, antioxidant status, blood pressure, lean body mass and gastric emptying. However, not all studies have shown positive results.

Ginseng is also a well-known medicinal herb in traditional Asian medicine and is considered an adaptogen. Panax ginseng Meyer (Araliaceae), grows in China and Korea and >30 ginseng saponins (ginsenosides) have been isolated as the main biologically active constituents that include anti-carcinogenic, anti-diabetic and anti-inflammatory effects as well as cardiovascular protection and neuro protection (Gadkariem, *et al.*, 2011).

In acute, sub-acute and chronic treated mice, biochemical studies revealed a significant decrease in the blood glucose level in all treated with Ginseng (Gadkariem, *et al.*, 2011) .Also,biochemical studies of mice treated with higher dose of Harpagophytum procumbens (Devil's claw) showed reduction in blood glucose level of mice in the treatment groups as compared to the control. (Naif, Al-Harbi, Riyadh, Arif and Shah, 2011).In addition, a multi centre control trial in France showed Chinese herbs are well-tolerated and effective in type 2 diabetes (Vary and Attali, 1995; Okezie, 2009). All these and others arouse the interest of indigenous people on herbal drugs.

Currently there is growing interest in herbal remedies due to side effects associated with therapeutic agents (oral hypoglycaemic agents and insulin) for the treatment of diabetes mellitus. The prevalence of herbal use for various medical conditions by patients in Nigeria is largely unknown. Few among Such medicinal food plants are;

2.2.8.2.1: Bush buck *(Gongronema Latifolium)*

Gongronema latifolium is a local herb called (*Utazi*) by the Eastern part (Igbo/Ibo) of Nigeria. The Western part of Nigeria calls this *Arokeke*. It's is a tropical rainforest plant mainly used as vegetable, medicine or spice by the people (Ugochukwu and Babady, 2002). It contains essential oil, saponins (Schiffers, 2000). *Gongronema latifolium* is a climbing shrub that reaches up to 5m long with woody base, hollow stems and fleshy roots.

Description of *Gongronema latifilium;* *Gongronema latifolium* has a characteristics soft/hairy stems with latex. The leaves are broad, heart shaped and slightly oval in appearance with a deeply cordate base. The flowers are originally small and bisexual with a characteristics yellowish-green appearance. *Gongronema latifolium* is a seasonal shrub that flowers mainly around July and September in Nigeria. This heart-shaped leaf is characterized by a distinguishable bitter taste especially when eaten fresh however the bitter taste tends to slightly disappear if the leaves get dried.

Benefits of *Gongronema latifolium* leaf: *Gongronema latifolium* belongs to the class of medicinal plants that are beneficial in preventing and treating certain diseases and ailments that are detrimental to human health. *Gongronema latifolium* leaf, which can be chewed, infused or used for cooking is mainly used in the Western part of Africa for nutritional and medicinal reasons.

Pharmacological studies speculate that bush buc*k* has both analgesic, antimicrobial, antibacterial, anti-ulcer, anti-sickling, anti-oxidant, anti-asthmatic, anti-pyretic, hypoglycaemic and anti-inflammatory properties (Blessing, 2015). Bush buck plant is highly medicinal in nature, which suggests why its health benefits cannot be overemphasized. Fundamental ingredients used for medicinal purposes are stored in the various parts of the plant such as; the fruits, seeds, leaves, root and bark (Blessing, 2015).

The vital medicinal ingredients stored in the various parts of the leaf can be extracted either through blending the fresh leaves, chewing the seeds, leaves or fruits, infusing either the dry or fresh leaves and decoction. However, these various methods of preparing and using bush buck leaves for medicinal purposes mainly depends on the part of the plant where the active medicinal ingredients are present. As a matter of fact bush buck can be used in the following ways

- The bush buck roots can be decocted and mixed with other medicinal plants for the treatment of sickle cell anemia.
- Infused bush buck leaves can be used as a home remedy for dysentery and intestinal worms.
- The leaves when chewed raw or infused with hot water can act as a fast relief for catarrh, congested chest, running nose and cough.
- History records that Ghanaian and Senegalese squeeze the bush buck leaves and use it to massage the joints of children with difficulty in walking and this practice helps those young children to start walking.
- Lactating mothers can use bush buck to control their body weight as well as maintain a healthy body system.
- The bush buck leaves can be macerated in alcohol and used for the treatment of viral hepatitis and bilharzia.
- The bush buck fruits can be used to cook soup, which can be eaten as a laxative.

- Researchers agree that the decoction of the bush buck leaves or stems can be beneficial in treating high blood pressure and diabetes.
- Infused bush buck leaves are very helpful in treating malaria.
- The bush buck latex from the fruits can be applied on the teeth that are affected by caries.

- Sierra Leone infuses the bush buck stems with lime juice before drinking it and this acts as a purgative to treat stomach ache and colic.
- People suffering from asthma can chew bush buck leaves or macerated roots to help relieve them from wheezing

  Source, Blessing, (2010).

Nutritional Benefits of *Gongronema latifolium* (Bush buck); Nutritionally, *G. latifolium* is an excellent source of protein and past studies show that *G. latifolium* leaves are suitable for use in food production due to its high amino acid contents. They are bitter-sweet in taste and often used as a local spice and vegetable for food preparations such as; *abacha*, *nkwobi*, unripe plantain porridge, *ugba*, local soups such as *nsala* (white soup), sauces, salads and *isiewu*. Africans such as Nigeria, Côte d'Ivoire and Ghana, use the stems and fruits as chewing sticks.

***Gongronema latifolium and* diabetic control;** *G. latifolium* has been traditionally used in the South Eastern part of Nigeria for the management of diabetes (Ugochukwu and Babbady, 2002). As vegetable *G. latifolium* is an excellent source of dietary fibre and there is serious speculation that it plays an important role in glucose metabolism. The aqueous extract further increased the activity of glutathione reductase while the ethanolic extract caused a significant increase in the activity of glutathione peroxidase and glucose-6-phosphate dehydrogenase and a significant decrease in lipid peroxidation. These results suggest that the extracts from *G. latifolium* leaves could exert their anti-diabetic activities through their antioxidant properties (Ugochukwu and Babbady 2002). The ethanol extract also significantly decreased triglyceride levels and normalized total cholesterol concentration (Ugochukwu and Babbady, 2002). The administration of Gongronema latifolium actually reduces the blood glucose levels in diabetic rats Effiong Eshiet, and Ajibola (2015). This suggests that the plant has hypoglycemic activities Effiong Eshiet, and Ajibola, (2015).

Alao they noted that Induction of diabetes in teir study caused significant increase in TC, LDL and HDL compared to NC but the intervention with GL however reduced these indices.

2.2.8.2.2. Egyptian cucumber *(Luffa aegyptiaca)*

Luffa aegyptiaca belongs to the family cucurbitaceae. *Luffa aegyptiaca* is also known as sponge gourd or Egyptian cucumber. It is an herbaceous plant and thrives commonly with twining tendrils. *Luffa aegyptiaca* plant is an annual vine, native to South Asia and Southeast Asia but grows predominantly as weed in most parts of Nigeria. It is known as *Awmpawng* in Mizo and *Bhûl* in Assamese. It is a large succulent tendril climber with slender, slightly hairy furrowed stem. The interior is cucumber like when immature, but quickly develops into a network of fibre surrounding large number of flat blackish seeds. The matured fruits are used for domestic purposes as sponge hence the name sponge gourd.

Etymology; The botanical specific epithet" *Aegyptiaca*" was given to this plant in the 16th century when European botanists were introduced to the plant from its cultivation in Egypt. In the European botanical literature, the plant was first described by Johann Veslingius in 1638, who named it "Egyptian cucumber". Veslingius also introduced the name "*Luffa"*.

Description and cultivation; *Luffa aegyptiaca* about-30-cm-long fruit resembles a cucumber in shape and size. *Luffa aegyptiaca* is best grown with a trellis support. It requires lots of heat and lots of water to thrive.

Health benefits and uses; *Luffa aegyptiaca* tender fruit is taken as vegetable. It is an excellent fruit in nature containing all the essential constituents required for good health for humans. *Luffa aegiptiaca* fruits has over 100 different chemical components, including mucilage, reducing sugars, resins, alkaloids, organic acids, tannins, saponins, and proteins. It also contains Mono unsaturated fatty acids, saturated fatty acids, fiber, flavonoids Niacin and Ascorbic acid which help to reduce hypercholesterolemia (Abdul, 2012).

The young fruit is eaten as a vegetable and is commonly grown for that purpose in tropical Asia (Abdul, 2012). Unlike the young fruit, the fully ripened fruit is strongly fibrous and inedible, and is used to make scrubbing bath sponges. Due to the use as a scrubbing sponge, it is also known by the common names dishrag gourd, rag gourd, sponge gourd, and vegetable-sponge. It is also called

smooth *Luffa* to distinguish it from the ridged L*uffa* (*Luffa acutangula*), which is used for the same purposes. Owing to its striking yellow flowers, *Luffa aegyptiaca* is occasionally grown as an ornamental.

The main uses of *Luffa aegyptiaca* are dyslipidemia, anti -diabetic, hepato protective, anti-hypertensive and diuretic (Abdul, 2012). As a food habit of British people *Luffa aegyptiaca* Mill fruits are extensively used along with egg preparations which possibly reduce the cholesterol from the egg. Oral administration of leaf extract at the dose of 100mg/kg body weight showed a significant decrease ($P<0.05$) in blood glucose level in 7^{th} and 14^{th} days treatment www.jocpr.co

There is no known history of toxicity of *Luffa aegyptica*.

2.2.8.2.3: Chaya plant *(Cnidoscolus aconitifilius)*

Cnidoscolus aconitifilius (CA*)*, belongs to the Euphorbiaceae family. *Cnidoscolus aconitifilius* is commonly known as chaya or tree spinach (EOL Search, 2010). It is a large, fast-growing leafy perennial shrub that is believed to have originated in the Yucatán Peninsula of Mexico (Grubben and Denton, 2004). The specific epithet, *Aconitifolius*, means "*Aconitum*" 'like leaves". It has succulent stems which exude a milky sap when cut. It can grow to be 6 meters tall, but is usually pruned to about 2 m for easier leaf harvest. The leaves are broad and may consist of 3 or more lobes with fleshy petioles.

It is a popular leaf vegetable in Mexican and Central American cuisines, similar to spinach. Some varieties have stinging hairs and require gloves for harvesting. Plants in the Chayamansa Group (syn. *Cnidoscolus chayamansa*) are the most widely cultivated, because they lack stinging hairs on the leaves. It is divided into four cultivars based on leaf morphology: 'Chayamansa' (most common) Grubben and Denton (2004).

Cultivation; Chaya is easy to grow, a tender perennial in the US, and suffers little insect damage. It is tolerant of heavy rain and has some drought tolerance. Propagation is normally by woody stem cuttings about 6-12 inches long, as seeds are produced only rarely. Early growth is slow as roots are slow to develop on the cuttings, so leaves are not harvested until the second year. Chaya leaves can be harvested continuously as long as no more than 50% of the leaves are removed from the plant, which guarantees healthy new plant growth. Chaya is one of the most productive green

vegetables http://people.umass.edu; http://www. Echotech .org/#Chay. Higher yields of greens could be obtained with chaya than any other vegetable (wikipedia, 2015).

Consumption and nutrient composition of chaya plant (*Cnidoscolus aconitifolius)*: The leaves should be cooked before being eaten, as the raw leaves contain a high content of toxic hydrocyanic acid http://people.umass.edu. Also, some varieties have stinging hairs and cooking destroys the stinging hairs. To be safely eaten, the required cooking time is 5-15 minutes (Grubben and Denton, 2004; Johnest, *2011)*. However, up to 5 raw leaves can be eaten a day (wikipedia, 2015). Chaya leaves were found to contain substantially greater amounts of nutrients than spinach leaves https://books.google.com. There is lack of proven information on the ability of chaya to control diabetes. That notwithstanding, Chaya is a good source of protein, vitamins, calcium, and iron and is also a rich source of antioxidants http://www.echotech.org/#Ch ay

Young chaya leaves and the thick, tender stem tips are cut and boiled as spinach. It is a tasty vegetable, In fact, levels of chaya leaf nutrients are two- to threefold greater than any other land-based leafy green vegetable http://people.umass.edu.; http://www.echotech.org/#Chay. Chaya leaves have a possible anti-diabetic effect (http://people.umass.edu.; Kuti and Konuri, 2004). Traditionally the leaves are immersed and simmered for 20 minutes and then served with oil or butter. The stock or liquid the leaves are cooked in can also safely be consumed as the cyanide is volatilized as hydrogen cyanide (HCN) during cooking. Cooking in aluminum cookware can result in a toxic broth, causing diarrhea http://www.echotech.org/#Chay.

Therefore, *Cnidoscolus aconitifolus* is among the many underexploited native leafy plants with potential as a traditional food source. With current renewal of interest in household gardens, attention is being focused on promoting some of these plants as leafy green vegetable among populations in the developing countries (FAO 1987).

Nutrient Composition of *Cnidoscolus aconitifolus*: The nutrient analysis of *Cnidoscolus aconitifolus* leaves revealed substantially greater amounts of nutrients than the spinach leaves. *Cnidoscolus aconitifolus* leaf is especially high in protein (5.7%), crude fiber (1.9%), calcium (199.4 mg/100g), potassium (217.2mg/100g), iron (11.4mg/100g), and carotene (0.085mg/100g).

In terms of average nutritive value, *Cnidoscolus aconitifolus* leaf is by far superior to other leafy green vegetables such as spinach, amaranth, Chinese cabbage and lettuce (Grubben 1978). While some edible leafy green vegetables are usually good sources of mineral and other micronutrients (Levander 1990), *Cnidoscolus Acontiitiflous* furnishes appreciable quantities of several of the essential mineral and other micronutrients necessary for human health maintenance.

Phytochemical contents of *Cnidoscolus aconitifolus;* Phytochemical analysis of the plant leaves showed mean concentrations (%) of tannins, saponins, cyanogenic glycosides, alkaloids, phenols, flavonoids and steroids at 0.14, 4.04, 0.003, 4.72, 0.19, 2.36, and 0.27, respectively (Akachukwu, Okafor and Ibegbunam, 2016) ; http://dx.doi.org/10.5455/jib.20140504023102. Also (Situ *et al.*, 2014) showed the nutrient composition of ***Cnidoscolus aconitifolus*** to be; moisture 2.86±0.02%, ash- 14.22±0.02%, crude fibre- 16.37 ±0.16%, crude fat- 7.39±0.19%; crude protein 14.7±0.03%, carbohydrate- 44.31±0.16% and energy- 1276±10%. Mineral elements recorded sodium- 269.40±0.02mg/100g, potassium- 2030.02±0.03 mg/100g, calcium- 616.00±0.10 mg/100g, magnesium- 2.00±0.02 mg/100g, manganese- 2.00±0.02mg/100g, copper- 0.03±0.10 mg/100g, iron- 20.60±0.20 mg/100g, zinc- 2.74±0.01 mg/100g, chromium- 0.06 ±0.10 mg/100g, lead- 0.07 mg/100g (Situ *et al.*, 2014).

Phytochemical investigation of the dry, aqueous and ethanolic leaf extracts of Cridoscolus *aconitifolius* showed the presence of alkaloids, tannins, phlobatannin, saponin and phenols (Mordi and Akanji, 2012). However, alkaloids, tannin and phenol occur in appreciable amount in ethanol extraction but in minute amount in water extraction but saponin are appreciable in both. In a similar studies carried by (Situ *et al.*, 2014), the phytochemical screening of chaya leaves revaled the presence of saponin, tannin, phlobatanin, alkaloid, phytate, phenol, cardiac glycoside and terpenoid. But flavonoid and anthraquinone were not detected.

Table 2.3 Comparisons of nutrient compositions of leaves of "chaya" (*Cnidoscolus chayamansa* Mc Vaughn) and spinach (*Spinacia oleraceae* L.) per 100g fresh weight.

Component	Chaya	Spinach
Water (%)	85.3	90.7
Protein (%)	5.7	3.2
Fat (%)	0.4	0.3

Crude fiber (%)	1.9	0.9
Total CHO (%)	4.2	3.8
Ash (%)	2.2	1.8
Calcium (mg/100g)	199.4	101.3
Phosphorus (mg/100g)	39.0	30.0
Potassium (mg/100g)	217.2	146.5
Iron (mg/100g)	11.4	5.7
Ascorbic acids (mg/100g)	164.7	48.1
Carotenoids (mg/100g)	0.085	0.014
Average nutritive value	14.94	6.38

Data for spinach USDA (1984). while that of Cnidoscolus is from Grubben (1978).

C*nridoscolus aconitifolius* and management of chronic diseases

One of the plant genera widely used traditionally for the treatment of many diseases is *Cnidoscolus aconitifolius.* Colloquially, the plant is referred to as Chaya (Donkoh, Kese and Atuahene, 1990). In the western part of Nigeria, it is called different names such as Efo Iyana, Igaja and Efo Jerusalem. In Niger Delta of Nigeria; it has been nick-named “Hospital Too Far” While in Enugu state, it is nick-named *obara kpu* (blood booster) because of its numerous traditional claims. It is a tasty vegetable, and is exceptionally high in protein, calcium, iron, vitamin A and is also a rich source of antioxidants making the plant necessary for good health. (http://www.echotech.org/technical/az/aztext/azch2veg.htm#chay). From previous studies, the plant is shown to have large quantities of vitamins and minerals. Many previous studies have shown the effect of mineral and vitamins on human systems. For example, potassium has been shown to be an important mineral nutrient in the control of hypertension and in the reduction of risks of stroke, calcium is important for bone formation. *Cnidoscolus aconitifolus* may have more to offer in health management.

For moderately diabetic mice, CA and chlorpropamide decreased the glucose levels by 25.6% and 16.3% respectively while for the severely diabetic mice CA decreased the blood glucose by 43.7% (Oladeinde *et al.,* 92007).

2.2.8.2.4: *Morinda*

Morinda is a genus of flowering plants in the madder family, Rubiaceae. The generic name is derived from the Latin words *morus* "mulberry", from the appearance of the fruits, and *indica,*

meaning "India". In traditional Japanese, Korean and Chinese medicine, *Morinda* is considered to be an herb with biological properties, although there is no confirmed evidence of clinical efficacy Wikipedia *(*November 2010*)*.Most species of this genus originate in the area of Borneo, New Guinea, Northern Australia and New Caledonia (Wikipedia, November 2010). Morinda is distributed in all tropical regions of the world. *Morinda* includes 80 species of trees, shrubs or vines. All *Morinda* species bear aggregate or multiple fruits that can be fleshy (like *Morinda lucida*) or dry. However, the comparatively small flowering and fruiting heads on long slender peduncles are distinctive characteristics of *Morinda lucida*.

Brimestone tree *(Morinda lucida Benth)*: *Morinda lucida* Benth. (Rubiaceae) originated from south East Asia and Australia. *Morinda lucida is* a tropical tree found in African rainforest and commonly known as brimstone tree. *Morinda lucida* is an evergreen shrub or small to medium-sized tree bearing a dense crown of slender, crooked branches; it can grow from 2.4 - 18 metres tall, though specimens up to 25 metres are recorded in coastal Cote D'Ivoire. The bole and branches are often crooked or gnarled. It is a multipurpose species yielding dyes, timber, fuel and traditional medicines. The roots are sold in local shops and markets, both as dyestuff and medicine, whilst the leaves and twigs are sold as a medicinal tonic for young children. The plant is occasionally grown in home gardens. It grows well in grassland, exposed hillsides, thickets, forests, often on termite mounds, sometimes in areas which are regularly flooded, at elevations from sea-level up to 1,300 metres.

In the South and Central Regions of Cameroon, *Morinda lucida* is commonly known as "akeng" and it is one of the most widely used plants in the environment for medicinal purposes (Zapfack and Ngobo, 2002). *Morinda lucida* is also an indigenous plant found in some parts of Nigeria such as Eastern part like Anambra and Enugu states where it is known as "Udara ohia". *Morinda lucida* is employed for centuries in Anambra State, Nigeria by traditional practitioners in the treatment of various ailments.

Edible and Ethno botanical properties of *Morinda lucida*: The bitter-tasting roots are used as flavouring for food and alcoholic beverages and are also popular in Nigeria as chewing sticks. Different parts of *Morinda lucida* Benth have been reported to possess medicinal properties. From the wood and bark of *Morinda lucida* 18 anthraquinones, tannins, flavonoids and saponosides have

been isolated (Zimudzi and Cardon, 2005). The leaf extract of the plant possess trypanocidal (Asuzu and Chineme, 1990), antimalarial activities (Makinde and Obih, 1985) aortic vaso relaxant effect (Ettarh and Emeka, 2004). The leaf extract of *Morinda Lucida* has also been associated with some reversal toxicity such as the reversible antispermatogenic activities in rats (Raji, Akinsomisoye and Salman, 2005). The leaves are used as "oral teas", which are usually taken orally for the traditional treatment of malaria, and as a general febrifuge, analgesic, laxative and anti- infections (Makinde and Obih 1985; Lawal, Sudhanshu and Tiwo, 2012). The leaves have also been reported to possess strong trypanocidal and aortic vasorelaxant activities (Asuzu and Chineme, 1990). Further studies have shown that leaf and stem bark of *M. lucida* possess anticancer (Sowemimo, *et al.,* 2007), hepatoprotective (Oduola, *et al.,* 2010), cytotoxic and genotoxic (Akinpelu and Onakoya, 2006), hypoglycemia and antidiabetic activity (Daziel, 1973).

Phytochemical composition of *Morinda lucida*

Plants have been used for ages in treatment of illness. Recently, a lot of attention is directed to medicinal plant. The phytochemical analysis of *Morinda lucida* was evaluated to ascertain some of the secondary metabolites that exhibit medicinal properties. The results of phytochemical screening of ethanol crude leaf extract of *Morinda lucida* revealed the presence of alkaloids, tannins and saponins. These secondary metabolites could be responsible for the observed medicinal properties of *Morinda lucida* by traditional herbalists

Morinda lucida* and glucose control*;

The ability of *M. lucida* at 5g /kg body weight to significantly reduce plasma glucose concentration of normal albino rats is indicative of a possible hypoglycemic activity. The leaf extract of *Morinda lucida* has also been reported to have a strong oral hypoglycemic property by increasing the utilization of peripheral glucose (Adeneye and Agbaje, 2008; Olajide, Awe, Makinde and Morebise, 1999). The bark of *Morinda lucida* is widely used in Cameroon as a decoction for treating diabetes mellitus. Administration of extracts for four weeks had significant reduction effect on glucose, creatinine, cholesterol and triglyceride at dose level of 5 g/ kg body weight (Adeneye and Agbaje, 2008). Several recent studies have aldso demodstrated decrease level of blood glucose following *Morinda lucida* administrtionn.

2.3 The use of Animal models in experimental studies

Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Streptozotocin (STZ) and alloxan have been extensively used to induce diabetes for various diabetes studies in laboratory animals (Calabresi and Chabner, 1985) cited as by (Dhanush *et al.*, 2014).

Rats are various medium-sized, long-tailed rodents of the super family Muroidea 'True rats" are members of the genus Rattus, the most important of which to humans are the black rat, Rattus rattus, and the brown rat, Rattus norvegicus. Many members of other rodent genera and families are also referred to as rats, and share many characteristics with true rats (Korinke, 2000). Rats are typically distinguished from mice by their size; rats are generally large muroid rodents, while mice are generally small muroid rodents (Mordes, Poussire, Blankenhorn and Gerinier, 2007). The muroid family is very large and complex, and the common terms rat and mouse are not taxonomically specific. Generally, when someone discovers a large muroid, its common name includes the term rat, while if it is small; the name includes the tent mouse. Scientifically, the terms are not confined to members of the Rattus and Muriod genera, for example, the pack rat and cotton mouse (Korinke, 2000).

Over the years, rats have been used in many experimental studies, which have added to our understanding of genetics, diseases, the effects of drugs, and other topics in health and medicine. Laboratory rats have also proved valuable in psychological studies of learning and other mental processes. The historical importance of this species to scientific research is reflected by the amount of literature on it, roughly 50% or more than that on mice (Korinke, 2000). Domestic rats differ from wild rats in many ways. They are calmer and less likely to bite, they can tolerate greater crowding, they breed earlier and produce more offspring, and their brains, livers, kidneys, adrenal glands, and hearts are smaller. Scientists have bred many strains or "lines" of rats specifically for experimentation. Most are derived from the Albino, Wistar rat, which is still widely used. Other common strains are the Sprague, Dawley, Fischer 344, Holtzman Albino strains, the Long-Evans, and Lister black hooded rats. Inbred strains are also available but are not as commonly used as inbred mice.

Rat strains are generally not transgenic, or genetically modified, because the genes that knockout and embryonic stem cell techniques that work in mice are relatively difficult in rats. This has disadvantaged many investigators, who regard many aspects of behaviour and physiology in rats as more relevant to humans and easier to observe than in mice and who wish to trace their observations to underlying genes. As a result, many have been forced to study questions in mice that might be better pursued in rats. In October 2003, however, researchers succeeded in cloning two laboratory rats by nuclear transfer. So rats may begin to see more use as genetic research subjects. Much of the genome of Rattus norvegicus has been sequenced (http //www.ensembi org/Rattus norvegicus. Laboratory rat is rat of the species Rattus norvegicus which is bred and kept for scientific research. Laboratory rats have sewed as an important animal model for research in psychology, medicine, and other fields. Laboratory rats share origins with their cousins the "fancy rats" in domestication.

In 18th century in Europe, wild Brown rats ran rampant and this infestation fueled the industry of rat-catching. Rat- catchers would not only make money by trapping the rodents, but also by turning around and selling them for food, or more importantly, for rat-baiting (Sukov and Barth, 1998). Rat-baiting was a popular sport which involved filling a pit with rats and timing how long it took for a terrier to kill them all. Over time, breeding the rats for these contests produced variations in color, notably the albino and hooded varieties.

The first time one of these albino mutants was brought into a laboratory for a study was in 1828, in an experiment on fasting (Ufele, 2011). Over the next 30 years rats were used for several more experiments and eventually the laboratory rat became the first animal domesticated for purely scientific reasons (Korinke, 2000). The best-known rat species are the Black rat (Rattus rattus) and the Brown rat (Rattus norvegicus). The group is generally known as the Old World rats or true rats, and originated in Asia. These rats are bigger than most Old World mice, which are their relatives, but seldom weigh over 500 grams (11 Ib) (Ufele, 2011).

The tern "rat" is also used in the names of other small mammals which are not true rats. Examples include the North American pack rats, a number of species loosely called kangaroo rats, and others rats such as the Bandicoot rat (Bandicota bengalensis) are mouse rodents related to true rats, but are not members of the genus Rattus. Male rats are called bucks, unmated females are called does,

pregnant or parent females are called dams, and infants are called kittens or pups. A group of rats is either referred to as a pack or a mischief (Ufele, 2011).

The common species are opportunistic survivors and often live with and near humans, therefore they are known as commensals. They may cause substantial food losses, especially in developing countries (Korinke, 2000). However, the widely distributed and problematic commensal species of rats are a minority in this diverse genus. Many species of rats are island endemics and some have become endangered due to habitat loss or competition with the Brown, Black or Polynesian rat.

Wild rodents, including rats, can carry many different zoonotic pathogens, such as Leptospira, Toxoplasma gondii, and Campylobacter jejeni. Black Death is traditionally believed to have been caused by the micro-organism Yersinia pestis, carried by the tropical rat flea (Xenopsylla cheopis) which preyed on Black rats living in European cities during the epidemic outbreaks of the Middle

Ages. These rats were used as transport hosts. Other zoonotic diseases linked to pest rodents include Classical swine fever and Foot- disease (Ufele, 2011). The average lifespan of any given rat depends on which species is being discussed, but many only live about a year due to predation.

2.3.1: Laboratory rat species mainly used in experimental studies

Wistar Rats

Wistar rats are an outbred strain of albino rats belonging to the species Rattus norvegicus. This strain was developed at the Wistar Institute in 1906 for use in biological and medical research, and is notably the first rat strain developed to serve as a model organism at a time when laboratories primarily used Musculus, or the common house mouse. More than half of all laboratory rat strains are descended from the original colony established by physiologist Henry Donaldson, scientific administrator Milton J. Greenman, and genetic researcher/embryologist Helen Dean King (Clause, 1998).

The Wistar rat is currently one of the most popular rat strains used for laboratory research. It is characterized by its wide head, long ears, and having a tail length that is always less than its body length. The Sprague Dawley rat and Long-Evans rat strains were developed from Wistar rats. Wistar rats are more active than other strains like Sprague Dawley rats (Sukov and Barth, 1998).

Sprague Dawley rat

The Sprague Dawley rat is an outbred multipurpose breed of albino rat used extensively in medical research. Its main advantage is its calmness and ease of handling (Davis, 1997). This breed of rat was first produced by the Sprague Dawley farms, later to become the Sprague Dawley Animal Company) in Madison, Wisconsin (Kuti, and Konuru, 2004).

The adult body weight is 250-300g for females, and 450-520g for males. The typical life span is 2.5 and 3.5 years respectively, (Mordes *et al.*, 2007). These rats typically have increased tail to body length ratio compared with Wistar rats

Bio breeding diabetes prone rats

Bio breeding Diabetes Prone rats (BBDP) is an inbred rat strain that spontaneously develops autoimmune Type I Diabetes like Non Obese Diabetic mice, BBDP rats are used as an animal model for Type 1 diabetes. The strain re-capitulates many of the features of human type 1 diabetes, and has contributed greatly to the research of type 1 diabetic pathogenesis (Kurtz and Morris, 1989).

Long-Evans rat:

Long-Evans rats are an outbred strain of rats belonging to the species Rattus norvegicus. This strain was developed by Drs. Long and Evans in 1915 by crossing several Wistar females with a wild gray male Long Evans. Long Evans rats are white with a black hood, or occasionally white with a brown hood. They are utilized as a multipurpose model organism, frequently in behavioral and obesity research (Mordes *et al.*, 2007).

Zucker rat

Zucker rat were bred to be a genetic model for research on obesity and hypertension. They are named after Lois M. Zucker and Theodore F. Zucker, pioneer researchers in the study of the genetics of obesity. There are two types of Zucker rat, a lean Zucker rat, denoted as the dominant trait(Fa/Fa) or (Fa/fa), and the characteristically obese (or fatty) Zucker rat, which is actually a recessive trait (fa/fa) of the leptin receptor, capable of weighing up to I kilogram (2.2lb)–more than twice the average weight (Ufele, 2011)

Obese Zucker rats have high levels of lipids and cholesterol in their blood, are resistant to insulin without being hyperglycemic, and gain weight from an increase in both the size and number of fat cells (Mordes *et al.*, 2007: Ufele, 2011)). Obesity in Zucker rats is primarily linked to their

hyperphagic nature. However, food intake does not fully explain the hyperlipidemia or overall body composition (Takaya *et al.,* 1996)

2.3.2 The use of Alloxan on animal model

Alloxan *(Mesoxalylurea ;2,4,5,6 (1-Pyrimidinetetrone;Mesoxalylcarbmide)*This is a diabetogenic agent which apparently acts through formation of superoxide radicals formed by redox cycling. Superoxide radicals are also formed by a variety of mechanisms in hyperglycemia (Ishibashi. 1981).Alloxan and its derivatives were selectively toxic to pancreatic beta cells, with other endocrine cells and exocrine parenchymal cells being well preserved, even at high concentration (Jorns, 1997). Alloxan is known as a selective B-cell cytotoxic substance, and there is so far little evidence for a direct toxic effect on the other islet cell types. It is therefore concluded that alloxan affects the secretory mechanism of not only the B-cell but also of the islet A2-cell, although this latter cell type is not primarily destroyed by the drug. The data furthermore support the concept of a relationship between glucose metabolism and the glucose-mediated glucagon release of the A2-cell(Ostenson,1980).Insulin-producing isletB-cells participate to a sizeable extent to the overall uptake of alloxan by the whole pancreatic gland, despite the fact that they account for no more than about one percent of the total pancreas mass (Malaisse, 2001).

Copper-oxygen complex derived from the reaction of H2O2 with Cu^{2+} participates in Cu^{2+}-dependent DNA damage by alloxan plus NADH and dialuric acid (Murata, 1998).

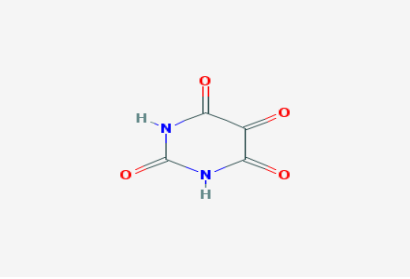


Fig 2. 1 Structure of Alloxa

History / Discovery of Alloxan; Alloxan was originally isolated in 1818 by Brugnatelli. The compound was discovered by Justus von Liebig and Friedrich Wöhler following the discovery of urea in 1828 and is one of the oldest named organic compounds that exist. And it was named in 1838 by Wöhler and Liebig. The name "Alloxan" emerged from an amalgamation of the words "allantoin" and "Oxalsäure" (oxaic acid). The name is derived from allantoin, a product of uric acid excreted by the fetus into the allantois, and oxaluric acid derived from oxalic acid and urea, found in urine. The alloxan model of diabetes was first described in rabbits by Dunn, Sheehan and McLetchie in 1943 (Lenzen, 2008).

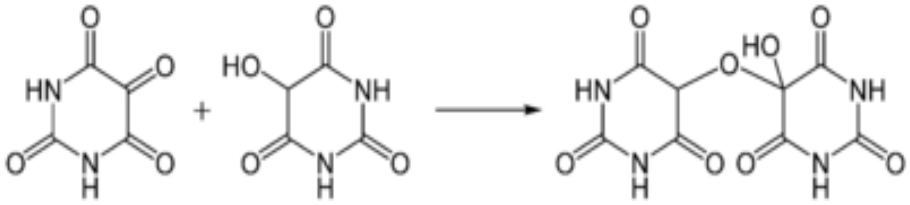


Fig. 2.2 Synthesis of alloxan

Synthesis of alloxan: The original preparation for alloxan was by oxidation of uric acid by nitric acid. In another method the monohydrate is prepared by oxidation of barbituric acid by chromium trioxide (Mrozikiewicz, *et al.*, 1994). Alloxan is a strong oxidizing agent and it forms a hemiacetal with its reduced reaction product dialuric acid (in which a carbonyl group is reduced to ahydroxyl group) which is called alloxantin.

2.3.3: Mechanism of Action of alloxan

Alloxan and streptozotocin are widely used to induce experimental diabetes in animals. The mechanism of their action in B cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of the both diabetogenic agents is mediated by reactive oxygen species; however, the source of their generation is different in the case of alloxan and streptozotocin. Alloxan and streptozotocin are toxic glucose analogues that preferentially accumulate in pancreatic beta cells via the GLUT2 glucose transporter. In the presence of intracellular thions, especially glutathione alloxan generates reactive oxygen species (ROS) in a

cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defense capacity, and the ensuing state of insulin-dependent 'alloxan diabetes.

However, the initial lesions on essential beta-cell structures are not known (Szkudelski, 2001). As a thiol reagent, alloxan also selectively inhibits glucose-induced insulin secretion through its ability to inhibit the beta cell glucose sensor glucokinase (Lenzen, 2008). Glucose transporters (GLUT2) and glucokinase (GK) are target molecules. A gradual decrement of both GLUT2 and GK mRNA expression was found in islets isolated from ALX-treated C57BL/6 mice (Szkudelski, 2001). The severity of disturbances in carbohydrate metabolism in rats with alloxan-induced diabetes depends on activity of antioxidant enzymes in the target organ (pancreas). The antioxidant compound probucol indirectly increased activity of antioxidant enzymes in the pancreas and prevent the development of alloxan-induced diabetes in rats (Lankin, 2004).

The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but when it is administered intraperitoneally (i.p.) or subcutaneously its effective dose must be higher (Federiuk *et al.,* 2004) as cited by Frode (2008). For instance, an intraperitoneal dose below 150 mg/kg may be insufficient for inducing diabetes in animal species (Katsumata *et al.*, 1992) as cited by Frode (2008).Alloxan does not cause diabetes in humans (Lenzen, 2008). Others found a significant difference in alloxan plasma levels in children with and without Type 1 diabetes (Mrozikiewicz *et al.*, 1994).

However, in the case of Streptozotocin, it enters the B cell via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity

and participates in DNA damage. As a result of the streptozotocin action, B cells undergo destruction by necrosis. (Bromme and Horm, 2001). In both conditions type 1diabetes is common.

Commercial use of alloxan; Alloxan is a raw material for the production of the purple dye murexide. Carl Wilhelm Scheele discovered the dye in 1776. Murexide is the product of the complex *in-situ* multistep reaction of alloxantin and gaseous ammonia. Murexide results from the condensation of the unisolated intermediate uramil with alloxan, liberated during the course of the reaction.

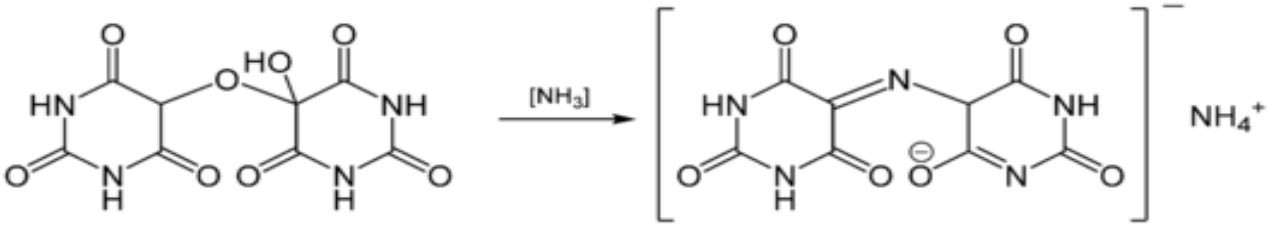


Fig. 2.3 Murexide dye (right) from reaction of alloxantin (left)

Safety and Hazard control measures (**Fire Fighting Measures)**

Small fire: Use dry chemical powder while for Large Fire use water spray, fog or foam. Do not use water jet.

Clean up Methods

Small Spill: Use browm and packer were used to put the spilled solid in a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority.

Large Spill; A shovel to put the material into a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and allow evacuating through the sanitary system.

Disposal Methods: The most favorable course of action is to use an alternative chemical product with less inherent propensity for occupational exposure or environmental contamination. Recycle any unused portion of the material for its approved use or return it to the manufacturer or supplier. Ultimate disposal of the chemical must consider: the material's impact on air quality; potential

migration in soil or water ; effects on animal, aquatic, and plant life; and conformance with environmental and public health regulations.

Eye contact; Check for and remove any contact lenses. In case of contact, immediately flus eyes with plenty of water for at least 15 minutes. Get medical attention if irritation occurs.

Skin contact; Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops

Inhalation: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Ingestion; DO NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately.

Handling and Storage; Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 8^{o} C (46.4^{o}F).

2.4 Biochemical indexes

2.4.1: Blood Glucose level

Several studies have been carried out to prove hypoglycaemic potentials of plant foods. These studies generally assume that the effects may be caused by the bioactive compounds present in the plants A number of medicinal/culinary herbs have been reported to yield hypoglycemic effects in patients with diabetes, examples of these include bitter melon, Gymnema, Korean ginseng, onions (Alan et al., 2003). As assured by Bailey and Day (1998), botanical products can improve glucose metabolism and the overall condition of individuals with diabetes not only by hypoglycemic effects but also by improving lipid metabolism, antioxidant status, and capillary function.

Alan *et al.* (2003), documented that intake of 1, 3, or 6 g of cinnamon per day reduces serum glucose (18 -29%) levels in people with type 2 diabetes and suggested that the inclusion of cinnamon in the diet of people with type 2 diabetes will reduce risk factors associated with diabetes and cardiovascular diseases. Futhermore, aqueous extracts of Fraxinus excelsior (FE) seed and Silybum marianum (SM) aerial part was investigated in diabetic rats incorporated with

streptozotocin and was proved to produce a significant decrease of blood glucose levels (Maghrani *et al.*, 2004).

Also, a single oral administration of the aqueous extract of Piper sarmentosum Roxb.at doses of 0.125g and 0.25 g/kg significantly lowered the plasma glucose levels in healthy rats (Pengvicha et *al*, 1998). Because insulin also plays a key role in lipid metabolism, we postulated that consumption of cinnamon would lead to improved glucose and blood lipids in vivo (Alan *et al*., 2003).

The speed of glucose capture by human lymphocytes has been observed in four studies (Estrada *et al.,* 1994; Piątkiewicz *et al.,* 2007; Piątkiewicz, 2010). In a study, glucose capture was measured in an indirect way by measurement of glucose depletion from incubation surface (Estrada *et al.,* 1994; Piątkiewicz, 2010) in other recent studies, cells radiolabelled with 2-deoxy-D-glucose were incubated (Piątkiewicz *et al.,* 2007; Piątkiewicz *et al.,* 2010). In these studies it was shown that glucose capture reaches its peak at the 30th minute of the examination. Later on, the plateau phase was observed with insulin concentration exceeding 100 Mu/ L (Piątkiewicz, 2010).

In quoted works, the examined cells were acquired from patients with type 1 diabetes mellitus, displaying insulin resistance, which is a probable cause of the necessity of employment of considerably higher concentrations of insulin required for the achievement of maximal deoxy-D-glucose capture. In humans (Rizza, Mandarino and Gerich, 1981) and in rats (Kraegen, James and Jenkins, 1985; Rosetti and Giaccari, 1990; Piątkiewicz, 2010) glucose capture in peripheral tissues (muscles and adipose tissue) can be accurately measured with the method of hyperinsulinemic euglycaemic clamp and radiolabelled glucose infusion. In these experiments,

the increase of insulin level in serum to100 Mu/L caused an increase of the hypoglycaemic state of the patient.

2.4.2 Serum Lipids

Several studies have been carried out to show relactionship between diabetes and lipid abnormality. Many of these studies showed that the control of diabetes authomatically resulted in near or complete normalization of the lipid profile of the diabetic human or animal model.

Lipids are the building blocks of fats and fatty substances found in animals and plants. They are microscopic layered spheres of oil, which, in animals, are composed mainly of cholesterol, triglycerides, proteins (called lipoproteins), and phospholipids (molecules made up of phosphoric acid, fatty acids, and nitrogen). Lipids do not dissolve in water and are stored in the body to serve as sources of energy.

Diabetes is generally accompanied with lipid metabolism abnormality known as diabetic dyslipidaemia which increase the risk for coronary heart disease (Dhanush *et al..,* 2012.) Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and phospholipids and changes in lipoprotein composition. One of the major pathogenesis of lipid metabolism disturbances in diabetes is the increased mobilization of free fatty acids from adipose tissue and secondary elevation of free fatty acid level in the blood due to insulin deficiency or insulin resistance. The excessive lipolysis in diabetic adipose tissue may lead to increased free fatty acids in circulation which enter the liver and are esterified to form triglycerides (Dhanush *et al.,* 2012). Therefore, the fatty acid compositions of various tissues are altered in both human and animal experimental diabetic studies (Dhanush *et al.,* 2012).

Diabetes mellitus carries a high risk of atherosclerosis, and cardiovascular disease, especially coronary heart disease (CHD) and stroke. Coronary heart disease (CHD) and stroke are the leading cause of death among patients with type 2 diabetes. The cluster of lipid abnormalities associated with type 2 diabetes is defined by a high concentration of triglyceride (TG) and small dense low density lipoprotein (LDL) and a low concentration of high density lipoprotein (HDL) cholesterol. Plasma LDL cholesterol levels are generally normal. Insulin resistance is believed to contribute to this atherogenic dyslipidemia by increasing the hepatic secretion of very low density lipoprotein (VLDL) and other Apo lipoprotein (apo) B-containing lipoprotein particles, as a result of increased free fatty acid flux to the liver (Berneis and Kraus, 2002).

Diabetic dyslipidemia may also be the result of a diminished suppressive effect of insulin on apo B secretion, either at the level of the regulation of apo B degradation, or inhibition of microsomal TG transfer protein activity (Malmstrom *et al.,* 1997). Through the action of cholesterol ester transfer protein, TGs are transferred from VLDL to HDL, creating TG-rich HDL particles, which are hydrolyzed by hepatic lipase and rapidly cleared from plasma. A similar cholesterol ester protein-

mediated transfer of TGs from VLDL to LDL contributes to the formation of small dense LDL particles (Berneis and Kraus, 2002). Other mechanisms, including impaired clearance of lipid and lipoproteins may also be involved. Therefore, diabetes tends to lower "good" cholesterol levels and raise triglyceride and "bad" cholesterol levels, which increases the risk for heart disease and stroke. This common condition is called diabetic dyslipidemia. Studies show a link between insulin resistance, which is a precursor to type 2 diabetes, and diabetic dyslipidemia, atherosclerosis and blood vessel disease. These conditions can develop even before diabetes is diagnosed (American Diabetic care, 2009). Recent studies on diabetes also bring into focus how to control lipid dysfunction associated with pooril controlled diabetes.

In a study by (Alan *et al.*, 2003) intake of 1, 3, or 6 g of cinnamon per day reduces serum triglyceride (23–30%), LDL cholesterol (7–27%), and total cholesterol (12–26%) levels in people with type 2 diabetes and suggested that the inclusion of cinnamon in the diet of people with type 2 diabetes will reduce risk factors associated with diabetes and cardiovascular diseases. There were also significant decreases in serum cholesterol levels in all three groups consuming cinnamon, and no changes were noted in the respective placebo groups Rashwan (1998) reported that supplementation of the diet of rabbits with fenugreek decreased total serum lipid level. In rats, curry leaf and mustard seeds decreased total serum cholesterol, LDL cholesterol, and VLDL cholesterol and increased HDL cholesterol levels (Alan *et al*., 2003).

Triglycerides; Triglycerides are composed of fatty acid molecules. They are the basic chemicals contained in fats in both animals and plants. Triglyceride is the most common type of fat in the body. Normal triglyceride levels vary by age and sex. A high triglyceride level combined with low HDL cholesterol or high LDL cholesterol is associated with atherosclerosis that increases the risk for heart attack, peripheral artery disease (PAD) and stroke.

Triglycerides interact with HDL cholesterol in such a way that HDL levels fall as triglyceride levels rise. High triglycerides may pose other dangers, regardless of cholesterol levels. For example, they may be associated with blood clots that form and block the arteries. High triglyceride levels are also associated with the inflammatory response -- the harmful effect of an overactive immune system that can cause considerable damage to cells and tissues, including the arteries.

Cholesterol; Cholesterol is a waxy substance that is made by the body and found in all animals and some animal- based foods but not in plants. Cholesterol includes HDL-C, or "good" cholesterol and LDL-C or "bad" cholesterol. In spite of the fact that people detest cholesterol cholesterol is an essential nutrient necessary for many functions, including: repairing cell membranes, manufacturing vitamin D on the skin's surface, producing hormones such as estrogen and testosterone, HDL removes LDL cholesterol from the walls of the arteries and returns it to the liver for disposal from the body and also help prevent oxidation of LDL. HDL actually appears to have its own antioxidant properties; it might fight inflammation and possibly helping cell connections in the brain that are important for learning and memory. Active ingredients necessary for the control of lipids can be found in other parts of the medicinal plants not just in the leaves. A significant reduction in the total serum cholesterol (TC) Low density lipoprotein chole sterol (LDL-C) levels and decreased activity of HMGCoA reductase was observed in alloxan rats feed ethanol extract obtained from seeds of Eugenia jambolana (Sharma, 2003).

Although the body acquires some cholesterol through diet, about two-thirds is manufactured in the liver. Its production is stimulated by saturated fat. Saturated fats are found predominantly in animal products, such as meat and dairy products, and are strongly associated with higher cholesterol levels. Tropical oils - such as palm, coconut, and cocoa butter - are also high in saturated fats (Daniels and Greer 2008).

Though cholesterol is important to overall health, but when levels are too high, depending on the type of cholesterol, it may become a source of danger. Cholesterol can be harmful by contributing to narrowed or blocked arteries. Unfortunately, people with diabetes are more prone to having unhealthy high cholesterol levels, which contributes to cardiovascular disease (CVD). Genetic factors, type 2 diabetes and certain drugs, such as beta-blockers and anabolic steroids also lower HDL cholesterol levels. Smoking, being overweight and being sedentary can all contribute to lower HDL cholesterol. The lowest incidence of heart disease is usually found among people with the lowest LDL levels. Lowering LDL is the primary goal of cholesterol drug and lifestyle therapy. Low-density lipoprotein (LDL) transports about 75% of the blood's cholesterol to the body's cells. It is normally harmless. However, if it is exposed to a process called oxidation, LDL can penetrate and interact dangerously with the walls of the artery, producing a harmful inflammatory response.

The association between reduced HDL cholesterol levels and increased risk of heart disease is well established, independently of TG levels and other risk factors. Subramanian, (2006) reported that aloe vera leaf gel extract decreased plasma levels of high-density lipoprotein–cholesterol and increased plasma levels of low-density lipoprotein–and very low-density lipoprotein–cholesterol in diabetic rats were restored to near normal levels following treatment

Ensuring normalization of lipid profile is very important in health management. HDL helps keep arteries open and reduces the risk for heart attack. High levels of HDL (above 60 mg/dL) may be nearly as protective for the heart as low levels of LDL. HDL levels below 40 mg/dL are associated with an increased risk of heart disease. In fact, the "low HDL cholesterol" or "hypo alpha" syndrome is the most frequent lipoprotein abnormality in coronary patients (Genest *et al.,* 1997). Studies consistently report a higher risk for death from heart disease with high total cholesterol levels (200 mg/dL and higher), thus, higher cholesterol, greater the risk. On average, every time a person's total cholesterol level drops by a point, the risk of heart disease drops by 2%.

Reducing LDL ("bad" cholesterol) and total cholesterol levels, while at the same time boosting HDL ("good" cholesterol) levels can prevent heart attacks and death in all people (with or without heart disease). If patient has diabetes, even without heart disease, medication may be considered for LDL cholesterol of 100 mg/dL The bark of *Morinda lucida* is widely used in Cameroon as a decoction for treating Diabetes mellitus. A significant ($p<0.001$) decrease was observed in the concentration of cholesterol, triglyceride, glucose and creatinine (Agbor *et al* 2015). In a similar study, diabetes induction caused significant increases (P=.05) in total cholesterol (TC) with 54.42% and low density lipoprotein (LDL) with 55.0% compared to the normal control (NC), while treatment with extracts of Gongronema latifolium significantly decreased (P=.05) these by (58.70% TC) and (71.70% LDL) respectively Effiong Eshiet, and Ajibola, (2015

There are no warning signs for high LDL and other unhealthy cholesterol levels. When symptoms finally occur, they usually take the form of angina (chest pain) or heart attack in response to the buildup of atherosclerotic plaque in the heart arteries.

Intravascular ultrasound studies demonstrate that patients with low HDL cholesterol and high TG levels have more extensive coronary atheroma than those with an isolated elevation of LDL cholesterol. Patients with reduced HDL cholesterol levels show intima-media thickness results similar to those with familial hypercholesterolemia (Baldassarre et al., 2002). While a high level of HDL cholesterol reduced plaque growth in subjects with pre-existing carotid atherosclerosis (Johnson *et al*., 2005).

The HDL particles induce the removal of cholesterol from cells, including those in atherosclerotic plaques, and carry them to the liver, but the mechanisms by which HDL confer protection from atherosclerosis include more than just reverse cholesterol transport. HDL particles seem to have anti-inflammatory and antioxidant properties, inhibiting the oxidation of LDL cholesterol and the expression of cellular adhesion molecules and monocyte recruitment.

The HDL may also reduce the risk of thrombosis by inhibiting platelet activation and aggregation. Recent studies, however, demonstrated that it may be the level of apoA-I, rather than the level of HDL cholesterol, that is important in reducing the risk of atherosclerosis (Vander Steeg *et al.*, 2008). In fact, high plasma HDL cholesterol and large HDL particles may be associated with increased coronary artery disease (CAD) risk when levels of apoA-I and apoB remain constant. In contrast, apoA-I remains negatively associated with CAD risk, even in high concentrations.

In a post hoc analysis of four prospective randomized intravascular ultrasound trials, a decrease in LDL cholesterol levels and an increase in HDL cholesterol levels were independent predictors of atheroma regression (Nicholls *et al.,* 2007). Statin therapy was associated with regression of coronary atherosclerosis when LDL cholesterol was substantially reduced, and HDL cholesterol was increased by >7.5%. The hypolipidaemic effect of *Gongronema latifilium* can be explained as a consequence of reduction in blood glucose Effiong, Eshiet, and Ajibola, (2015). Thus the plant extract was capable of ameliorating hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats and could be a potential source for isolation of new orally active agent(s) for anti - diabetic therapy Effiong Eshiet, and Ajibola, (2015) Activation of peroxisome

proliferator–activated receptor (PPAR) -α has numerous effects that lead to an improvement in dyslipidemia. These include the induction of enzymes mediating fatty acid oxidation, stimulation

of lipid import into the cell in part by induction of lipoprotein lipase, and suppression of apo C-III, which interferes with the clearance of TG-containing lipoproteins. The result is a reduction of TG levels, increase in HDL cholesterol levels, and reduction of small dense LDL levels. These changes have a favorable effect on endothelial function and hematologic parameters. There are many major dietary approaches for protecting health, such as the Mediterranean diet, which emphasizes fruits, vegetables, and healthy types of fats (Daniels and Greer 2008). According to New York Times (2012), the following are advocated.

- Choose fiber-rich food (whole grains, legumes, and nuts) as the main source of carbohydrates, along with a high intake of fruits and vegetables.
- Walnuts in particular have cholesterol-lowering properties and are good sources of antioxidants and alpha-linoleic acid.
- Avoid saturated fats (found mostly in animal products) and Trans fatty acids (found in hydrogenated fats and many commercial products and fast foods).
- Choose unsaturated fats (particularly omega-3 fatty acids found in vegetable and fish oils). For dairy products, choose low fat over high fat.
- For proteins, choose soy protein, poultry, and fish over meat. Studies have found that soy does not help improve cholesterol. However, it is still a heart healthy food choice.
- Fish is particularly heart protective. People who don't or won't eat fish can take a daily fish oil supplement. Omega-3 fatty acid fish oil supplements contain docasahexaenoic (DHA) and eicosapentaenoic (EPA) acids, which have significant benefits for the heart, particularly for lowering triglyceride levels. Fish oil supplements are also available in prescription form (Lovaza).
- Controlling weight, quitting smoking, and exercising are essential companions of any diet program. Niacin, like the stains, has been used to treat elevated blood cholesterol levels as well as elevated triglyceride levels (Medicine Net.com, 2011).

From puberty on, men tend to have lower HDL (“good” cholesterol) levels than women. One reason is that the female sex hormone estrogen is associated with higher HDL levels. Because of this, premenopausal women generally have lower rates of heart disease than men.

After menopause, as estrogen levels decline, women catch up in their rates of heart disease. Throughout the menopausal years, HDL levels decrease and LDL ("bad" cholesterol) and triglyceride levels increase. For men, LDL and triglyceride levels also rise as they age and the risks for heart disease increase as well. There is some evidence that high triglyceride levels carry more risks for women than men. Heart disease is the main cause of death for both men and women (New York Times, 2012).

2.4.3. Liver and liver enzymes

The liver is the largest gland in the body, and one of the most important organs for the overall health of the body. It serves many vital functions, like production of bile, albumin and more importantly, the detoxification of chemicals and drugs that pass through the body (Bassaventhappa, 2010). The liver, like the salivary gland and pancreas, is an outgrowth of the digestive tract, a very large and versatile organ endowed with metabolically active cells which independently coordinate virtually all biochemical transformations. Accordingly, the regenerating power of these cells is tremendous and involved metabolic, secretary, excretory, storage, detoxification, protective and synthetic functions, among others (Chatterjea and Shinde, 2002). Many plasma proteins are synthesized in the liver and their plasma levels therefore depend on the balance between synthesis and catabolism and/ or loss from the body (Chatterjea and Shinde, 2002). The cells in the liver contain proteins called enzymes that drive these chemical reactions. When liver cells are damaged or destroyed, the enzymes in the cells leak out into the blood, where they can be measured by blood tests.

These liver enzymes and proteins generally are very important biomarkers in the body. The liver enzymes are utilized in the diagnosis and assessment of normal function or otherwise of the tissues, organs and the body as a whole. Major or minor changes in the integrity of cellular membranes in tissues or organs have culminated in changes in enzyme activities. For instance, alanine amino transferase (ALT) and Aspartate aminotransferase (AST) are useful in detecting alterations in liver disease - hepatocellular damage or increased permeability of liver cells while ALT and gamma glutamyl transferase (GGT) are implicated in extra-hepatic or intra-hepatic obstruction (Chatterjea and Shinde, 2002). induction and alteration in the membrane permeability (Fakurazi, Hairuszah and Nanthini, 2008)]. Increased aminotransferases and alkaline phosphatase was the clear manifestation of cellular leakage and loss of functional integrity of the cell (Saroswat, Visen, Patnalik and Dhawan, 1993).

An elevated level of these enzymes markers of hepatic necrosis in animals indicates tissue damage. Injury to the hepatocytes changes their transport function and membrane permeability, causing leakage of enzymes from the cells (Nsari and Asif ,2012). Consequently, the significant release of these enzymes and their increased activity in animals can be interpreted to be as a result of liver cell destruction.

Hepatocellular damage releases ALT and AST into the bloodstream. ALT is found primarily in the liver; AST is also found in skeletal muscles and erythrocytes. Therefore, elevations in ALT levels generally are more specific for hepatic injury. At times, the AST/ALT ratio can suggest certain disease patterns. For instance, a ratio greater than 2 suggests alcoholic liver disease, whereas nonalcoholic fatty liver disease is usually associated with a ratio of less than 1. A ratio greater than 4 may suggest Wilson disease (Ahmed and Siddiqi, 2006). When the AST/ALT ratio does not suggest an etiology, asymptomatic elevation of liver tranmsaminase levels can be categorized into common hepatic, less common hepatic, and extra hepatic causes.

Mild elevations in levels of the liver enzymes alanine transaminase and aspartate transaminase are commonly discovered in asymptomatic patients in primary care (Ahmed and Siddiqi, 2006). Liver enzymes can serve as markers of hepatocellular injury e.g. aspartate aminotransferase (AST), alanine aminotransferase (ALT) or of an obstruction in the bile flow cholestasis e.g. alanine phosphatase (ALP) and gamma-glutamyl transferase (GGT). Although these enzymes are elevated in liver disease, the elevation can also be secondary to enzyme induction without hepatic pathology (Ahmed and Siddiqi, 2006). Increases in both aminotransferases (AST and ALT) are found in liver diseases with ALT activity being much higher than AST activity (Gray and Howorth, 1980; Zilva and Pannal, 1984).

There was a significant high liver enzyme activity in diabetic rats fed Vernonia amygdalina compared to the non-diabetic (Atangwho, *et al.,* 2007). From the results the increase in enzyme activity were not dose dependent as was the case also with whole liver protein level. For instance, at a dose level of 300mg/kg body weight, in diabetic rats, the AST, ALT, ALP and GGT activities were decreased significantly when compared to the control, whereas a test dose of 400mg/kg body weight, increased significantly ALP and GGT activities when compared to the control (Atangwho, *et al.,* 2007).

Although this appears difficult to explain, but it clearly emphasizes the sensitivity and relevance of dose margins and precision in toxicity studies (Atangwho, *et al..*, 2007). Recent studies have demostated the effects of medicinal plants in controlling increased liver cel activities. For example, the amino transferases (ALT and AST) parameters activities which increased by 66.83% and 72.87% in the diabetic control (DC) rats became reduced upon treatment with Gongronema latifolium Effiong Eshiet, and Ajibola, (2015).

2.4.3.1 Liver Function Tests

The liver enzyme tests are a group of blood tests that detect inflammation and damage to the liver. They can also check how well the liver is working. Liver enzyme level depends on the extent of the initial liver damage. Permanent Liver damage leads to complete liver failure. Liver enzyme test includes ALT, AST, alkaline phosphatase; true liver function tests (LFTs) include PT, INR, albumin, and bilirubin.

Aspartate aminotransferase (AST), formerly called SGOT; the AST enzyme is also found in muscles and many other tissues besides the liver.

- Alanine aminotransferase (ALT), formerly called SGPT; ALT is almost exclusively found in the liver.
- If ALT and AST are found together in elevated amounts in the blood, liver damage is most likely present
- When bile flow is slow or blocked, blood levels of certain liver enzymes rise: Alkaline phosphatase 5 nucleotidase ,Gamma-glutamyl transpeptidase (GGT)

If alkaline phosphatase and/or 5 nucleotidase and GGT are elevated, a problem with bile flow is most likely present. Bile flow problems can be due to a problem in the liver, the gallbladder, or the tubes connecting them. Since alkaline phosphatase is also found in bone, it can be elevated in certain medical conditions that affect bone, as well.

2.5 Toxicity studies

Hepatotoxins are toxic substance that can damage the liver. Some toxins are known for having properties that can bring about liver damage and that always lead to liver toxicity. Though liver toxicity can occur in any age, young people are more susceptible to adverse drug or substance

reactions and liver damage due to their immature liver metabolism, functionality and excretory functions. Usually, hepatotoxicity is associated with other clinical manifestations of drug allergy (fever, rash and eosinophilia). However, an individual may be more likely to develop liver toxicity symptoms that are related to a particular drug or substance than another might under seemingly similar circumstances. This type of reaction is called an "idiosyncratic reaction" and can sometimes lead to unpredictable injury (Aucker, 1996; Bassaventhappa 2010).

In comparison to other organs, the liver is more susceptible to adverse toxicity reactions due to its location and to its central role, in the metabolism of toxic chemicals and drugs. Liver toxicity is most commonly associated with adverse drug reactions and over dosage of some substances. The severity of the liver toxicity is also determined by age, nutritional status, concurrent diseases, hereditary factors, other drugs being used, and previous exposure to the same or similar drugs or substance. The extent of the liver injury also depends on drug concentration, duration and frequency of drug or substance exposure, and current liver health status.

Several studies have been carried out to describe the effect of toxicity of drugs, plant extracts and food substances in the liver cells. The results of acute toxicity showed no mortality and general behaviour changes when fed kernel extract of sclera carya birrea (Mohammed and Sharif, 2011). However, during acute toxicity experiment, mice treated with higher dose of Harpagophytum procumbens (Devil's claw) were found to have reduced loco motor activity as compared to the control animal (Naif *et al.,* 2011).

The ALT and AST are liver specific enzyme markers of necrotic injury and cholestasis (Speech and Liehr, 1983). The significant increase in AST and ALT following the administration extract of sclerocarya could be due to damage to the hepatic cell or heart attack (Hearly, Sambaiah and Cole, 1995) and may have been induced by some phytocompounds of the kernel extract of sclerocarya. The increase could be attributed to enzyme activation by the phytochemical constituents of the kernel (Mohammed and Sharif, 2011). This knowledge is usually applied in monitoring the safety of xenobiotic such as trial drugs (including medicinal plant extracts), since the liver is preoccupied with the function of biotransformation of xenobiotics (Crook, 2006).

In a study, aloe vera(Liliaceae) extracts did not produce any mortality throughout the study period of 15 days even when the limit dose was maintained at 400mg/kg and 600mg/kg of body weight. So, testing the extracts at a minimum dose was practically non-toxic (Rajalakshmi, Jayachitra, Gopal and Krithiga, 2014).

Furthermore, one male animal was found to develop forelimb inflammation and snout alopecia during chronic toxicity studies. All other animals were normal and comparable to the control animals. (Naif *et al.*,2011). At the end of the treatment, even the visceral condition and the vital organs of animals were found to be normal and comparable to the control. (Naif *et al.*,2011).

The result of acute oral toxicity (LD_{50}) of Morinda lucida leaf extract was found to be greater than 6400mg/kg body weight as no mortality was recorded in any group of experimental rats. In an acute oral toxicity study by Adeneye and Agbaje (2008) Morinda lucida leaf extract was documented to be non-lethal in rats at 2000mg/kg body weight.

Niacin can damage the liver. It can cause mild transient elevations in blood levels of AST and ALT, jaundice, and, in rare instances, liver failure. Liver toxicity with niacin is dose-dependent; toxic doses usually exceed 2 grams per day Patients with pre-existing liver diseases and those who drink alcohol regularly are at higher risk for developing niacin toxicity. The current LD_{50} values based on acute oral toxicity recommended by the Globally Harmonized System of Classification and Labeling of Chemicals are as follows: Category 1, ≤ 5mg/kg; category 2, > 5mg/kg≤50mg/kg [they are both labeled as danger: fatal if swallowed]; category 3, >50mg/kg≤300mg/kg [danger: toxic if swallowed]; category 4, >300mg/kg ≤ 2000mg/kg [warning: harmful if swallowed], category 5, >2000mg/kg≤5000mg/kg [warning: may be harmful if swallowed] and LD_{50} >5000mg/kg [not classified: no specified label]. Hence, the LD_{50} of greater than 6400mg/kg is an indication that the extract may be safe for human consumption, confirming the belief of the herbalists that *Morinda lucida* leaf extract is not harmful (Oduola *et al.,* 2010). However, LD_{50} should not be regarded as a biological constant, since differing results are obtained on repetition or when the determinations are carried out in different laboratories (Lorke, 1983) due to many variables such as animals' species and strain, age, gender, diet, bedding, ambient temperature, and time of the day. Hence, there are considerable uncertainties in extrapolating LD_{50} value obtained for specie to other species,

consequently, recognizing LD_{50} test as providing, at best, only a ballpark estimate of human lethality has been advocated Zbinden and Flury-Roversi (1981).

Symptoms of liver toxicity; Symptoms can vary depending on the duration of exposure and the type of toxin. The following symptoms may be observed in liver toxicity:

- Loss of appetite
- Vomiting
- DiarrhealJaundice (often progressive)
- Weakness
- Fluid in the abdominal cavity (ascites) - this symptom is often indicative of advanced disease
- Coma
- Hemorrhages
- Petechia (minute red or purple spots on the surface of the skin as the result of tiny hemorrhages of blood vessels in the skin)
- Ecchymosis (the escape of blood from ruptured blood vessels into the surrounding tissue, forming a purple or black-and-blue spot on the skin)

2.5.1 Management of toxicity.

Special dietary support is generally prescribed for patients with toxicity. Immediate treatment is withdral of the substance coupled with the use of antidote where necessary. Supportive and acurate nutrition is essential for energy levels and a successful recovery. The already damaged liver is very vulnerable for some time after treatment, and needs to be treated with great care. The consequence of chronic liver failure is death (Bassaventhappa, 2010).

2.6: Summary of literature review on nutrient and phytochemical composition of leafy vegetables decoction from *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida*, and their effects on blood glucose, lipid profile, liver enymes, pancreatic and liver cells of rat models.

Several studies have been done in this field. Out of 115 medicinal plants collected and used in Nigeria, 100 were found to be useful in management of diabetes (Udoamaka, Ezuruike and Preito,

2014). Therefore, herbs have been reported as one of the remedies used for treatment by patients with diabetes in Zimbabwe, Nigeria, Vietnam, Oman, India and China (Elizues *et al.,* 2013).
Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity (Okanla Oyewal and Akinyanju, 1990). For example, medicinal plants enhance the glucose uptake by GLUT4 translocation and were proven by in vitro glucose uptake model (Rajeswaria and Sridevi, 2014).The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of the bioactive constituents of plant are alkaloids, tannins, flavonoids, saponins and phenolic compounds (Ekhaise, Soroh and Falodun, 2010).

Recently (Nwaoguikpe, 2010) documented the presence of some vitamins such as vitamin A 345.50 ± 0.0 IU), Vitamin C (228.40 ± 0.0 mg), Vitamin E (37.30 ± 0.01 mg), vit.B1 (1.0 ± 0.00 mg), vit.B2 (3.10 ± 0.00 mg),fe (11.0 ± 0.0 mg) and niacin (0.41 ± 0.0 mg) in analyzed sample of Vernonia amygdalina (bitter leaf) extract Also, the phytochemical analysis of *Cnidoscolus aconitifilius* plant leaves showed mean concentrations (%) of tannins, saponins, cyanogenic glycosides, alkaloids, phenols, flavonoids and steroids at 0.14, 4.04, 0.003, 4.72, 0.19, 2.36, and 0.27, respectively, (Akachukwu, Okafor and Ibegbunam, 2016).

Similarly, from the wood and bark of *Morinda lucida* 18 anthraquinones, tannins, flavonoids and saponosides have been isolated (Zimudzi and Cardon, 2005). The phytochemical screening of CA also revealed the presence of some bioactive compounds in the plant. Seven bioactive constituents were tested, out of which four tested positive in CA. Analysis of tannins, alkaloids, flavonoids and saponins were positive, while phlobatannins, anthraquinones and cardiac glycosides were completely absent in the extract http://dx.doi.org/10.5455/jib.20140504023102

The plant extract of Vernonia amygdalina (bitter leaf) administered to alloxan induced diabetic rats orally at concentrations of 2%, 4%, 6%, 8% and 10% before meals for 5, 10 and 15 days respectively was shown to lower the blood glucose Nwaoguikpe, 2010).

In a similar study by Ugochukwu and Babbady (2002) ethanol and methanol extracts of *Gongronema latifilium* significantly decreased triglyceride levels and normalized total cholesterol concentration. There was also a remarkable decrease in blood glucose level from the mean value of

4.44 ± 0.2 to 1.66 ± 0.2 mmol/L. The decrease in the blood glucose level of the rats following the administration of the plant extract suggests that the plant extract possesses anti-diabetic, anti-

hyperglycemic and hypoglycemic effects on alloxan induced diabetic rats (Ugochukwu and Babbady, 2002).

According to Abdul (2012) the main uses of *Luffa aegyptiaca* are dyslipidemia, anti -diabetic, hepato protective, anti-hypertensive and diuretic. Similarly, Adeneye and Agbaje (2008) documented that the bark of *Morinda lucida* is widely used in Cameroon as a decoction for treating diabetes mellitus. Administration of extracts for four weeks had significant reduction effect on glucose, creatinine, cholesterol and triglyceride at dose level of 5 g/ kg body weight (Adeneye and Agbaje, 2008).

Results showed significant decrease in total cholesterol, TG, LDL levels and it was more with bark and flower extracts than other parts and there was significant increase in HDL cholesterol compare to diabetic control. The underlying mechanism explained for lipid lowering activity was due to inhibition of lipid absorption by saponins and tannins present in the medicinal plants (Venu et al., 2013). In a study by Agbor *et al*.(2012), the biochemical parameters showed a significant ($p<0.05$) increase in ALT and AST at the dose level of 5 g kg^{-1} in both two weeks and four weeks examination.

Morinda lucida extract had a significant (5<0.05) dose reducing effect on creatinine and triglycerides at 2 weeks administration while at four weeks extract had significant reduction effect on glucose creatinine, cholesterol and triglyceride at dose level of 5 g kg^{-1}. In the same study, the histo-pathological examination revealed steatosis in the liver at 5 g kg^{-1} *Morinda lucida* extract administration. Thus *M. lucida* aqueous extract is well tolerated at low dose administration but may be toxic at dose level of 5 g kg^{-1} at sub-administration.

Though, it is obvious that numerous studies have been done to analyze the nutrient and phytochemical composition of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifil-ius* and *Morinda lucida* as well as their effects on different disease conditions especially chronic disease as such as diabetes and hypertension but all concentrated on the use of extracts. It is known that humamans do not consume extracts so works on extracts may not be applicable to human

beings. To bridge that gap, this work was intended to evaluate the nutrient and phytochemical composition of leafy vegetables decoction from *Gongronema latifilium, Luffa aegyptiaca,*

Cnidoscolus aconitifilius and *Morinda lucida,* and determine their effects on blood glucose, lipid profile, liver enymes, pancreatic and liver cells of alloxan induced diabetic male Wistar rats.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3. 1 Research design

The research design was experimental / intervention. This involves focuses on establishing cause and effect relationship. In such studies the researcher plays active roles by carrying out interventions and observing the effects of the interventions on the dependent variables of interest. The design is characterized by manipulation of independent variables, control over the experimental situation and randomization including random selection of subjects and assignment into experimental and control groups. In this design, the researcher plays an active role by introducing the interventions on the independent variables and takes decision about the full nature of the intervention or treatment. This includes the method of manipulating the independent variables, the group to receive which intervention or treatment, the extent of the intervention or treatment and the period to administer the treatment and whom to administer the treatment.

3.1.2: Procurement of materials:

Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius and *Morinda lucida* leafy vegetables were used for the study. They were collected from their natural habitats at Umuabi - Udi local Government Area of Enugu state. Matured leaves were used for the study. The leaves were authenticated by a Taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka and a voucher specimen kept in the herbarium for further reference. Samples were prepared by the reaesarcher within one hour of collection.

Recruitment and training of Research Assistants.

Four research assistants were engaged. One Nurse, one Laboratory Scientist, one laboratory technician and one Nutritionist were coopted. The research assistants were trained on how to master and use the code numbers and how to catch and handle the rats. They were also tutored on how to measure and administer correct treatments.

3.1.3: Preparation of the samples.

The researcher paid preliminary visits to the President of defunct Umuabi General Assemble and Umuabi Progressive Union, respectively. Through them the researcher contacted the village heads and leaders. With the help of the village heads and leaders the researcher contacted the users of plant remedies in management of diabetes. Many of the users were un-cooperative, very secretive and repelled participating in the research. However, with the help of the village heads and leaders the researcher finally got three users per plant. The twelve users agreed to participate in the research on condition that they will be visited individually to demonstrate the preparation methods. The condition was accepted by the researcher.

The researcher then visited the three users per plant and watched them individually preparing the green leafy vegetables decoction. After the initial visits the researcher made arrangement with the individual users on when to come with some necessary equipment for better reportage. The equipment were measuring jug, weighing scale and stop watch. On each user's appointment day the researcher visited the user with measuring jug, weighing scale and stop watch in company of research assistants.

On each visiting day, the researcher with the help of the research assistants measured the quantity of each green leafy vegetable used using a weighing scale, the volume of water used for boiling it with measuring jug and the time taken to boil it using a stop watch. At the end of the whole visits it was observed that different quantities or measures of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables respectively, were used by the three different users. Generally, they were separately picked to remove extraneous materials and washed with deionized water. Also, they were separately prepared by boiling in water for some minutes and the decoction sieved out for consumption.

From the three users, the measures of green leafy vegetables used, the quantity of water used, as well as the boiling time differed slightly. The method of preparation was demonstrated by the researcher before each user to ensure that the samples for the study will be properly prepared. The samples were then prepared using the method of preparation use by the users.

Table 3.1 Preparation of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction by the three users.

Name of leaf	Variable	User 1	User 2	User 3	Average use
Gl	QL U	3.2kg	3kg	2.8kg	3kg
	VWU	3litres	4litres	3.5 litres	3.5litres
	BT	12mins	9mins	10mins	10.3mins
	QC	280	300	315	298.3ml
Le	QL U	4.7kg	4,3kg	4kg	4.3kg
	VWU	4,5litres	4.3litres	4 litres	4,3litres
	BT	10mins	11mins	10mins	10.3mins
	QC	320ml	300ml	295ml	305ml
Ca	QL U	3.6	3,2kg	3kg	3.3kg
	VWU	3 litres	3.5litres	3 litres	3.2litres
	BT	9mins	12mins	10mins	10.3mins
	QC	305ml	300ml	285ml	296ml
Ml	QL U	4.1kg	4,3kg	4.2kg	4.2kg
	VWU	4.7litres	4.6litres	4.5 litres	4.6litres
	BT	10mins	9mins	10mins	9.7mins
	QC	380ml	300ml	315ml	332ml

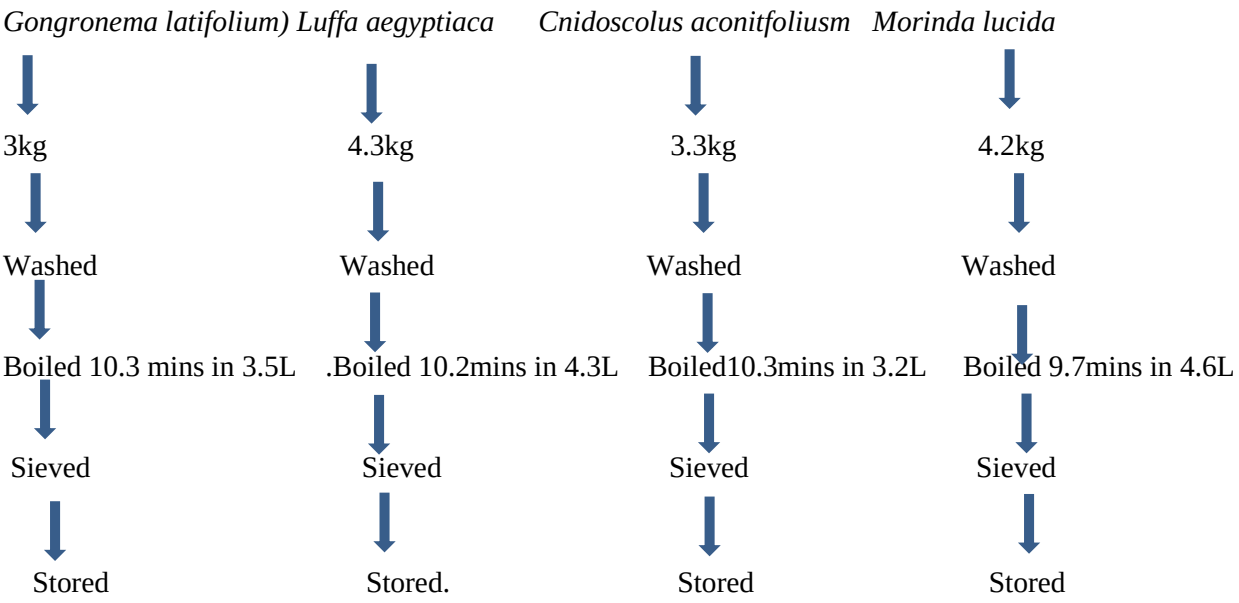
Key
Gl = *Gongronema latifolium* ,
La= *Luffa aegyptiaca* ,
Ca= *Cnidoscolus aconitfolius* ,
Ml= *Morinda lucida*
QLU = Quantity ofleafy vegetables used,
VWU =Volume of water used for boiling
BT = Boiling time,
QC= Quantity consumed

3.1.4: Preparation of samples for rat study

The Researcher having observed little variations in the quantities of green leafy vegetables used, the amount of water used in boiling the leafy vegetables as well as the boiling time by the users for each leafy vegetable decided thus, for each green leafy vegetable, (*Gongronema latifilium,Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida,* respectively, the average quantity of leaves, average amount of water used for boiling it as well as the average boiling time was used to prepare the samples for rat study and analysis.

Thus, 3kg, 4.3kg, 3.3.kg and 4.2kg of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetables, respectively, were used. They were separately picked to remove extraneous materials and washed with deionized water. They were separately boiled with local tripod ****ekwu**** using aluminum pot They were separately prepared by boiling in 3.5L, 43L, 3.2L and 4.6L for *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables, respectively, for 10.3min, 10.3 mins, 10.3min and 9.7min also for *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida*leafy vegetables, respectively. The end of boiling was determined by the leave changing color from green to brown. The decoctions were sieved out separately stored in refrigerator for rat study and analysis

Flow chart for preparation of samples for rat study and analysis.



NB. For each sample 1 litre was used for analysis while the rest was used for rat study.

3.2.0 Chemical analysis

All chemicals and reagents used in this study were of analytical grade and were products of May and Baker, England, Merck, Germany, Sigma – Aldrich, Germany, British Drug Honses (BDH), UK and Kieselgel GmbH, Germany.

3.2.1 Proximate composition

Proximate composition (moisture, protein, fat, ash, crude fibre and total carbohydrate) of the vegetables decoction was determined using AOAC (2010) approved methods.

Moisture determination

Moisture was determined according to the standard methods of Association of Official Analytical Chemist (AOAC, 2010). Stainless steel oven dishes were cleaned and dried in the oven for 1 hour to achieve a constant weight. They were cooled in a desiccator and then weighed. 2ml of each sample was weighed into each dish and dried in the oven until a constant weight was achieved. Then each weighed dish and sample was cooled in a desiccator and weighed. The % moisture was calculated thus:

$$\% \text{ Moisture} = \frac{(W2 - W3)}{(W2 - W1)} \times \frac{100}{1}$$

Determination of protein

Protein was determined using (AOAC, 2010). Two (2ml) of each sample was placed in a Kjeldahl flask. Anhydrous sodium sulphate (5g of Kjeldahl catalyst) was added to each flask. Concentrated H_2SO_4 (25m1) was added with few boiling chips in each. The flasks were heated in the fume chamber until the sample solution become clear. Each sample solution was allowed to cool to room temperature, then transferred into 250 ml volumetric flask and made up to volume with distilled water.

The distillation unit was cleaned, and each apparatus set up. Five milliliters of 2% boric acid solution with few drops of methyl red indicator was introduced into distillate collectors (100ml conical flask). Each conical flask was placed under the condenser. Then 5m1 of each sample digest pipetted into each, apparatus, and washed down with distilled water. Five milliliters of 60% sodium hydroxide solution was added to each digest. The content of each receiving flask were titrated with 0.049MH to a pink colored end point.

Calculation:

$$\frac{\%\ \text{Total Nitrogen (titre- Blank)}\ X\ \text{Normality of acid}\ X\ N2}{\text{Weight of sample}}$$

Nitrogen factor = 6.25

Protein = % total N x 6.25

Fat determinations

Fat was extracted according to AOAC (2010) soxhlet extraction method. Five hundred (500ml) capacity round bottom flasks were filled with 300ml petroleum ether and fixed to the soxhlet extractor. 2ml of each sample was placed in labeled thimbles. The extractor thimbles were sealed with cotton wool. Heat was applied to each apparatus for six hours. The thimbles were removed with care. The petroleum ether then recovered for reuse. The flasks free of ether were removed and dried at 105^{o} C for 1 hour in an oven. The flasks were cooled in a desiccator and weighed.

$$\%\ \text{fat} = \frac{\text{weight of fat}}{\text{Weight of sample}} \times \frac{100}{1}$$

Ash determination

Ash determination was carried out using AOAC (2010) procedure.two (2ml) of each sample was placed in silica dishes which had been ignited, Cooled and weighed. The dishes and samples were ignited first gently and then at 550 ^{o} C in amuffle furnace for 3 hours until a grey ash was obtained. Each dish and contents was cooled in a desiccator and weighed.

$$\%\ \text{Ash} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times \frac{100}{1}$$

Crude fibre determination

Crude fibre was determined using the method described by AOAC (2010).Five(5ml)of each sample was weighed into 50m1 beakers and fat was extracted with petroleum ether by stirring, settling and decanting three times.The extracted samples were air dried and each transferred to a 600ml dried beaker.Then 200m1of 1.25% sulphoric acid and few drops ofanti-forming agent were added to each beaker. Each beaker was placed on digestion apparatus with pre adjusted hot plate and boiled for

30minutes, rotating beaker periodically to keep solid from adhering on the sides of the beaker. After 30min, eaach mixture was allowed to stand for 1min, and then filtered through a Buchner funnel. Without breaking suction, the insoluble matter was washed with boiling water until it become free of the acid. The residues were washed back into the original flask by means of a wash bottle containing 200m1 of 1.25% sodium hydroxide solution. Each was again boiled briefly for 30minutes, with similar precautions as before. After 30minutes, each was allowed to stand for 1min and then filtered immediately under suction. The residues were washed with boiling water, followed by 1% hydrochloric acid and finally with boiling water until they become free of acid. Then washed twice with alcohol and then with ether for three times. The residues were transferred into ash dishes and dried at 100°C to a constant weight. Incineration to ash was done at 600°C for 30minutes, cooled in a desiccator and weighed. The difference in weight between oven dry weights after incineration was taken as the fibre content of each sample. This was expressed as a percentage weight of the original sample taken for analysis. Crude fibre (%) = oven dried sample -weight of sample incineration X 100

Weight of sample taken 1

Carbohydrate determination

The carbohydrate content was obtained by difference according to (Oyenuga, 1968) as follows; % carbohydrate =100 - (% moisture + % protein%, % fat, % ash, and % crude fibre). The total carbohydrate of each sample was the difference in value.

3.2.2 Vitamin determination.

Determination of pro vitamin A (B - carotene)

The procedure of Jakutowicz, Tomick, and leokadia (1997) was used.One (1ml) of each sample was weighed. Then the proteins were first precipitated with 3m1 of absolute ethanol before the extraction of vitamin A with 5m1 of heptane. The test tubes containing these were shake vigorously for 5mins. On standing, 3ml from heptane layer of each was taken up in a cuvette and read at 450nm against a blank of heptane. The standard was prepared, read at 450nm and vitamin A calculated.

Thiamin (B_1)

The procedure of Deepark – Koche (2011) was used. Five (5ml) of each sample was homogenized in 50m1 ethanol sodium hydroxide. Its 10ml filtrate was added to 10mI potassium dichromate and the absorbance was recorded at 360nm in a spectrophotometer after the development of colour. The absorbance obtained from each sample was converted to thiamin concentration by means of curve generated using different standard concentrations.

Riboflavin (B_2).

The procedure of Deepark – Koche (2011) was used. Five (5ml) of each sample was mixed with 100ml ethanol for 1hour. To 10ml of the filtered each sample was added 10mI 5% potassium permanganate and 10ml 3% hydrogen peroxide and they were allowed to stand on hot water bath for 30minutes. To each was added 2ml of 40% sodium sulphate. Then each volume was made up to 50ml. Each sample was centrifuged at 1500 rpm and the supernatant taken for spectrophotometric reading at 510nm. The absorbance obtained from each sample was converted to riboflavin concentration by means of calibration curve generated using different standard concentrations.

Vitamin niacin (B_3).

The procedure of Deepark – Koche (2011) was used. Five ml (5ml) of each samp1e was treated with 50ml of N sulphuric acid for 30minutes. Then 0.5 ml ammonia solution was added to it and then filtered. To 10ml of each filtrate was added 5m1 of 0.5% potassium cyanide. Each was further acidified with 5ml of 0.02N sulphuric acid. The absorbance of each resultant solution was recorded at 420nm. The absorbance obtained from each sample was converted to Niacin concentration by means of a calibration curve generated using different standard concentrations.

Determination of vitamin C

Vitamin C determination was done using the method described by AOAC, 2010

Procedure

Each sample (1.0 ml) and 1.0 ml of blank was added to test tubes. Then, 1 ml of 10 % TCA and 0.5 ml chloroform were added and centrifuged for 15 minutes. After that, 1 ml of each supernatant was pipetted into a test tube and 0.4 ml of combined reagent was added. Each was boiled for 1 hour,

cooled and 2 ml of H_2S0_4 was added. Each was allowed to stand for 30 minutes. The absorbance of each sample was measured at 540 nm.

Vitamin C = $\frac{\text{Abs of sample – abs of – blk}}{\text{Slope}}$

3.2.3 Mineral determination

Iron determination

Iron determination was done using the method described by AOAC, 2010 10 ml aliquot of each ash solution was pipetted into a tube. After 5 min, to each 5 ml buffer solution was added and 1 ml Ortho Phenanthroline solution also added and diluted to volume. Absorbance of each solution was determined at 510 nm. From absorbance readings Fe content present in each aliquot of ash solution was determined by referring to standard curve.

Calculations:

Iron content of sample (mg Fe/100ml sample =

$$\frac{\text{Quantity of Fe in aliquot of ash solution}}{\text{Total volume of iron from calibration curve (ash solution)}} \times \frac{100}{1}$$

Determination of selenium.

Selenium determination was done using the method described by Li, Zhai and fan (2006).

10 millimeter ash solution was pipited into a 30ml test tube. One milliliter 2% potassium iodide solution was added to the test tube. This was in turn followed by the addition of 1ml HCL and the mixture was gently shaken until the appearance of a yellow colour indicating the liberation of iodine. Then 0.5ml of 0.02% Safranine solution and 2ml acetate buffer solution of pH 4 were both added. The absorbant of the resultant solution was measured at 532nm against a reagent blank.

Calculation

Se (ug/100ml) = $\frac{\text{abs sample xconc. standard}}{\text{Abs of standard x weight of sample}}$

Determination of potassium

Determination of potassium was done using the flame photometry

Method

Set up the flame photometr. Aspirate the blank solution and set the zero. Aspirate the standard into the photometer. Plot a standard curve of potassium concentration against intensity. Aspirate the sample solution into the flame and record the reading. From the graph, read off the sample potassium concentration.

Calculation

Multiply the potassium concentration of the sample from the graph by 10.

Phosphorus determination

Phosphorus was determined using the atomic absorption spetrophometer (AAS) method of AOAC (2000). A total of 0.5ml of each sample was weighed into digestion tube. Then 5ml of 68% nitric acid was added and allowed to stand overnight. The digestion tube was placed in the digestion block and the temperature set at 120^{o}C to digest for one hour. The mixture was removed and the tube was allowed to cool. A total of 5ml of 30% hydrogen peroxide (H_2O_2) was added and heated to 700^{o}C. The treatment was repeated until the digest was colourless. The temperature was increased to 1800^{o}C and continued digesting until almost dryness and the digest was allowed to cool. A total of 10ml of 10% nitric acid was added and the dissolved digest was transferred quickly to a 50ml volumetric flask. The solution was used to determine phosphorus through atomic absorption spectrophotomer

Zinc determination

This was determined by dithizone method as described by AOAC (1997).

Procedure

Five ml of the digested sample was pipetted into a test tube and 5ml of acetate buffer added. One ml of sodium thiosulphate solution was added and mixed after which 10ml solution of dithizone solution was added into it. The solution was shaking vigorously for 40 minutes. The absorbance reading was taken at 530nm.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Dilution factor}}{\text{ml of sample used.}}$$

3.2.4 Phytochemical tests

3.2.4.1 Qualitative phytochemical analyses

Phytochemical tests were carried out using standards procedures described by (Soforowa, 1993; Trease and Evans, 1989; Harborne, 1973).m

Tests for tannin: For each sample, 0.5ml was boiled in 20ml of distilled water in a test tube and then filtered. A few drops of 0.1% of ferric chloride were added to each and it was observed for brownish green or blue black coloration.

Test for alkaloids; Two **(**2ml) of each sample was extracted for 2mins using 1% sulphuric acid in a 50ml conical flask on a water bath. Each sample mixture was centrifuged and the supernatant pipetted off into a small conical flask. 0.1ml of each sample was treated with Meyer's reagent which gave a cream precipitate indicating the presence of alkaloids.

Tests for flavonoid: Two **(**2ml) of each sample was prepared.five (5ml) of dilute ammonia, followed by few drops of concentrated sulphuric acid was added to each. Each was observed for a yellow coloration which disappeared on standing.

Carotenoid; carotenoid was screened using the method of kobola and Siriamornpu, (2011). The mobile phase consists of acetonenitrate (solvent A) dichloromethane (solvent B) and methanol (solvent c) at this flow rate of 1ml/ minute at this flow rate one sample took 30 minutes to pass through the column and the absorb ant was measured at a wavelength of 450nm.

Tests for glycoside; five (5ml0 of each sample was treated with 2ml glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml conc. Sulphuric acid. Each was then observed for a brown ring at the interface which indicates the presence of glycoside. Each was also observed for a violet ring below the brown in the acetic acid layer and a greenish ring gradually throughout the thin layer.

Tests for terpenoids five **(**5ml) of each sample was treated with 2 ml of chloform and concentrated sulphuric acid which was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Tests for steroids; Two (2 ml) of each sample was boiled in 20ml distilled water in a water bath and filtered. 10ml of each filtrate was mixed with 5ml distilled water and shaken vigorously for a stable persistent froth. The froting was mixed with 3 drops of olive oil and shaken vigorously; then observed for formation of emulsion.

Test for anthraquinone; 5 ml of each sample was boiled with 50 ml of dilute sulphuric acid. 5ml of each filtrate was shaken with 10ml of benzene, filtered and 5 ml of 10% ammonia added to each filtrate. The presence of pink or violet colour in the ammoniacal (lower) layer indicates the presence of anthraquinone.

Test for phenol Five **(**5ml) of each sample was filtered; to each 2 ml of 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of phenol.

3.2.4.2 Quantitative Phytochemical Analyses

Alkaloid determination

Alkaloid determination was done using method described by Harborne (1973). Five (5 ml) of each sample was weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. Each was filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium was added drop wise to each until the precipitation was complete. Each whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

Flavonoid determination

This was was done using the method described by Bohm and Kocipal-Abyazan (1994):

Ten (10 ml) of each sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. For each sample, the whole solution was filtered through whatman filter paper

No 42 (125mm). Each filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Sponin determination:

The method of Obadoni and Ochuko (2001) was used. Twenty (20m)l of each sample was used. Each sample was heated over a hot water bath for 4hours with continuous stirring at 55oC. The mixture was filtered and the residue re-extracted with another 200ml 20%. The combined extracts were reduced at 40ml over water bath of 9 ^{o} C. The concentrate was transferred into a 250ml separate tunnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the other layer was discarded. The purification process was repeated. 50 ml of n-butanol was added. The combined n-butanol was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, each sample was dried in the oven to a constant weight; the saponin content was

Determination of total phenols

Total phenol was done using the method of Olivera, (2003).

Fat free samples of each were boiled with 50ml of ether for the extraction of the phenolic component for 15min. Five (5ml) of each was pipette into a 50ml flask, and then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5ml of concentrated amylalcohol were also added to each. Each sample was made up to mark and left to react for 30min for colour development. Each was measured at 505nm.

Determination of tannin

This was done using the method described by Boham and Kocipal- Abyazan (1994) .One (1ml) of each sample was extracted with 300ml diethyl either for 20 hours at room temperature. The residue from each was boiled for 2 hours with 100ml distilled water, cooled and filtered. Each extract was adjusted to a volume of 100ml in a volumetric flask. Then, the tannin content of each sample was determined colorimetrically using Folin-Denis reagent by measuring the solution’s absorbance at 760nm, using tannic acid as standard.

Determination of glycoside

Glycoside was done using the method described by Onwuka (2005). Five (5ml) of each sample was weighed into conical flask and 50 ml of distilled water was added after which each flask was corked. The mixture was allowed to stand overnight. Then it filtered for glycoside determination for each sample.

Procedure for glycoside determination:

One (1ml) of each sample filtrate was transferred into a test tube and 4ml alkaline pictrate was added, and then placed in a water bath for 5 minutes. After colour development (reddish-brown colour), the absorbance from each was read with spectrophometer at 490nm. The blank solution was prepared using 1ml distilled water and 5ml alkaline picrate solution. The concentration of glycoside for each sample was determined using a standard.

Concentration of glycoside: $\frac{\text{absorbance of test sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Weight of sample used}}$

Determination of carotenoids;

The method according to Harbone 1973 was used. 5ml of each sample was homogenized in 1% methanol solution using lab blende. Each homogenate was filtered to obtain the initial crude extract. 20ml of ether was added to each filtrate to take up the carotenoid. 20ml of distilled water was also added and the mixtures shaken very well separating carotenoid. 20ml of distilled water was evaporated to dryness at low temperature 30-50 degrees Celsius. Each dry extract was then saponified with ethanoic potassium hydroxide and left overnight in a dark cupboard. The next day, the carotenoids extract (either layer) of each sample was dried in desiccators. Each was then treated with a light petroleum sprit and allowed to stand overnight in a freezer ($-10^{o}C$). The next day, the precipitated steroid from each sample was removed by centrifugation and the carotenoids extract was evaporated to dryness in a weighed evaporating dish. Each dish was cooled in desiccator, weighed and carotenoid expressed as a parentage of the sample weight.

3.3 Acute toxicity test (lethal Dose, LD_{50}) of the plants.

Acute toxicity or lethality of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetables decoction was carried using Lorke (1984) with some modification. Acute toxicity of the leafy vegetables decoction was carried out using 72 mice. The mice were grouped into twenty-four (24) of 3 mice per group. Acute toxicity was carried out in two phases.

In the first phase 1ml, 2 ml and 3 ml respectively, of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetables decoction kg/body weight respectively, was given to the first 12 groups (36 mice) in this order; A_1, A_2 and A_3 received 1ml, 2 ml and 3 ml of *Gongronema latifilium* / kg body weight respectively. B_1, B_2 and B_3 received 1ml, 2 ml and 3 ml of *Luffa aegyptiaca* / kg body weight respectively. C_1, C_2 and C_3 received 1ml, 2ml and 3ml of *Cnidoscolus aconitifiliius* kg body weight, respectively while D_1, D_2 and D_3 received 1ml, 2ml and 3ml of *Morinda lucida* / kg body weight respectively. They were observed for 24hours no mortality or behavoural change was observed in any of the groups within the 24 hours.

In the second phase 4ml, 5.ml and 6ml respectively, of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetables decoction kg/body weight respectively, was given to the second 12 groups (remaining 36 mice) in this order; A_4,A_5 and A_6 received 4ml, 5 ml and 6 ml of *Gongronema latifilium* / kg body weight respectively. B_4, B_5 and B_6 received 4 ml, 5 ml and 6 ml of *Luffa aegyptiaca* / kg body weight respectively. C_4, C_5 and C_6 received 4 ml, 5 ml and 6 ml of *Cnidoscolus aconitifiliius* /kg body weight, respectively while D_4, D_5 and D_6 received 4ml, 5ml and 6ml of *Morinda lucida* / kg body weight respectively. The mice were observed closely for 24 hours for behavioural changes or death.

Within the period, no behavoural or physiological changes were observed in any of the groups treated with 4 ml of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetables decoction kg/body weight, respectively. Groups A_5, B_5, C_5 and D_5 given 5 ml of *Gongronema latifolium, Luffa aegyptiaca, Cnidoscolus aconititifolius* and *Morinda lucida* green leafy vegetables decoction /kg body weight respectively, showed behavioural changes. In the groups treated with 6ml only one death occurred and it was recorded in group B_6 that received *luffa aegyptiaca* 6ml /kg body weight. Group A_6 treated with 6ml of *Gongronema*

latifolium 6 ml /kg body weight showed one rat with one big toe oedema. C_6 that received *Cnidoscolus acontititifolius* 6ml/kg body weight had one weak rat while Group D_6 given *Morinda lucida* 6 ml /kg body recorded one very weak rat. Based on the result the LD_{50} the rat dose was determined.

Table 3.2 First phase of acute toxicity test of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *M. lucida* green leafy vegetables decoction using 36 mice

Grp	No of rats	Vegetable decoction	Amount /kg b.w	Reaction
A_1	3	*Gongronema latifolium*	1ml	No reaction
A_2	3	*Gongronema latifolium*	2ml	No reaction
A_3	3	*Gongronema latifolium*	3ml	No reaction
B_1	3	*Luffa aegyptiaca*	1ml	No reaction
B_2	3	*Luffa aegyptiaca*	2ml	No reaction
B_3	3	*Luffa aegyptiaca*	3ml	No reaction
C_1	3	*Cnidoscolus aconitifilius*	1ml	No reaction
C_2	3	*Cnidoscolus aconitifilius*	2ml	No reaction
C_3	3	*Cnidoscolus aconitifilius*	3ml	No reaction
D_1	3	*Morinda lucida*	1ml	No reaction
D_2	3	*Morinda lucida*	2ml	No reaction
D_3	3	*Morinda lucida*	3ml	No reaction

Table 3.3 Second phase of acute toxicity test of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *M. lucida* green leafy vegetables decoction using 36 mice

Grp	No of rats	Vegetables decoction	Amount /kg b.w	Reaction
A_4	3	*Gongronema latifolium*	4ml	No reaction
A_5	3	*Gongronema latifolium*	5ml	1 weak
A_6	3	*Gongronema latifolium*	6ml	1 big toe oedoma
A_4	3	*Luffa aegyptiaca*	4ml	No reaction
A_5	3	*Luffa aegyptiaca*	5ml	2 sluggish
A_6	3	*Luffa aegyptiaca*	6ml	1 died
A_4	3	*Cnidoscolus aconitifilius*	4ml	No reaction
A_5	3	*Cnidoscolus aconitifilius*	5ml	1 not alert
A_6	3	*Cnidoscolus aconitifilius*	6ml	1weak
D_4	3	*Morinda lucida*	4ml	No reaction
D_5	3	*Morinda lucida*	5ml	2 sluggish
D_6	3	*Morinda lucida*	3ml	1 very weak

3.4. 0 Animal study

3.4.1 Ethical consideration

All animals for the experiment were handled according to the international guiding principles for research involving animals as permitted by university of Nigeria Ethical Committee concerning the use of laboratory animals.

3.4.2 Animal sourcing and housing

The experimental animals for the study were seventy-five (75) healthy adult male wistar rats weighing 230g -250g, aged 3 months. They were purchased from the Faculty of Veterinary Medicine, University of Nigeria Nsukka, and were kept in metallic laboratory cage in the Department of Nutrition and Dietetics, University of Nigeria, Nsukka. The animals were maintained under standard (room) temperature (28 o C), humidity and 12 h light/dark cycle. They were allowed to acclimatize for 5 days and fed with vital feeds (grown maxx) and distilled water *ad libitum* during the study period. They were housed in groups of five in metallic laboratory cages specially designed to separate feaces and urine. The rat dose for the treatments (leafy vegetables

decoction and oral hypoglycaemic drug) were calculated based on the body weight using the LD_{50} results as a guide thus;

$$\frac{\text{dosage}}{\text{1kg (1000g)}} \times \frac{\text{weight of rat}}{1}$$

3.4.3 Experimental design

The study period was twenty-nine (29) days, 5 days for acclimatization, 1 day for inducing diabetes, 2 days for establishment of diabetes and 21 days for giving the experimental treatment. Data including weight were collected from all the groups on day 6. When diabetes was established on day 8 the baseline data were collected and treatment commenced with the green leafy vegetable decoctions. Groups A, B, C and D were given 2 ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction /kg body weight, respectively.

Also, groups E, F G and H were treated with 3ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetable decoction /kg body weight, respectively. Rats in groups I, J, K and L were given 4ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetables decoction /kg body weight, respectively. Rats in group M (positive control) were induced and then treated with pharmacological standard dose of Glibenclamide 0.5 mg/kg body weight (Hassan, Mohammed and Alyemeni, 2011), while rats in group N (negative control) were induced but not treated at all to see if they will recover spontaneously. Finally, rats in group O (normal control) were neither induced nor treated and were given rat chow vital feeds (grown maxx) and distilled water only during the experimental period.

The doses of the green leafy vegetables decoction administered to the rats were determined from the result of the LD_{50}. The treatments (green leafy vegetables decoction and oral hypoglycaemic drug) were given orally every day using a syringe. During the study period (29) days blood samples were collected on days 6, 8 and 29 to determine some biochemical parameters (blood glucose level, lipids and liver enzyme activities).

After collecting blood samples on day 29, the rats were sedated with chloroform and sacrificed by decapitating them and the pancreas and liver harvested for histo-pathological examination. The result of the biochemical indices on day 8 served as the baseline data.

3.4.4 Inducing diabetes

After the acclimatization period of 5 days, the rats were fasted overnight, and then induced diabetes using alloxan on day 6. Freshly prepared aqueous solution of alloxan monohydrate was injected intra peritoneally (IP) to the rats at a single dose of 150mg/kg body weight. The rats were allowed free access to 5% glucose solution to avoid possible effect of hypoglycemia. After 48 hours (2 days) of inducing diabetes, blood was taken from each rat to confirm diabetes. The determination was done using Trinders glucose-oxidase method (Lott and Turner, 1975). The result was reported as mg/dl. Rat with blood glucose concentration of l30mg/dl and above was considered diabetic (Etuk, 2010).

3.4.5 Feeding trial

Wistar rats (n = 75) were divided into fifteen (15) groups of 5 rats per group labeled A to O.
Rats in groups A, B, C and D were given 2ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction /kg body weight, respectively**.** Groups E, F G and H received 3ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetable decoction /kg body weight, respectively. Rats in groups I, J, K and L were given 4 ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetables decoction /kg body weight, respectively. Rats in group M (positive control) were treated with Glibenclamide 0.5 mg/kg body weight. Rats in group N (negative control) were induced but not treated at all. Rats in group O (normal control) were neither induced nor treated and were given rat chow and distilled water only during the experimental period.

NB. The treatments (green leafy vegetables decoction and oral hypoglycaemic drug) were given orally every day using syringe while distilled water and rat chow vital feeds (grown maxx) were given *ad libitium* to all groups.

Table 3.4 Experimental diet

Name of group	No of rats	Experimental diet	quantity / kg b,w
A	5	Rat chow, water + *Gongronema latifilium*	2ml
B	5	Rat chow, water + *Luffa aegyptiaca*	2ml
C	5	Rat chow, water + *Cnidoscolus aconitifilius*	2ml
D	5	Rat chow, water + *Morinda lucida*	2ml
E	5	Rat chow, water + *Gongronema latifilium*	3ml
F	5	Rat chow, water + *Luffa aegyptiaca*	3ml
G	5	Rat chow, water + *Cnidoscolus aconitifilius*	3ml
H	5	Rat chow, water + *Morinda lucida*	3ml
I	5	Rat chow, water + *Gongronema latifilium*	4ml
J	5	Rat chow, water + *Luffa aegyptiaca*	4ml
K	5	Rat chow, water + *Cnidoscolus aconitifilius*	4ml
L	5	Rat chow, water + *Morinda lucida*	4ml
M	5	Rat chow, water + Glibenclamide	0.5mg/kg
N	5	Rat chow + water only	
O	5	Rat chow + water only	

3. 4. 6 Blood sample collection;

Nucleo capillary tube was carefully inserted into the lateral canthus to puncture the retro bulbar plexus to enable outflow of about 2ml of blood into a clean glass test tube. The blood samples were kept at room temperature (27 - 28^{o}C) for 30 minutes to clot. Afterwards the test tubes containing the clotted blood samples were centrifuged at 300 revolutions per minute for ten minutes using a table centrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was carefully aspirated with syringe and needle into a clean sample bottle for the biochemical analysis. Blood samples were collected on days 6, 8 and 29.

3.5.0 Biochemical indices determination

3.5.1 Determination of blood glucose

Determination of blood glucose was by the Trinders glucose-oxidase principle (Lott and Tuner, 1975 using the one Touch Basic (lifescan Milpitas, CA) instrument.

Method

The test strip was inserted in the meter. The meter turns on automatically. A small drop of blood was put on the top white edge of the test strip. The test strip automatically draws the blood into the reaction cell where the reaction takes place. The blood glucose level was read on the meter.

3.5.2 Lipids determination

Determination of Serum High Density Lipoprotein

Dextran sulphate-mg (II) method for the in-vitro determination of HDL-cholesterol in serum, using Quimica Clinica Applicada (QCA) HDL test kit was used to determine HDL (Alberts, Warnick and Cheung, 1978).

Method

Accurately 0.03m1 of the serum sample was added to a clean labeled 1ml test tube. A drop of the precipitant solution was added to 1ml test tube. It was mixed and allowed to stand for 15 minutes at room temperature, centrifuged at 300 revolutions per minutes for 10 minutes.

Approximately 1ml of cholesterol working reagent was added to a set of cleaned test tubes. Two of the test tubes were labeled Standard 1 and 2 while the other was label blank apart from the test samples that were labeled according to group names and numbers (samples A, B, C to O). Approximately 0.01ml of the supernant derived from centrifugation of the precipitant-serum sample mixture was added to the test tube. It was mixed well and allowed to stand for 10 minutes at room temperature. Also 0.01ml of the standard was also added to each of the test tubes labeled standard I and 2. It was mixed well and allowed to stand for 10 minutes at room temperature. Reading was taken at absorbance of the samples and standard against the reagent blank at 52 5nm.

Cholesterol content of each sample was calculated using the following formular:

Calculations

The HDL cholesterol concentration in the sample were calculated using the following general formula:

$$\frac{A_{sample}}{A_{standard}} \text{ x } 52.5 = \text{mg/dl HDL - Cholesterol}$$

The result was expressed in mg HDL-cholesterol/dl.

Low density lipoprotein-cholesterol concentration was determined using QCA commercial kit, method of (Assman, *Jabs, Kohnert, Nohe & Schriewer* 1984)

Principle

Low density lipoprotein–cholesterol (LDL–cholesterol) was determined from the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethyleneglycol monomethyl ether.

LDL-cholesterol = Total cholesterol – cholesterol in the supernatant

Reagents

Content	**Initial Concentration of Solutions**
1. Precipitation Reagent:-	
Polyvinyl sulphate	0.7 g/L
EDTA Na_{2s}	5.0 Mm
Polyethyleneglycol monomethyl ether	170 g/L
Stabilizers	

Procedure

Precipitation reaction

The precipitation solution (3 drops or 0.1 ml) was carefully measured into test tubes labeled accordingly. The serum sample (0.2 ml) was added to the labeled test tubes. The contents were thoroughly mixed and left to stand for 15 minutes at room temperature (20–25^{o}C). Then, the mixture was centrifuged at 2,000 × g for 15 minutes and the cholesterol concentration in the supernatant was determined.

Cholesterol determination

The concentration of the serum total cholesterol was determined according to the QCA CHOD–PAP method (Albers, Warmick & Cheng, 1978).

Calculations

The LDL–cholesterol concentration in the sample was calculated using the following general formula:

LDL–cholesterol (mg/dl) = Total cholesterol (mg/dl) – 1.5 × supernatant cholesterol (mg/dl).

Determination of triacylglycerol concentration (Randox Enzyme Kit)

Principle (Trinder, 1969)

The concentration of triacylglycerols were determined after enzymatic hydrolysis with lipases. The indicator showed quinoneimine formed from hydrogen peroxide, 4 – aminophenazone and 4 – chlorophenol under the catalytic influence of a peroxidase.

$$\text{Triacylglycerols} + H_2O \xrightarrow{\text{Lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{phosphate} + O_2 \xrightarrow{\text{GPO}^*} \text{dihyroxy acetone} + \text{Phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + 4H_2O$$

GPO = Glycerol –3– phosphate oxidase

Reagents

Contents	Initial Concentration of Solution
Buffer	
Pipes buffer	40.0mmol/1, pH 7.6
4 – Chlorophenol	5.5mmol/1
Magnesium ions	17.5mmol/1
Enzyme Reagent	
4 – Aminophenazone	0.5mmol/1
ATP	1.0mmol/1

Lipases	≥1.5U/ml
Glycerol kinase	≥0.4U/ml
Glycerol – 3 – phosphate oxidase	≥ 1.5U/ml
Peroxidase	≥0.5U/ml
Standard	2.29mmol/1(200mg/dl)

Procedure

Three sets of tubes labelled reagent blank (B), standard (ST), and sample (S) were set up. The enzyme reagent (15ml) was reconstituted with 15ml of the buffer solution and the new solution stored in the refrigerator. An aliquot of the serum sample, 10.0µl was pipetted into the test tube labeled S while 10.0µ of the standard was pipetted into the test tube labeled ST. Then, 1.0ml of the reconstituted enzyme reagent was added to each of the three sets of test tubes. The contents of the test tubes were mixed and incubated in a water bath for 5 minutes at 37^0C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured at 500nm against the reagent blank within 60 minutes. The concentration of triacylglycerols in the serum samples were calculated using the formula:

$$\text{TAGs concentration} = \frac{A_{Sample}}{A_{S\,\tan dard}} \times 2.29\text{mmol}/1$$

Determination of the total serum cholesterol

Determination of total cholesterol was done using Quimica Clinica Applicada (QCA) (Allain, Poon, Chan, chmond and Fu, 1976).

Principle

The total cholesterol determination uing QCA enzyme kit based on the assey principle that total cholesterol were determined after enzymatic hydrolysis and oxidation The indicator coloured quinonicderivative were formed from hydrogen peroxidite and 4 aminoantipyrine in the presence of p – hydroxybenzoic acid and peroxidase

Cholesterol - esters $+H_2 0 \xrightarrow{\text{cholesterol\_\_esterase}}$ Cholesterol + fatty acid

Cholesterol + $H_20 + 0_2 \xrightarrow{\text{cholesterol}}$ Cholesterol + H_20_2

$2H_20$+ 4- Aminoantipyrine + p – Hydroxybenzoicacid $\xrightarrow{\text{peroxidase}}$ coloured quinnonoic derivative $+4H_20$.

Procedure

Blank (BL), sample (SA) and standard (ST) were put in three labelled text tubes.0.01ml of the serum sample was pippeted into the sample (SA) test tube. Also 0.01ml of the standard (ST) was introduced into the standard test tube with a corresponding addition of 1ml of working reagent into each of the test tube. The solution in the different sets of test tubes were well mixed and allowed to stand for 5 minutes at 37ºC or 10 minutes at room temperature. The absorbance was read at the wavelength of Hg 546nm

Calculation

The total cholesterol concentration in the sample was calculated using the following general formular:

$\frac{SA\ 0.D \times 200}{ST\ 0.D\ \ 1}$ = mg/dl of total cholesterol

Where

SA = sample

ST = standard

OD = optical density

200 = constant

To convert to SI units: (mg/100dl) x 0.0259mm0L/L

3.5.3 Determination of liver enzymes

Determination of the aspartate aminotransferase (AST) activity

The activity of aspartate aminotransferase was assayed by the method of Reitman and Frankel (1957) as outlined in the Randox kit used.

Procedure

0.1 ml each serum sample was pipetted into a test tube and only 0.1 ml of distilled water was pipette into blank test tubes. 0.5 ml of Reagent one (R_1) containing phosphate buffer, L – aspartate and beta – oxoglutarate was pipetted into both the blank and each serum sample test tube respectively. Each entire reaction medium was well mixed and incubated for 30 minutes in a water

bath at 37 ^{0}C. Immediately after incubation, 0.5 ml of Reagent two (R_2) containing 2,4-dinitrophenylhydrazine was added to each blank and the serum sample test tubes and allowed to stand for exactly 20 minutes at 25 ^{0}C. Finally, 5.0 ml of 0.4 N sodium hydroxide solutions was added to both each blank and serum sample test tubes respectively and mixed thoroughly. Then the absorbance read at a wavelength of 546 nm after 5 minutes.

$$AST = \frac{\text{Abs of sample} - \text{abs of} - \text{blank}}{\text{Slope}}$$

Determination of the alanine aminotransferase (ALT) activity

The activities of alanine aminotransferase was assayed by the method of Reitman and Frankel (1957) as outlined in Randox Kit.

Procedure

The serum sample (0.1 ml) was pipetted into the samples test tube and 0.1 ml of distilled water pipette into blank test tube, 500 µl of the ALT substrate buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate (R_1) were added. The entire reaction media were well mixed and incubated for 30 minutes in a water bath at 37 ^{0}C and pH 7.4. Immediately after incubation, 0.5 ml of Reagent two (R_2) containing 2, 4-dinitrophenylhydrazine was added to the blank and sample test tubes. These were thoroughly mixed and allowed to stand for exactly 20 minutes at 25 ^{0}C. Finally, 0.5 ml of sodium hydroxide solution was added to both the blank and serum samples test tubes respectively and mixed thoroughly. The absorbance was read at wavelength of 546 nm after 5 minutes.

$$ALT = \frac{\text{Abs of sample} - \text{abs of blank}}{\text{Slope}}$$

Alkaline phosphatase (ALP)

Assay of serum alanine phosphate (ALP) activity was determined using randox labratory reagent kit, Uk, 29 4qy based on method developed by Ritman and frankel (1957).

Procedure

For each sample, 0.5ml of alkaline phosphatase substrate was dispense into labeled test tubes and equilibrate to 37°C for three (3) minutes. At timed intervals, 0.05mL (50 u1) of each standard,

control, and sample was added to its respective test tube. Then it was mix gently. Deionized water was used as sample for Reagent Blank. Incubation was done for exactly ten (10) minutes at 37°C. Following the same sequence as in Step 2, 2.5 mL Alkaline Phosphatase Color Developer was added at timed intervals. Then it was mixed well. The wavelength of the spectrophotometer was set at 590 nm. Zero with Reagent Blank. (Wavelength range 580-630). Absorbance of samples was read and recorded.

Calculations

The activity of alkaline phosphatase in the sample was calculated using the following formula:

$$\frac{A\ Sample}{A\ standard} \times C_{Standard} = C_{sample}$$

Equivalent enzyme activity of standard =50 U

3.6 Histo- pathological examination.

This was carried out as described by Bancroft and Stevens, (2002).

Procedure:

At the end of the experiment, the liver and pancreas were obtained from dissected rats and fixed in 10% neutral buffered formalin to arrest metabolic activity in the tissues, avoid autolysis and protein precipitation thus preventing enzymatic digestion of dead tissues. The fixed tissues were passed through several changes of alcohol, 70% alcohol for 24 hours and 90% alcohol for 12 hours and through absolute alcohol to remove water from the fixed tissues and allowed complete infiltration of tissues by paraffin. The tissues were then passed through xylene for 3 hours to prevent shrinkage and tissue brittleness in paraffin. After tissue processing, tissues were embedded or blocked out using the leukhand embedded mould. The L pieces were arranged on an aluminum base to form a rectangle. The molten paraffin was then poured into the moulds and the selected surfaces of the tissues embedded with the aid of a pair of blunt end forceps and allowed to set. The embedded tissues were separated into different blocks and then attached to wooden blocked with the aid of an electric spatula. The blocks were then trimmed using a rotary microtome and knife. At the end of each trimming, the blocks were arranged on ice trays in order to cut thin sections using the rotary microtone at a thickness of 5 micron. Sections were then collected with the help of a camel hair brush and then placed on the slide. Sections were flood picked with 20% alcohol in other to spread

out fold on the sections and then floated out on a water bath with a temperature 5-10 degree centigrade below the melting point of the wax used.

The sections were picked and floated on a water bath and then picked with a pre-labeled slide. The slides were dried on a hot plate at a temperature of a 5-10 degree centigrade above the melting point of wax used. These were left on the hot plate for 15 minutes. The staining methods employed in staining the sections were haematoxylin and eosin method to demonstrate general tissue structure as follows. They were dewaxed in two bathes of xylene for 2minutes each and hydrated in descending grades of alcohol, absolute: 90%, 70% for 2 minutes each and then wash in water. They were then stained with haematoxylin for 15minute after which excess stains were washed with tap water and differentiated in 1% acid alcohol briefly.

Washing in running tap water and bluing for 10 minutes was done followed by counter staining in 1% aqueous eosin for 3 minutes before washing off in water. Dehydration through 70% alcohol, 90% alcohol and absolute for 2 minutes each was followed by clearing in 2 baths of xylene for 2 minutes. The slides were then mounted in cover slips with DPX devoid of air bubbles. The specimens were examined and photomicrographs were captured using a Moticam Images plus 2.0 digital camera (Motic China Group Ltd. 1999–2004).

3.7 Statistical analysis

Data collected were coded, keyed into the computer and analysis was done with statistical package for service solution (SPSS) version 23. The Results of the study were analysed using descriptive statistics. Differences were significance at $p < 0.05$.

CHAPTER FOUR

4.0 PRESENTATION OF RESULTS

Table 4.1 shows the proximate composition of bush buck plant *(Gongronema latifilium)* Egyptian cucumber (*Luffa aegytiaca),* chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree *(Morinda lucida)* leafy vegetables decoctions per 100ml as consumed.

The table shows that the moisture content of the samples was very high. It ranged from 98.64 % in *Morida lucida* to 99.61% in *Gongronema latifilium.* The table also revealed that the protein content of the decoctions was low. *Morinda lucida* had the highest protein value (0.23g/100ml), and the least was in *Luffa aegyptiaca* (0.07g/100ml). However, *Cnidoscolus aconitifilius* and *Gongronema latifilium* had protein values of 0.11g/100ml and 0.14g/100ml, respectively, the ash content was low. *Morinda lucida* had (0.31g/100ml.), *Luffa aegyptiaca* had (0.22g/100ml) *Gongronema latifilium* (0.12g/100ml) and *Cnidoscolus aconitifilius* (0.10g/100ml).

The carbohydrate content varied. The carbohydrate content was 0.82g/100ml, 0.53g/100ml, 0.49g/100ml and 0.12g/100ml for *Morinda Lucida, Luffa aegyptiaca, Cnidoscolus aconitifilus* and *Gongronema latifilium,* respectively.

Table 4 1: Proximate composition of bush buck plant *(Gongronema latifilium)* Egyptian cucumber (*Luffa aegytiaca)*, chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree *(Morinda lucida)* leafy vegetables decoction per 100ml as consumed.

Name	Moisture (%)	Protein (g)	Fat (g)	crude fibre	Ash (g)	CHO (g)
Gl	99.61 ± 0.04	0.14 ±0.01	trace	trace	0.12 ±0.1	0.13 ±0.02
La	99. 18 ± 0, 07	0,07 ±0,01	trace	trace	0,22 ± 0.01	0, 53 ± 0,05
Ca	99.30 ± 0.08	0.11 ±0.01	trace	trace	0.10 ±0.01	0.49 ± 0.07
Ml	98.64± 1.2	0.23± 0.02	trace	trace	0.31 ± 0.02	0. 82 ± 0.12

Mean ± SD of three determinations

Key

Gl = Gongronema latifilium

La = Luffaaegytiaca

Ca = Cnidoscolus acoitifilius

Ml = Morinda lucida

Table 4.2 shows the mineral composition of bush buckplant *(Gongronema latifilium),* Egyptian cucumber (*Luffa aegytiaca),* chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree *(Morinda lucida)* leafy vegetables decoction per 100ml as consumed.

The table shows that iron content was low. It ranged from 0.37mg/100ml in *Luffa aegyptiaca* to 0. 66mg/ 100ml in *Morinda lucida*. The table also shows that *Morinda lucida* had the highest value of phosphorus (6.69mg/100ml), followed by *Luffa aegyptiaca* (4.59mg/100ml), *Gongronema latifilium* (3.69mg/100ml) and *Cnidoscolus aconitifilus* (2.08mg/100ml). Selenium result shows, *Cnidoscolus aconitifilius* (6.15 IU/100ml) and *Gongronema latifilius* (18 IU/100ml*)*. However, *Luffa aegyptiaca* and *Morinda lucida* recorded trace, respectively.

The table revealed that potassium content was high. The potassium content was 77.67mg/100ml, 55.54mg/100ml, 55.02mg/100ml and 54.73mg/100ml for *Gongronema latifilium, Cnidoscolus aconitifilium, Morind lucida* and *Luffa aegyptiaca,* respectively. Zinc values were low with the least found in *Cnidoscolus aconitifilius* 0.05mg/100ml

Table 4.2: Mineral composition of bush buck plant *(Gongronema latifilium),* Egyptian cucumber (*Luffa aegytiaca),* chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree *(Morinda lucida)* leafy vegetables decoction per 100ml as consumed.

Name	Iron (mg)	Phosphorus (mg)	Selenium(iu)	Potassium (mg)	Zinc (mg)
Gl	0.48 + 0.02	3.69 +0.15	1.18 ± 0.05	72.67 ± 0.75	0.08 ± 0.01
La	0.37 +0.01	4.59 ±0.100	trace	54.73 ± 0.59	0.08 ±0.00
Ca	0.43 ±0.01	2.08 ±0.04	6.15± 0.06	55.54 ±0.47	0.05 ±0.00
Ml	0.66 ±0.00	6.69± 0.22	trace	55.02 ± 0.23	0.07 ±0.01

Mean ±SD of three determinations

Key

Gl = Gongronema latifilium

La = Luffa aegytiaca

Ca = Cnidoscolus aconitifilius

Ml = Morinda lucida

Table 4.3 shows the vitamin composition of bush buck plant *(Gongronema latifilium),* Egyptian Cucumber *(Luffa aegytiaca),* chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree *(Morinda lucida)* leafy vegetables decoction per 100ml as consumed.

The table revealed that the samples contain an appreciable quantity of beta carotene. The highest amount of beta carotene was found in *Cnidoscolus aconitifilus* (63.03mg/100ml), followed by *Morinda lucida* with 58.50mg/100ml, *Gongronema latifilium* (55.63mg/100ml) and *Luffa aegyptiaca (*54.68mg/100ml). The highest level of thiamin was found in *Morinda lucida* (0.07mg/100ml), followed by *Gongronema latifilium (*0.03mg/100ml), however, *Luffa aegyptiaca* and *Cnidoscolus Aconitifilius* had trace of thiamin, respectively.

Riboflavin content varied. The riboflavin content was 0.14mg/100ml, 0.12mg/100ml, 0.07mg/100ml and 0.04mg/100ml for *Morinda lucida, Luffa aegyptiaca, Gongronema latifilium* and *Cnidoscolus aconitifilus* respectively. The niacin contenst were *Morinda lucida* (4.09mg/100ml), *Luffa aegyptiaca* (2.53mg/100ml), *Gongronema latifilium* (0.87mg/100ml) and *Cnidoscolus aconitifilus* (0.48mg/100ml).

For ascorbic acid, the table shows Gongronema *latifilium* ranking highest with ascorbic acid content of (7.70mg/100ml) and *Cnidoscolus aconitifilus* least (6.07mg/100ml).

Table 4.3: Vitamin composition of*bush buck*plant *(Gongronema latifilium),* Aegyptian cucumber (*Luffa aegytiaca),* chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree *(Morinda lucida)* leafy vegetables decoction per 100ml as consumed.

Name	Beta carotene(mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Ascorbic Acid (mg)
Gl	55.64 ±2.85	0.03 +0.01	0.07±0.00	0.87 ±0.04	7.70± 0.04
La	54.68±0.44	trace	0.12 ±0.00	2.53 ±0.02	6.12 ±0.11
Ca	63.03 ±0.68	trace	0.04 ±0.00	0.48 ±0.01	6.07 ±0.06
Ml	58.50 ±0.37	0.07 +0.01	0.14±0.02	4.09 ±0.01	6.87 ±0.06

Mean ± SD of three determinations

Key

Gl = Gongronema latifilium

La = Luffa aegytiaca

Ca = Cnidoscolus aconitifilius

Ml = Morinda lucida

Table 4.4 shows the phytochemical qualitative analysis of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida leafy* vegetables decoction / 100ml as consumed.

The table shows that *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction contain alkaloid, saponin, phenol, glycoside, tannin, carotenoid and flavonoid, respectively, however, anthrocynide, anthroqunine, terpernoid and steroid were not detected.

Table 4.4 Qualitative phytochemical analysis of bush buck plant *(Gongronema latifilium),* Egyptian cucumber (Luffa *aegytiaca),* chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree*(Morinda lucida)* leafy vegetables decoction per 100ml as consumed.

Name	Alk. %	Sap. %	Phe.mg	Gly.mg	Tan.mg	Car.mg	Fla. %	Ant.	Anthr	Ter.	Ste.
Gl	+	+	+	+	+	+	+	—	—	—	—
	+	+	+	+	+	+	+				
La	+	+	+	+	+	+	+	—	—	—	—
	+	+	+	+	+	+	+				
Ca	+	+	+	+	+	+	+	—	—	—	—
	+	+	+	+	+	+	+				
Ml	+	+	+	+	+	+	+	—	—	—	—

key;

Gl = Gongronema latifilium

La= Luffa aegyptiaca

Ca =Cnidoscolus aconitifilius

Ml =Morinda lucida

Table 4.5 presents the phytochemical quantification of bush buck *(Gongronema latifilium)* Egyptian cucumber (*Luffa aegytiaca),* chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree *(Morinda lucida)* leafy vegetable decoction /100ml as consumed.

The data revealed that alkaloid content of the samples was moderate *Luffa aegyptiaca* had the highest alkaloid (0.99%) and *Gongronema latifilium* had the least (0.31%).

Saponin values were 0.13%, 0.39%, 0.42% and 0.75% for *Cnidoscolus aconitifilus, Luffa aegyptiaca, Gongronema latifilum* and *Morinda lucida* respectively. Phenol content was highest in *Luffa aegyptiaca* (12.60mg/100ml) and least in *Gongronema latifilium* (5.10mg/100ml). Other values were *Morinda lucida* (10.67mg/100ml), *Cnidoscolus aconitifilus* (6.17mg/100ml).

For glycoside, *Morinda Lucida* had highest glycoside content (4.90mg/100ml). This was similar to the glycoside content of *Luffa aegyptiaca* (4.47mg/100ml). However, *Cnidoscolus aconitifilius* ranked least with (1.93mg/100ml). The tannin values were 2.36mg/100ml, 2.31mg/100ml and 2.00mg/100ml for *Cnidoscolus aconitifilus, Gongronema latifilium* and *Luffa aegyptiaca,* respectively, *Morinda lucida* had (1.00mg).

The value for carotenoid was high. The value ranged from 306.67mg/100ml in *Cnidoscolus aconitifilus* to 936/100ml, 66mg in *Luffa aegyptiaca*. The flavonoid content varied and was highest in *Morinda lucida* (2.44mg/100ml) followed by *Luffa aegyptiaca* (1.70mg/100ml), *Gongronema latifilium* (1.17mg/100ml) and *Cnidoscolus aconitifilus* (0.42mg/100ml).

Table 4.5 Phytochemical Quantification of bush buck plant *(Gongronema latifilium)* Egyptian cucumber (*Luffa aegytiaca),* chaya plant *(Cnidoscolus aconitifilius)* and brimstone tree *(Morinda lucida)* leafy vegetables decoction per 100ml as consumed.

Name	Alkanoid (%)	Saponin (%)	Phenol (mg)/	Glycoside (mg)	Tannin (mg)	Carotenoid (mg)	Flavonoid(%)
GL	0.31±0.01	0.42±0.02	5.10±0.102.	60±0.26	2.31±2.08	686.67±25.17	1.17±0.14
LA	0.99±0.07	0.39 ±0.05	12.60±0.53	4.47±0.12	2.00 ±2.65	936.67±64.29	1.70+0.24
CA	0.42±0.08	0.13±0.03	6.17 ±0.25	1.93±0.25	2.36 ±2.52	306.67±32.15	0.42+0.01
ML	0.47±0.02	0.75±0.04	10.67±0.58	4.90 ±0.36	1..00±1.00	746.67±66.58	2.44+0.19

Mean ± SD of three determinations

Key

Gl = Gongronema latifilium

La = Luffa aegytiaca

Ca = Cnidoscolus aconitifilius

Ml = Morinda lucida

Table 4.6 shows the effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on blood glucose of the different rat groups

The table revealed that only the untreated group (negative control) had sustained increased blood glucose, baseline (218mg/dl), end line (324 .80mg/dl) with percentage increase of (48.58%). Groups A, B, C and D treated with 2ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolius aconitifilius* and *Morinda lucida*/kg body weight respectively showed slight percentage decreases in blood glucose.

However, group F treated with *Luffa aegyptiaca,* 3ml/kg body weight had highest percentage decrease in blood glucose (69.64%) followed by group E treated with *Gongronema latifilium* 3ml /kg body weight that had 68.88%, then group I that received Gongronema *latifilium* 4ml/kg body weight that recoded 68.45% and group J that was treated with *Luffa aegyptiaca* 4ml/kg body weight that had 68.20%. All other groups also had decreased blood glucose level. However, group O (normal control) maintained relatively the same blood glucose throughout the experimental period.

Table 4.6: Effect of *G. latifilium, L. aegyptiaca, C. aconitifilius* and *M. lucida* leafy vegetables decoction as consumed on blood glucose (Glu.) level of the different rat group

Grp & Rx / kg body weight		Glu.mg/dl baseline (bl) after ind. diabetes	Glu. mg/dl endline (el) after treatment	Diff. b/w bl and el Decrease/Increase	% diff.
A	Gl-2ml	277.40±86.69	104.00±4.24	173.40 ↓	62.51
B	La-2ml	273.60±74.94	102.20±8.04	169.40 ↓	61.92
C	Ca-2ml	264.20±43.67	142.60±38.82	121.60 ↓	46.03
D	Ml-2ml	308.60±58.79	145.80±7.29	182.80 ↓	55.63
E	Gl-3ml	307.80±62.27	95.80±9.34	212.00 ↓	68.88
F	La-3ml	325.40±52.35	98.80±10.64	226.60 ↓	69.64
G	Ca-3ml	265.60±64.24	108.80±8.07	156.80 ↓	59.04
H	Ml-3ml	327.80±50.88	107.40±4.67	220.40 ↓	67.24
I	Gl-4ml	303.00±62.07	95.60±5.55	207.40 ↓	68.45
J	La-4ml	313.20±50.94	99.60±5.90	213.60 ↓	68.20
K	Ca-4ml	276.00±68.89	103.60±5.18	172.40 ↓	62.46
L	Ml-4ml	300.40±37.19	108.40±3.85	192.00 ↓	63.91
M	Gc 0.5	228.40±43.53	96.60±7.67	131.80 ↓	57.71
N	Untred	218.60±36.49	324.80±56.24	-216.20 ↑	48.58
O	Rat	71.60 ±3.18	72.00 ±3.65	-0.2 ↑	0.27

Mean ± SD,

Key;

↑ = Increase from the baseline

↓ = Decrease from the baseline

Rx = treatment given / kg b.w

Gl = treated with Gongronema latifilium

La = treated with Luffa aegytiaca

Ca =treated with Cnidoscolus aconitifilius

Ml =treated with Morinda lucida

Gc = (treated with glibenclimide 0.5mg) (Positive control)

Untred = Induced not treated. (Negative control)

O =Normal control

Table 4.7 presents the effect of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on high density lipoprotein cholesterol (HDL – C) of the different rat groups

The table shows that baseline high density lipoprotein cholesterol was lower than the end line high density lipoprotein in all the groups except the untreated group (negative control) that had decreased high density lipoprotein cholesterol. However, the table shows that the percentage increase in high density lipoprotein varied.

The percentage increase in high density lipoprotein cholesterol ranged from 14.20% to 72.15%. Groups F and E that were treated with *Luffa aegyptiaca* 3ml/kg body weight and *Gongronena latifilium* 3ml/ kg body weight, respectively, recorded highest increase in high density lipoprotein cholesterol represented by 72.15% and 65.95%, respectively. However, the least increase in high density lipoprotein cholesterol was recorded in group D that was treated with *Morinda lucida* 2ml/ kg body weight that had 14.20%.

Table 4.7: Effect of *G. latifilium, L.aegyptiaca, C. aconitifilius* and *M. lucida* leafy vegetables decoction as consumed on high density lipoprotein cholesterol (HDL–C) of the diff. rat group

Grp & Rx /kg body weig	HDL baseline (bl) ng/dl) after ind diab.	HDL endline (el) mg/dl) after tretment	Diff. b/w Bl and El Increase/Decrease	% diff.
A Gl-2ml	38.60±7.40	58.40±9.21	19.80 ↑	51.30
B La-2ml	32.80±3.96	45.80±6.22	13.00 ↑	39.63
C Ca-2ml	32.60±4.22	40.60±4.77	8.00 ↑	24.54
D Ml-2ml	36.60±4.88	41.80±4.15	5.20 ↑	14.20
E Gl-3ml	38.80±3.11	62.00±5.92	24.20 ↑	65.95
F La-3ml	31.60±1.82	56.80±11.19	25.20 ↑	72.15
G Ca-3ml	38.60±1.67	57.00±10.00	18.40 ↑	47.67
H Ml-3ml	33.40±3.85	49.60±4.77	16.20 ↑	48.50
I Gl-4ml	35.20±3.70	56.76±5.18	21.56 ↑	63..64
J La-4ml	39.20±2.86	63.00±4.36	23.80 ↑	60.71
K Ca-4ml	38.40±1.52	56.40±4.98	18.00 ↑	48.88
L Ml-4ml	35.40±4.22	50 20±3.96	14.80 ↑	41.81
M Gc 0.5	40.20±5.22	55.40±4.22	15.20 ↑	37.81
N Untred	38.60±2.41	25.60±3.58	- 13.00 ↓	33.68
O Rat chow	53.80± 3.72	54.00 ±4.20	0.4 ↑	0.37

Mean ±SD

Key;

↑ = Increase from the baseline

↓ = Decrease from the baseline

Rx = treatment given / kg body weight

Gl = treated with Gongronema latifilium

La = treated with Luffa aegytiaca

Ca =treated with Cnidoscolus aconitifilius

Ml =treated with Morinda lucida

Gc = (treated with glibenclimide 0.5mg) (Positive control)

Untred = Induced not treated (Negative control)

O =Normal control

Table 4.8 presents the effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on low density lipoprotein cholesterol (LDL -C) of the different rat group

The table reveals that only the untreated group (Negative control) had sustained increase in low density lipoprotein cholesterol (LDL -C). The other groups (A to M) showed remarkable decreases in low density lipoprotein cholesterol after treatment. However, the values varied.

Group E treated with *Gongronema latifilium* 3ml/ kg body weight, I treated with *Gongronema latifilium* 4ml/ kg body weight, and H treated with *Morinda lucida* 3ml/ kg body weight had high decreased values represented by 80.88%, 80.38% and 80.19%, respectively.

Also the table revealed that the least value among the treated groups (29.51%) was recorded by group C treated with *Cnidoscolius aconitifilius* 2ml/ kg body weight

Table 4.8: Effect of *G.latifilium, L.aegyptiaca, C.aconitifilius* and *M.lucida* leafy vegetables decoction as consumed on Low density lipoprotein cholesterol (LDL-C) of the diff. rat group

Grp & Rx/kg body weight	LDL baseline (bl) mg/dl after ind. Diabetes	LDLendline (el) mg/dl) after tretment	Diff. b/w bl and el Increase/Decrease	% diff.
A Gl-2ml	94.40±8.88	22.40±7.13	72.00 ↓	76.27
B La-2ml	75.00±10.49	22.40±7.54	52.60 ↓	70.13
C Ca-2ml	70.40±7.13	50.60±6.15	20.80 ↓	29.51
D Ml-2ml	86.60±4.45	56.00±7.78	30.60 ↓	35.33
E Gl-3ml	91.00±5.92	17.40±4.98	73.60 ↓	80.88
F La-3ml	83.80±3.19	19.00±3.16	64.80 ↓	77.33
G Ca-3ml	78.80±7.69	18.20±3.49	60.60 ↓	76.90
H Ml-3ml	83.80±3.35	16.60±2.41	67.20 ↓	80.19
I Gl-4ml	83.60±7.92	16.40±2.70	67.20 ↓	80.38
J La-4ml	73.60±9.94	19.80±5.93	53.80 ↓	73.10
K Ca-4ml	81.60±5.90	17.40±2.41	64.20 ↓	78.68
L Ml-4ml	88.80±5.40	20.60±2.41	68.20 ↓	76.80
M Gc 0.5	72.00±13.42	19.20±3.03	52.80 ↓	73.33
N Untred	75.40±6.69	88.60±3.85	-13.20 ↑	17.51
O Rat chow	19.00± 2.86	19.10± 3.26	-0.1 ↑	0.5

Mean ± SD

Key;

↑ = Increase from the baseline

↓ = Decrease from the baseline

Rx = treatment given / kg body weight

Gl = treated with Gongronema latifilium

La= treated with Luffa aegytiaca

Ca =treated with Cnidoscolus aconitifilius

Ml =treated with Morinda lucida

Gc = treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treated (Negative control)

O = Normal control

Table 4.9: shows the effect of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on triglycerides of the different rat group

The table shows that the triglyceride level of all the treated groups varied. In all the treated groups the baseline data were higher than the end line data. Group K treated with *Cnidoscolus aconitifilius* 4ml/ kg body weight had highest decrease in triglyceride level, baseline (142.80mg/dl) end line (92.60mg/dl) representing 35.15% decrease in triglyceride level.

Ranking second to it is group M treated with Glibenclamide 0.5mg /kg body weight with percentage decrease of 33.56%. The least percentage decrease in triglyceride level was recorded in group D treated with *Morinda Lucida* 2ml/kg body weight that had 16.01%.

The table also revealed that the results of decrease in triglyceride levels of the groups treated with 3ml of *Cnidoscolus aconitifilius, Luffa aegyptiaca Morinda lucida and Gongronema latifilium*/ kg body weight, respectively slightly varied. The values were 28.34%, 27.03%, 25.84% and 22.46% for *Cnidoscolus aconitifilius, Luffa aegyptiaca*, *Morinda lucida* and *Gongronema latifilium,* respectively. Group O (Normal control) recorded almost the same result from the beginning to the end of the experiment.

Table 4.9: Effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on triglycerides (Trig.) levels of different rat groups

Grp & Rx / kg body weight	Trig. mg/dl baseline (bl) after ind. diabetes	Trig.endline (el) (treatment mg /dl/(	Diff. b/w bl and el Increase/Decrease	% diff.
A Gl-2ml	135.40±5.81	95.80±4.82	39.60 ↓	29.25
B La-2ml	121.80±4.15	92.60±6.91	29.20 ↓	23.97
C Ca-2ml	148.20±18.58	116.00±5.83	32.20 ↓	21.73
D Ml-2ml	137.40±6.07	115.40±4.77	22.00 ↓	16.01
E Gl-3ml	123.80±4.82	96.00±4.47	27.80 ↓	22.46
F La-3ml	120.60±5.36	88.00±5.12	32.60 ↓	27.03
G Ca-3ml	121.40±3.44	87.00±6.93	34.40 ↓	28.34
H Ml-3ml	124.60±4.88	92.40±8.17	32.20 ↓	25.84
I Gl-4ml	122.20±7.89	98.40±4.56	23.80 ↓	19.48
J La-4ml	118.80±2.41	87.80±2.00	31.00 ↓	26.09
K Ca-4ml	142.80±14.81	92.60±4.77	50.20 ↓	35.15
L Ml-4ml	122.80±11.39	90.20±10.18	32..60 ↓	26.54
M Gc 0.5	149.00±22.80	99.00±6.48	50.00 ↓	33.56
N Untred	128.40±8.88	157.00±5.00	-28.60 ↑	22.27
O Rat chow	64.90± 4.20	64.50± 2.80	-0.3 ↓	0.62

mean ± SD

Key;

↑ = Increase from the baseline

↓ = Decrease from the baseline

Rx = treatment given / kg / body weight

Gl = treated with Gongronema latifilium

La = treated with Luffa aegytiaca

Ca =treated with Cnidoscolus aconitifilius

Ml =treated with Morinda lucida

Gc = treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treated (Negative control)

O =Normal control

Table 4.10 presents the effect of *Gongronema latifiliu, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on total cholesterol profile of different rat groups.

The table shows that all the treated groups had decreased values of total cholesterol after treatment. The table shows that the decreases in total cholesterol valued varied. The highest decrease in total cholesterol among the treated group 44.99 % was recorded by group I treated with *Gongronema latifilium* 4 ml /kg body weight which had baseline (129.80mg/dl) end line (71.40mg/dl).

Furthermore, the least percentage decrease in total cholesterol was recorded by group C treated with *Cnidoscolus aconitifilius* 2ml / kg / body weight which had 11.09 %.

Table 4.10: Effect of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on total cholesterol (T.C) of different the rat groups

Grp & Rx /kg body weight		T.Cmg/dl baseline (bl) after ind. diabetes	T.C mg/dl end line (el) treatment	Diff. b/w bl and el Increase/Decrease	% diff.,
A	Gl-2ml	123.20±5.45	98.80±4.60	24.40↓	19.80
B	La-2ml	121.20±4.76	102.40±10.71	18.80.↓	15.51
C	Ca-2ml	124.40±2.97	110.60±7.40	13.80 ↓	11.09
D	Ml-2ml	121.60±6.99	103.20±3.63	18.40 ↓	15.13
E	Gl-3ml	122.80±6.46	71.80±8.26	51.00 ↓	41.53
F	La-3ml	128.80±9.65	74.80±3.35	54.00 ↓	41.93
G	Ca-3ml	120.00±2.35	78.80±2.97	41.20 ↓	34 .33
H	Ml-3ml	125.00±8.94	78.40±6.43	46.60 ↓	37.28
I	Gl-4ml	120 .80±8.32	71.40±8.41	49 .40 ↓	40.88
J	La-4ml	119.60±6.35	73.40±2.41	46.20 ↓	38.62
K	Ca-4ml	120.00±8.60	72.40±2.61	47..60 ↓	39.67
L	Ml-4ml	128.20±5.93	76.80±5.26	51.40 ↓	29.95
M	Gc 0.5	131.60±4.83	77.20±8.79	54.40 ↓	41.34
N	Untred	131.40±1.95	142.20±6.18	-10.80↑	8.22
O	Rat chow	73.50 ± 4.20	73.40± 2.10	0.10 ↓	0.14

Mean ± SD

Key;

↑ = Increase from the baseline

↓ = Decrease from the baseline

Rx = treatment given / kg b.w

Gll= treated with Gongronema latifilium-

La = treated with Luffa aegytiaca

Ca =treated with Cnidoscolus aconitifilius

Ml =treated with Morinda lucida

Gc = treated with glibenclimide 0.5mg (positive control

Untred = Induced not treated (negative control)

O =(Normal control)

Table 4.11: shows the effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on aspartate amino transferase (AST) level of the different rat groups

The table shows that all the groups had a reduction in aspartate amino transferase activity after treatment except the untreated group (Negative control) that showed an increase in aspartate amino transferase activity at the end of the experiment. The untreated group (Negative control) had baseline (70.20 Iu/l) end line (83.40 Iu/l) representing 18.57% increase in aspartate amino transferase activity. Group C treated with 2ml of *Cnidoscolus aconitifilus* /kg body weight had highest reduction (37.50 %) in aspartate amino transferase activity after treatment. This was followed by groups G and L that were given 3ml and 4ml respectively, of *Cnidoscolus aconitifilus* that recorded *37.35%* and 36,73% , respectively, while group A treated with 2ml of *Gongronema latifilium* /kg body weight had the least reduction (5.84%) in aspartate amino transferase activity .

Table 4.11: Effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on aspartate amino transferase (AST) of the different rat groups

Grp & Rx /kg body weight		AST Iu/L baseline (bl) after ind. diabetes	AST Iu/l endline (el) after treatment	Diff. b/w Bl and El Increase/Decrease	% diff.
A	Gl-2ml	58.20±2.86	54.80±3.11	3.40 ↓	5.84
B	La-2ml	65.40±3.58	53.00±7.38	12.40↓	18.96
C	Ca-2ml	70.40±9.45	44.00±3.16	26.40↓	37.50
D	Ml-2ml	64.00±3.16	43.40±2.97	20.60↓	32.19
E	Gl-3ml	67.40±5.55	46.20±3.11	21.20 ↓	31.34
F	La-3ml	62.40±2.07	44.60±3.85	17.80 ↓	28.53
G	Ca-3ml	68.00±4.30	42.60±3.05	25.40 ↓	37.35
H	Ml-3ml	66.20±4.66	48.60±1.52	21.60 ↓	32.63
I	Gl-4ml	61.60±9.53	42.00±3.24	19.60 ↓	30.84
J	La-4ml	69.20±5.02	47.20±7.79	22.00 ↓	31.79
K	Ca-4ml	69.20±4.21	43.60±3.91	25.60 ↓	36.99
L	Ml-4ml	64.80±3.27	41.00±2.35	23.80 ↓	36.73
M	Gc 0.5	63.80±4.15	48.60±6.07	15.20 ↓	23.82
N	Untred	70.20±7.09	83.40±3.29	-13.20 ↑	18.57
O	Rat chow	43.30± 1.20	44.10± 3.50	0.2 ↓	0.46

Mean ± SD

Key;

↑ = Increase from the baseline

↓ = Decrease from the baseline

Rx = treatment given / kg body weight

Gl= treated with Gongronema latifilium

La= treated with Luffa aegytiaca

Ca =treated with Cnidoscolus aconitifilius

Ml =treated with Morinda lucida

Gc = treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treated (Negative control)

O = Normal contro

Table 4.12 shows the effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on alkaline phosphatase (ALP) of the different rat groups

The table shows that the percentage decrease in alkaline phosphatase activity varied. The percentage decrease ranged from 14.93% in group M treated with glibenclanide 0.5mg / Kg body weight to 29.59% in group E treated with *Gongronema latifilium 3ml*/kg body weight Groups H treated with *Morinda lucida* 3ml/kg body weight, L treated with *Morinda lucida* 4ml /kg body weight and I treated with *Gongronema latifilium* 4ml/kg body weight had slightly varied decreases in alkaline phosphatase activity with 28.57%, 27.50% and 27.04% respectively, however, the untreated group (Negative control) showed an increase in alkaline phosphatase activity of 6.20 representing (7.45%) at the end of the experiment. The normal control (O) group did not record an appreciable decrease in ALP activity (0.31%).

Table 4.12: Effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on alkaline phosphatase (ALP) of the different rat groups

Grp & Rx /kg body weight	ALP Iu/l baseline (bl) after ind. diabetes	ALP. Iu/l end line (el) (bl) after treatment	Diff. b/w Bl and El Increase/Decrease	% diff.,
A Gl-2ml	87.00±2.24	73.80±5.31	13.20 ↓	15.17
B La-2ml	88.60±6.23	74.00±3.67	14.60 ↓	16.48
C Ca-2ml	83.20±3.63	68.20±5.12	15.00 ↓	18.03
D Ml-2ml	88.40±3.85	67.60±3.05	20.80 ↓	23.53
E Gl-3ml	87.20±5.81	61.40±6.99	25.80 ↓	29.59
F La-3ml	83.20±8.67	64.00±2.74	19.20 ↓	23.08
G Ca-3ml	80.60±2.97	63.20±4.55	17.40 ↓	21.59
H Ml-3ml	88.20±5.40	63.00±2.00	25.20 ↓	28.57
I Gl-4ml	85.80±3.90	62.60±4.67	23.20 ↓	27.04
J La-4ml	81.40±4.34	65.00±3.87	16.40 ↓	20.15
K Ca-4ml	80.60±2.97	61.20±3.03	19.40 ↓	24.07
L Ml-4ml	88.00±3.16	63.80±3.42	24.20 ↓	27.50
M Gc 0.5	84.40±4.04	71.80±5.93	12.60 ↓	14.93
N Untred	83.20±4.21	89.40±2.97	-6.20 ↑	7.45
O Rat chow	63.90± 4.25	63.70± 3.5	0.20 ↓	0.31

Mean ± SD

Key;

↑ = Increase from the baseline

↓ = Decrease from the baseline

Rx = treatment given / kg body weight

Gl = treated with Gongronema latifilium

La = treated with Luffa aegytiaca

Ca =treated with Cnidoscolus aconitifilius

Ml =treated with Morinda lucida

Gc = treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treated (Negative control)

O = Normal control

Table 4.13 presents the effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on alanine amino transferase (ALT) of the different rat groups

The table shows that the highest percentage decrease in alanine amino transferase activity following treatment was recorded in group H treated with *Morinda lucida* 3 ml/kgbody weight. This was closely followed by group G that was treated with *Cnidoscolus aconitifiliu* 3ml /kg body weight. The values were 26.76% and 26.10% for groups H and G, respectively. The least value (12.91%) was recorded by group A treated with *Gongronema latifilium* 2ml/kg body weight.

All other groups except the untreated group (Negative control) had decreased alanine amino transferase activity. The table revealed that untreated group (Negative control) had increased alanine amino transferase activity with baseline (76.60 Iu/l), end line (83.40 Iu/l), representing (10.32 %) increase.

Table 4.13: Effect of *Gongronema latifilium, luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction as consumed on alanine amino transferase (ALT) of the different rat groups.

Grp & Rx/ kg body weight		ALT Iu/l baseline (bl) after ind. Diabetes	ALT Iu/l endline (el) after treatment	Diff. b/w bl and el Increase/Decrease	% diff.,
A	Gl-2ml	72.80±1.92	63.40±3.85	9.40 ↓	12.91
B	La-2ml	79.20±4.60	62.20±3.03	17.00 ↓	21.46
C	Ca-2ml	73.40±3.58	60.20±8.93	13.20 ↓	17.98
D	Ml-2ml	72.80±2.39	62.60±4.22	10.20 ↓	14.01
E	Gl-3ml	75.00±5.48	59.80±1.48	15.20 ↓	20.27
F	La-3ml	73.40±3.85	59.20±6.98	14.20 ↓	19.35
G	Ca-3ml	72.80±5.07	53.80±3.63	19.00 ↓	26.10
H	Ml-3ml	74.00±4.53	54.20±4.49	19.80 ↓	26.76
I	Gl-4ml	73.60±7.92	60.40±1.14	13.20 ↓	17.93
J	La-4ml	75.60±4.77	59.20±6.61	16.40 ↓	21.69
K	Ca-4ml	71.20±2.68	56.40±5.37	14.80 ↓	20.79
L	Ml-4ml	73.80±4.21	53.60±5.32	20.20 ↓	22.37
M	Gc 0.5	73.40±3.29	55.80±3.35	17.60 ↓	23.98
N	Untred	75.60±3.51	83.40±3.85	-7.80 ↑	10.32
O	Rat chow	53.10±2.58	53.20±1.30	-0.10 ↑	0.19

Mean ± SD

Key;

↑ = Increase from the baseline

↓ = Decrease from the baseline

Rx = treatment given / kg body weight

Gl = treated with Gongronema latifilium

La = treated with Luffa aegytiaca

Ca =treated with Cnidoscolus aconitifilius

Ml =treated with Morinda lucida

Gc = control treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treated (Negative control)

O = (Normal control)

Figure 4.1 shows photomicrographs of sections of liver from the experimental rat groups treated with 2ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius, and Morinda lucida* /kg body weight respectively.

The hepatocytes of all the groups showed mild evidence of pathologhy. The liver cells have not fully recovered. Photomicrographs A,B,C and D showed that in all the groups the central veins were congested and the sinusoids dilated (black arrow).

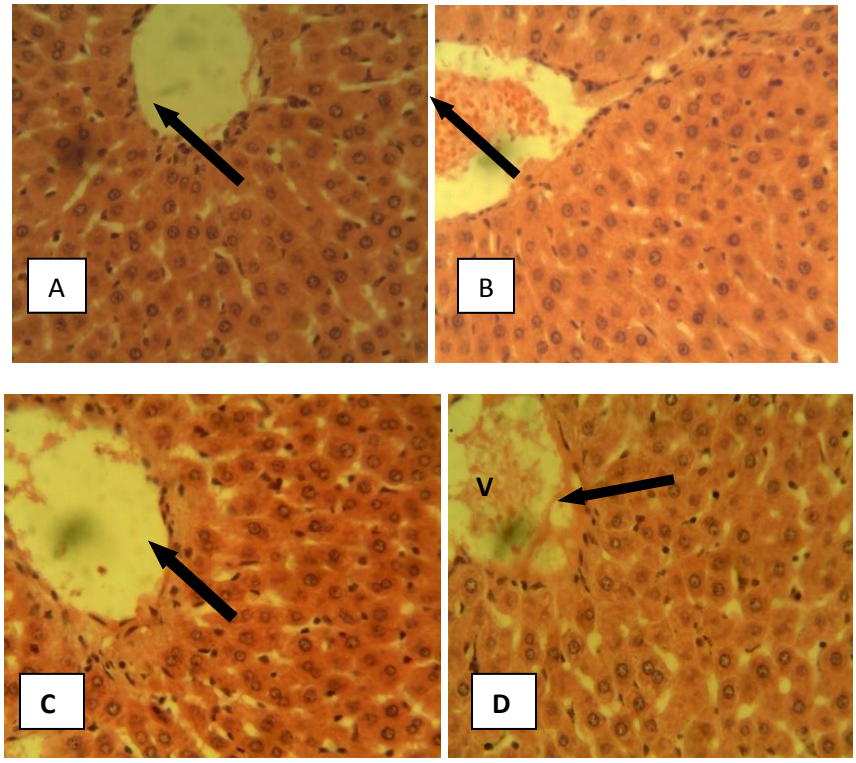


Figure 4.1 Photomicrograph of sections of liver from the experimental rat groups (A *Gongronema latifilium*-2ml/kg body weight B, *Luffa aegyptiaca*-2ml/kg body weight, C *Cnidoscolus aconitifilius*-2ml /kg body weight, and D *Morinda lucida*- 2ml/kg body weight). H and E x 400

Figure 4. 2 shows the photomicrograph of sections of liver from the experimental rat groups treated with 3ml of *Gongronema latifilium, Luffa aegyptiaca*, *Cnidoscolus aconitifilium* and *Morinda lucida* /kg body weight, respectively.

The photomicrograph showed no remarkable evidence of pathology during the experimental period. The photomicrographs E, F, G and H showed well defined central veins (V). However, photomicrograph G for the group treated with *Cnidoscolus aconitifilius* showed the presence of few kuppfer cells (black arrow) .

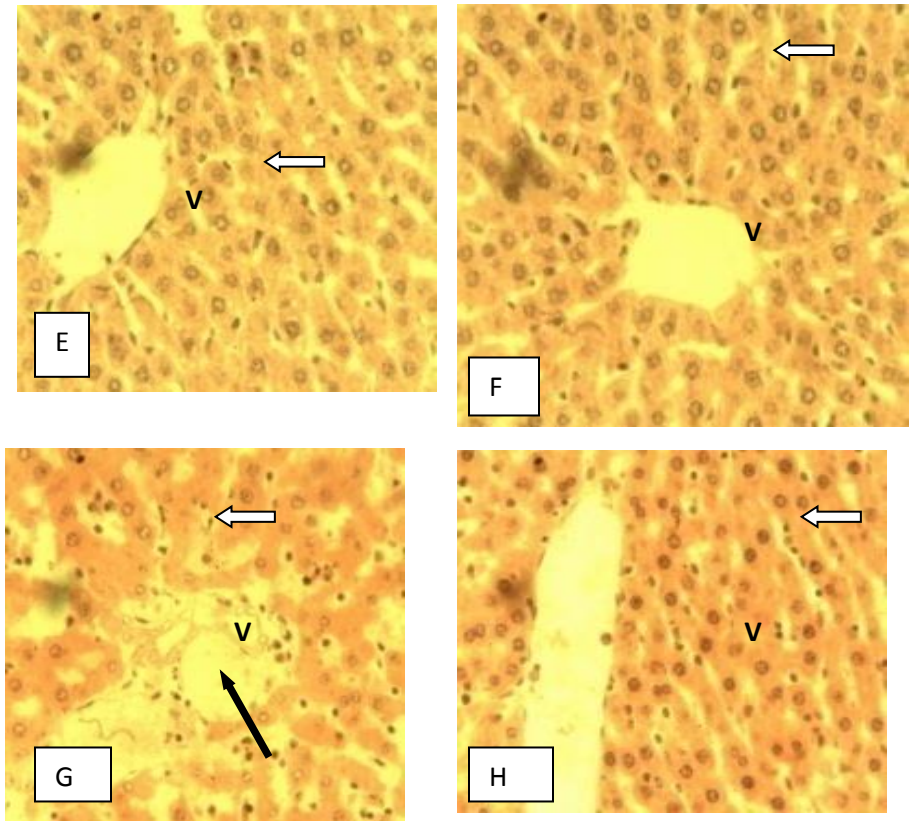


Figure 4.2 Photomicrograph of sections of liver from the experimental rat groups (E) *Gongronema latifilium* 3ml/kg body weight, F *Luffa aegyptiaca* 3ml/kg body weight, G *Cnidoscolus aconitifilius* 3ml/kg body weight and H *Morinda lucida* 3ml/kg body weight.) H and E x 400.

Figure 4.3 Photomicrograph of sections of liver from the experimental rat groups treated with 4ml *Gongronema latifilium*, *Luffa aegyptiaca*, *Cnidoscolus aconitifilius* and *Morinda lucida* /kg body weight, respectively.

Photomicrographs I, J, k and L showed that those treated with 4ml of *Gongronema latifilium*, *Luffa aegyptiaca*, *Cnidoscolus aconitifilius Morinda lucida* respectively, had apprently normal hepatocytes with slightly dilated sinusoids (black arrow).

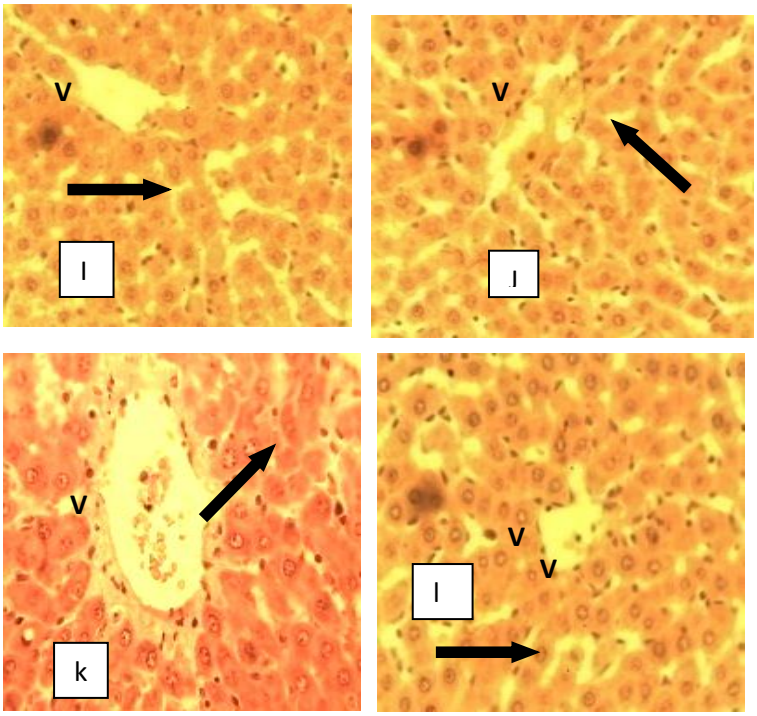


Figure 4.3 Photomicrograph of sections of liver from the experimental rat groups (I treated with *Gongronema latifilium*- 4ml/kg body weight, J treated with *Luffa aegyptiaca*- 4ml/kg body weight K treated with *Cnidoscolus aconitifilius*-4ml /kg body weight, L treated with *Morinda lucida*- 4ml/kg body weight) H and E 400

Figure 4.4 shows the photomicrograph of sections of liver from experimental rat groups treated with glibenclamide 0.5mg/kg body weight (positive control), untreated diabetic group (negative control) and untreated non-diabetic group (normal control).

The hepatocytes in the positive control (Photomicrograph M) are slightly normal, howevwe, the photomicrograph showed slightly congested central vein (White arrow).

The liver of the negative control was not preserved at all during the time frame. Negative control showed evidence of severe pathology. Photomicrograph N showed that the negative contol group had severe congested central vein with severe hepatocyte necrosis.

The lliver of the normal control was well preserved and showed no evidence of pathology at all. The hepatocytes in the nomal control are normal. Photomicrograph O showed that the hepatocytes in normal control are normal with clear central vein (white arrow).

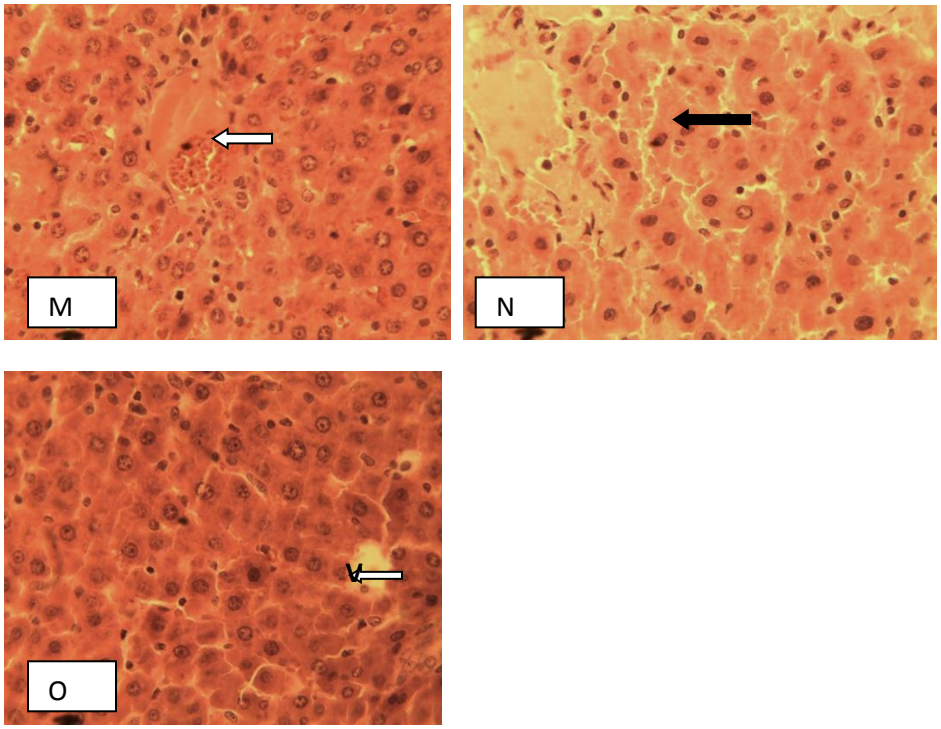


Figure 4.4 Photomicrograph of sections of liver from experimental rat groups (M treated with glibenclamide 0.5mg/kg body weight, N untreated diabetic group, O untreated non-diabetic group). H and E 400

Figure 4.5 Photomicrographs of sections of exocrine pancreas and endocrine pancreatic islets of langerhans from experimental rat groups treated with 2ml of *Gongronema latifilium, Luffa aegyptiaca*, *Cnidoscolus aconitifilius* and *Morinda lucida* / kg body weight, respectively.

The photomicrographs showed slightly dilated capillaries no serious remarkable histologic change in all the groups.

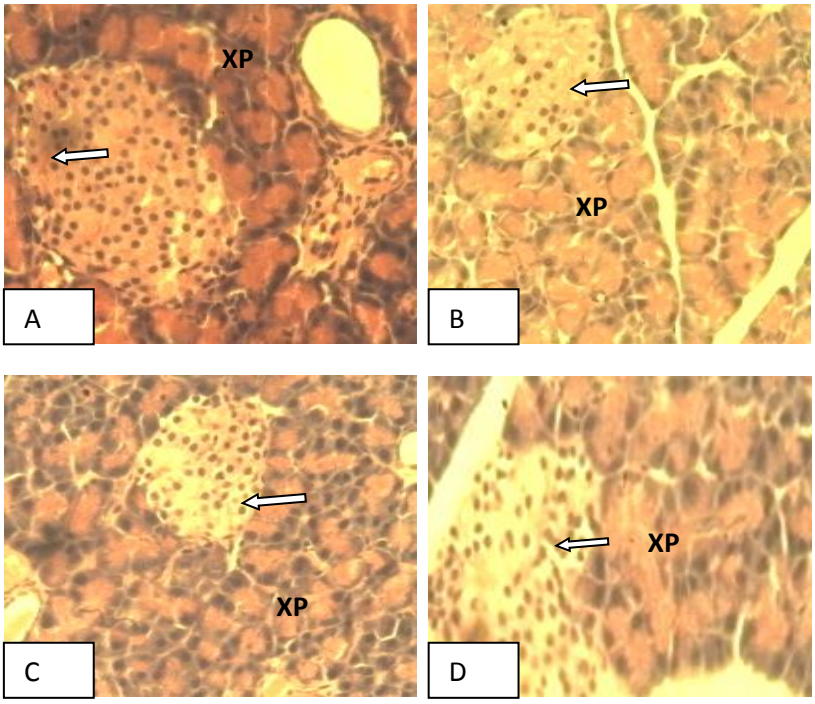


Fig 4.5 Photomicrograph of sections of exocrine pancreas and endocrine pancreatic islets of langerhans from experimental rat groups (A treated with *Gongronema latifilium*-2ml/kg body weight, B treated with *Luffa aegyptiaca*- 2ml/kg body weight, C treated with *Cnidoscolus aconitifilius*-2ml /kg body weight, D treated with *Morinda lucida*- 2ml/kg body weight). H and E x 400.

Figure 4.6 Photomicrograph of sections of the exocrine pancreas and endocrine pancreatic islets of langerhans from experimental rat groups; treated with 3ml of Gongronema *latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida*l / kg body weight, respectively.

The photomicrograph showed apparently normal endocrine pancreatic islets of langerhans in all the groups (white arrow).The pancreatic islets of langerhans of all the groups were apprently found to be well preserved.

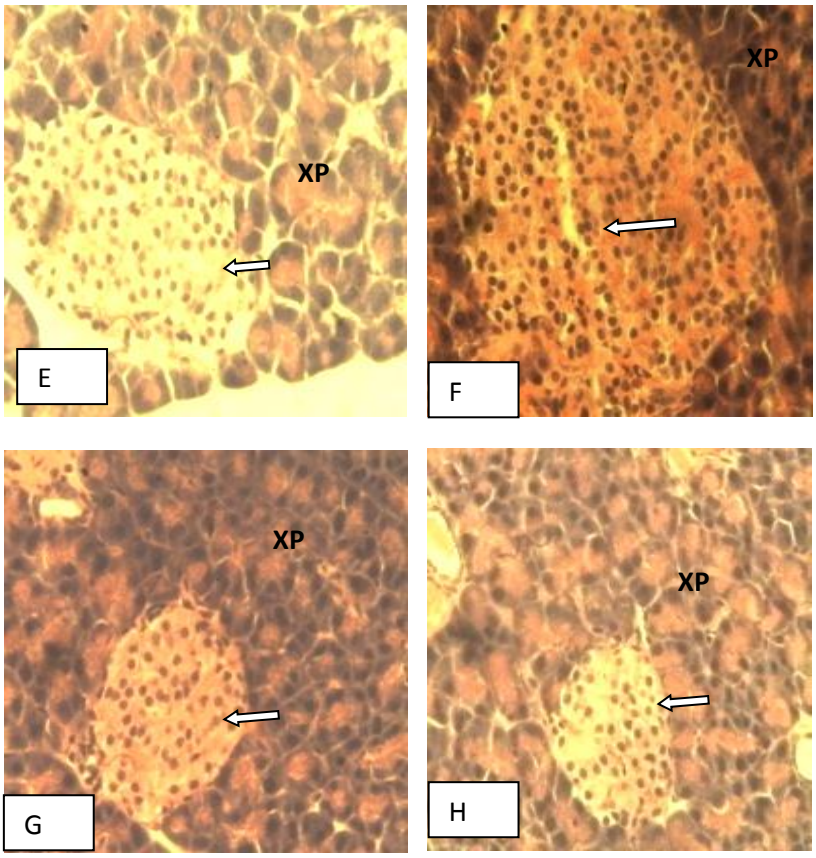


Figure 4.6 *Photomicrograph of sections of exocrine pancreas and endocrine pancreatic islets of langerhans from experimental rat groups; (**E** treated with Gongronema latifilium-3ml/kg body weight, **F** treated with Luffa aegyptiaca- 3ml/kg body weight, **G** treated with Cnidoscolus aconitifilius-3ml /kg body weight, **H** treated with Morinda lucida- 3ml/kg body weight). H and E x* 400.

Figure 4.7 Photomicrograph of sections of exocrine pancreas and endocrine pancreatic islets of langerhans from experimental rat groups treated with 4ml of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolius aconitifilius* and *Morinda lucida* / kg body weight, respectively.

The photomicrograph showed that the pancreas of the groups treated with 4ml of *G. Latifilium, L. aegyptiaca, C. Aconitifilius* and *M. lucida* leafy vegetables decoction / kg body weight, respectively showed apparently normal endocrine pancreatic islets of langerhans in all the groups (white arrow)

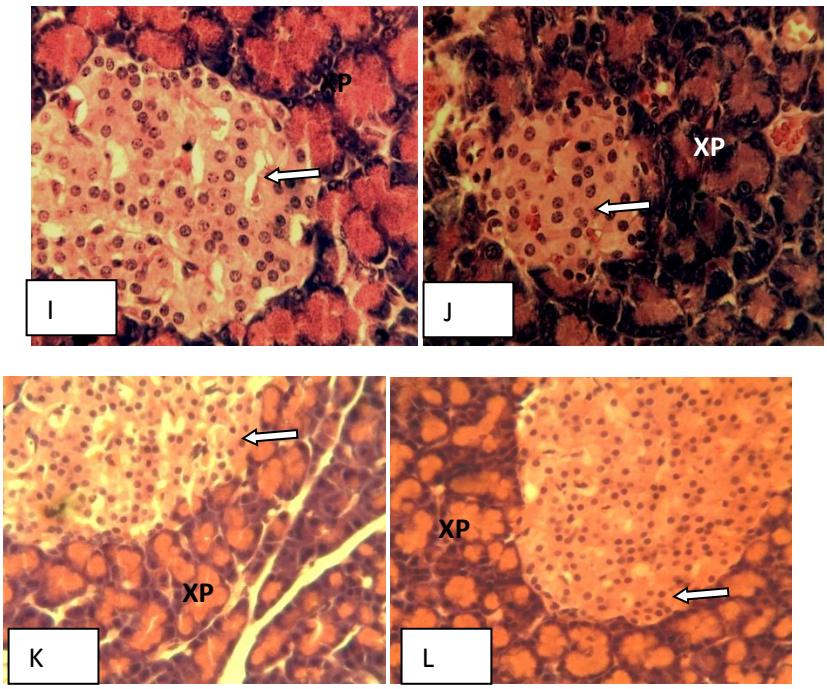


Figure 4.7 Photomicrograph of sections of the pancreas from experimental rat groups I treated with *Gongronema latifilium*- 4ml/kg body weight, J treated with *Luffa aegyptiaca*- 4ml/kg body weight, K treated with *Cnidoscolus aconitifilius*-4ml /kg body weight, L treated with *Morinda lucida*- 4ml/kg body weight) H and E x 400.

Figure 4. 8 Photomicrographs of sections o exocrine pancreas and endocrine pancreatic islets of langerhans from experimental rat groups treated with glibenclamide 0.5mg/kg, body weight (positive control), untreated diabetic group, (negative control and untreated non-diabetic group (normal control).

Photomicrograph M shows that the positive control had apparently normal endocrine pancreatic islets of langerhans. The photomicrograph N shows that the pancreas of the negative control displayed severe pathology. The photomicrograph shows that the negative control had congested and dilated capillaries of pancreatic islets of langerhans (black arrow).

Then, photomicrograph O shows that the normal control had a well preserved islets of langerhans. It showed normal endocrine pancreatic islets of langerhans.

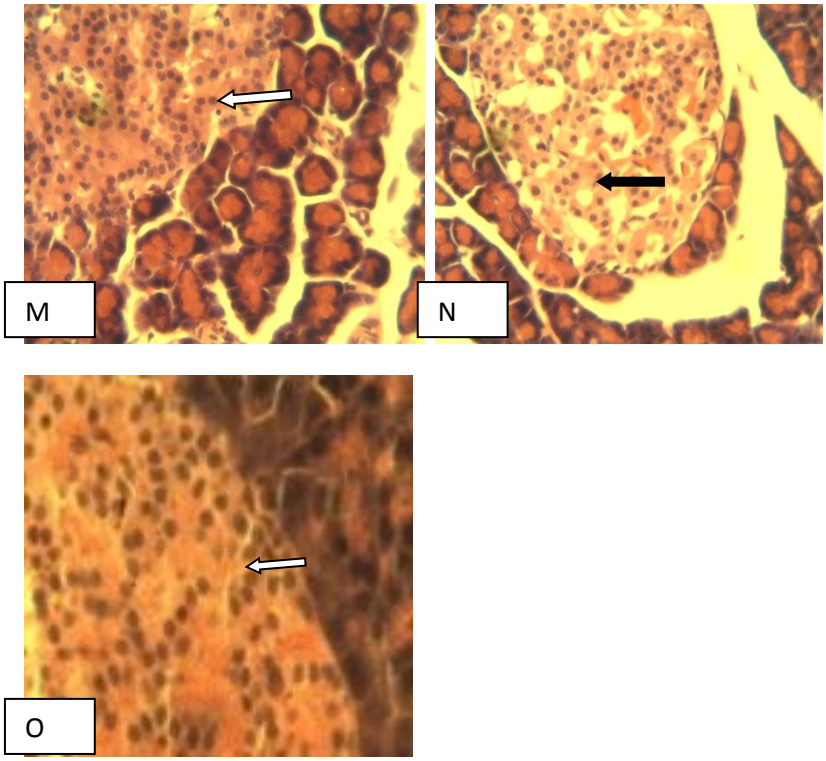


Figure 4. 8 Photomicrograph of sections of the pancreas from experimental rat groups **M** Glibenclamide 0.5mg/kg, body weight, **N** untreated diabetic group, **O** untreated non-diabetic group. H and E. 400

CHAPTER FIVE

5.0 DISCUSSION OF FINDINGS, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion of findings

Proximate composition of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoction.

The result of the proximate composition of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoction has shown that they are composed of significant amounts of essential food nutrients. Some of these food nutrients were in amounts comparable to those of leafy vegetables which are commonly consumed for good nutrition.

Moisture: The high moisture content (98.61% - 99.61%) of the vegetable decoctions is in line with Agbaire *et al.* (2013) who stated that though bulk of the weight of traditional leafy vegetables is water, they represent sometimes a veritable natural pharmacy of minerals, vitamin and phytochemical compounds such as alkaloids, flavonoids, glycosides and tannins. The high moisture content provides for greater activity of water soluble enzymes and co-enzymes needed for metabolic activities of these leafy vegetables (Iheanacho and Udebuani, 2009; Ononugbo, 2012). Also, moisture is generally important in the transportation of water soluble vitamins and for optimum functioning of the cells. This appears to suggest that the high moisture of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoction will help transport the water soluble vitamins and also maintain the intergrity of the cells. Also, the higher the moisture the more diluted other nutrients are. So it cannot be a surprise that the quantities of some of the nutrients were not very high though comparable with amount in some green leafy vegetables.

Protein: The protein value of the vegetables decoction were low and ranged from 0.07g - 0.23g/ 100 ml. The investigated leafy vegetables decoction though not good sources of protein will add to the protein value of patients using it for the treatment of diabetes.

Fat: The use of vegetable is not an excellent way of increasing fat intake because vegetables are not good sources of fat. The investigated samples recorded traces for fat. The level of fat in the samples is of serious health benefit especially in diabetes, cardiovascular disorders and obesity with attendant lipid dysfunction. It may be advocated that *Gongronema latifilium, Luffa aegyptiaca,*

Cnidoscolus aconitifilius, and *Morinda lucida* leafy vegetables decoctions be used in management of diabetes, obesity and coronary heart diseases because they have very low fat and low fat is needed in these conditions where exceess fat is usually the problem. Foods with high fat values are known to aggravate the conition.

Ash; the ash content was moderate (0.10 – 0.31/100ml). This suggests that the non-volatile inorganic constituents remaining after ashing could be moderate. However, for *Cnidoscolius aconitifilius* the value in the present study was lower when compared to the ash **v**alue of *Cnidoscolus aconitifilius* fresh leaf (2. 2g/100g). The difference might be attributed to the effect of boiling.

Carbohydrate: low carbohydrate was identified in all the investigated samples. The study confirms previous reports that vegetables are known to contain little amount of carbohydrate. The carbohydrate level of the decoctions ranged from (0.13g - 0.83g/100ml). The low levels of carbohydrate in the studied samples make them ideal for the management of diabetes and obesity. It is very important to note that the polysaccharides found in green leafy vegetables play important roles. The Polysaccharides are known to help protect pancreatic islets and beta cells (Jia, Gao and Tang, 2003).

It may be assumed that the investigated plants' decoctions are effective in management of diabetes partly due to their low carbohydrate content. Low carbohydrate content of vegetable is important physiologically. (Ene - Obong, 2008)**.** Perhaps one of the most appealing benefits of dark green leafy vegetables is their low total cholesterol, sodium, calorie, and carbohydrate contents and their low glycemic index**.**

Mineral composition of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetable decoctions

An appreciable amount of mineral elements was found to be present in *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoctions. The levels of iron (0.37mg – 0.66mg/100ml), phosphorous (2.08mg – 6,69mg/100ml), potassium (54,72mg -72.67mg/100ml) selenium (1.18 -6,15iu/100ml) and zinc (0.05mg - 0.08mg) in the leafy vegetables decoction respectively as obtained in the present study indicate that nutritional benefits would be derived in addition to utilization of the leafy vegetables decoction for medicinal

purposes. It may be proposed that the leafy vegetables decoction if consumed may help prevent micronutrient deficiency and provie anti – oxidant effect among the people.

Vitamin composition of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoctions

Beta carotene; The high value (55.64mg - 63.03mg/100ml) of beta carotene (the precausor for vitamin A) in all the samples analysed is suggestive that the decoctions may be of help in vitamin A deficiency disorders. It may be predicted that the studied samples may play a big role in general body maintainance due to high level of beta carotene. Vitamin A is also very important in synthesis and differencition of cells including the beta cells responsible for insulin production.

Thiamin; The vitamin analysis revealed low concentration of thiamin (0.03 - 0.07mg/100ml) riboflavin (0.04 -0.14mg /100ml) in the *Gongronema latifilium* and *Morinda lucida* vegetables decoction respectively. This was due to the high moisture level of the decoction. It is a known fact that the higher the moisture the less the nutrient density including vitamins.

Surprisingly, both *Luffa aegyptiaca* and *Cnidoscolus aconitifilius* had traces for thiamin. The traces for thiamin in *Luffa aegyptiaca* and *Cnidoscolus aconitifilius* may be the function of the structure of the thiamin they contain. Though thiamin is heat labile, it may be assumed that the structure of thiamin contained in *Luffa aegyptiaca* and *Cnidoscolus aconitifilius* was so fragile that it could not withstand the effect of heating at all.

One may presume that considering the functions of thiamin, the leafy vegetable decoctions especially *Luffa aegyptiaca* and *Cnidoscolus aconitifilius* are not good sources of the nutrient when consumed. That being the case, persons taking *Luffa aegyptiaca* and *Cnidoscolus aconitifilius* leafy vegetables decoction to control diabetes must include good sources of thiamin in their daily meals.

In the present study, the niacin values were between (0.48mg – 4.09mg/100ml). The niacin values were of great interest in diabetic management. Niacin is a typical anti – oxidant and also aids in glucose metabolism. The consumption of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoctions that contain niacin is of public health importance because it is an easy and cheap way of perventing cancer and other chronic diseases.

This confirms why *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoctions are used in traditional medicine.

vitamin C; In the present study, vitamin C values (6.07mg - 7.700mg/100ml) of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoctions were higher compared to *Gongronema latifilium* extract that had Vitamin .C 0.083mg/100g. The difference may be attributed to the different methods used in preparing the samples. The level of vitmin C in the vegetables decoctions is of nutrition importance. Vitamin C has an antioxidant property and also required for the maintenance of normal connective tissues, wound healing and also facilitates the absorption of dietary iron from the intestine. The absorption and bioavailability of iron contained in the decoctions may be very high due to high value of vitamin C in the decoction. Based on these functions, its proposed that *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoctions may be used in management of ailments especially iron deficiency aneamia due to poor iron absorption.

Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius, and *Morinda lucida* leafy vegetables decoctions contain typical antioxidants which include vitamin C, beta carotene, niacin (B_3), riboflavin (B_2) and mineral (zinc, selenium, and iron). The presence of these elements may be the reason for the usefulness of the leafy vegetables decoction in therapeutic applications.

The Phytochemical composition of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetable decoctions

The phytochemical constituent of *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction revealed the presence of alkaloid, saponin, phenol, glycoside, tannin, carotenoid, terpenoids and flavonoids. However, the result was partially similar to that of Bamisaye *et al.* (2013) who reported the presence of saponin, glycoside, carotenoid, terpenoids and flavonoids in aqeous extract of *M. lucida.* In the same study they reported the presence of anthraquinones in the alcohol extract of the same *M. Lucida.* However, they noted that tannin and phenols were not identified in both extracts. In the present study phenol and tannin were identified. The difference could be due to the effect of geograghical location and different processing methods used.

Flavonoid; The quantitative analysis of the vegetables decoction revealed moderate levels (0.42 - 2.44%) of flavonoid. The flavonoid content was lower than 3.06% reported by Akroum (2011). The difference may be attributed to the form used and the processing method. Flavonoids have antioxidant and detoxification activities and many health promoting effects (Akroum, 2011). These qualities (antioxidant and detoxification activities) make *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetable decoctions very important in the management of diabetes and other chronic diseases.

Tannin; Tannins in the study were indicated to be present but in low concentration in all samples (1.23 – 2.36mg/100ml). The low level of tannin in the present study is line with Mordi and Akanji (2012) who noted that alkaloids, tannin and phenol occur in low amount in ethanol extraction but in minute amount in water extraction but saponin is appreciable in both. The anti – nutrient effect of tannin is noticed at a range of 3 – 8 g/100g (Chung, 1998). The low level of tannin in the preset study is permissible for it to act as anti –oxidant and not as anti –nutrient. Tannins have potentials anti-viral activity (Cheng *et al.,* 2002) as well as potential prophylactic and therapeutic effect against cancer cells, but via different mechanisms, as well as for soothing relief, skin regeneration and diuresis of the mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea (Agbaire *et al*., 2013). That being the case, tannin may have played a role in restoring the gereral health status of the experimental animals.

Glycoside value of (4.93mg -6.00mg/100ml) identified in the leafy vegetables decoction is of health importance especially to people suffering from heart diseases. Glycosides are natural substances that act on the heart by regulating its contractions without increasing the amount of oxygen in the heart muscle (Ayoola and Adeyeye, 2010). The presence of glycoside makes the studied leafy vegetable decoction very useful in management of congestive cardiac failure.

The high level of carotenoid (306.67 - 936.67mg/100ml) in the vegetables decoction could be useful to people suffering from degenerative diseases such as aging cataracts and Hodgkins lymphoma. The value of carotenoid may be the reason for the use of Gongronema *latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoction in traditional remedies for diabetes. This suggests that the high carotenoid contents of the studied plants" derived foods

may help in the prevention of lifestyle associated diseases such as diabetes and coronary heart diseases.

Anthraquinones was not identified in *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoction. Anthraquinones are real metabolic poisons Agbaire *et al.* (2013) and the absence of anthraquinones in the vegetable decoctions somehow ensures consumers the inexistence of risk associated with the consumptions of the leafy vegetables decoction. The present result agrees with (Agbaire *et al.*, 2013) who reported the absece of anthraquinones in gbolo. The absence of anthraquinones in *Gongronema latifilium, Luffa aegyptiaca, Cnidoscolus aconitifilius,* and *Morinda lucida* leafy vegetables decoction may be the reason why it is freely used in traditional remedies.

Effects of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction on blood glucose.

The blood glucose of the different rat groups treated with different levels of the vegetables decoction decreased (46.03% - 69.64%). The decrease is comparable to that of the positive control (57.71%). The sustained high value (324.8mg/dl) representing 48.58% increase in blood glucose of the negative control confirms the blood glucose lowering effects of the vegetables decoction.

The result of the present study is in line with Ugochukwu and Babaddy (2002) who documented a remarkable decrease in blood glucose level from the mean value of 4.44 ± 0.2 to 1.66 ± 0.2 mmol/L after administration of ethanol and methanol extracts of *Gongronema latifilium* for three weeks. The decrease in blood glucose observed in the present study may be attributed to the effect of phytochemicals present in the vegetable decoctions. For example, Okaka *et al.* (1992) noted that when alkaloids are ingested by animals; they affect glucagon, thyroid stimulating hormones and inhibit certain enzymatic activities. The high alkaloid value of the vegetables decoction may have played a role in reducing the amount of glucagon secreted by the pancreas to bring about a reduction in blood glucose.

Furthermore, several natural flavonoids and saponins are known for their multi biological function including anti-diabetic effects (Kenjiro *et al.*, 2006; Alyemeni *et al.*, 2011). Other activities associated with flavonoids include: anti-allergic, anti- cancer, anti-inflammatory, anti-fungal, anti-viral, anti-diabetic and anti-malarial (Morel, 2011). The identification of flavonoid and saponins in

the vegetables decoction explains, in part, why the decoction reduced blood glucose in the diabetic animal model.

The appreciable decrease in blood glucose values (46.03% - 69.64%) may also be due to the peresence of phenol in the leafy vegtables decoction. Hanhineva, Torronen, bondia-Pons (2010)

reported that phenol may inhibit carbohydrate digestion and glucose absorption in the intestine, stimulate insulin secretion from the panceas, modulate glucose release from the liver, activate insulin receptor and glucose uptake in insulin sensitive tissue, modulate intracellular signaling pathways and gene expression. The synergistic action of all the phytochemicals, vitamins, minerals and other nutrients present in the samples may also be responsible for the observed lowering of the blood glucose after treatment with the leafy vegetables decoction.

From the present study, the blood glucose lowering effect of the leafy vegetable decoctions is comparable to that of the group given glibenclamide 0.5mg/kg body weight (positive control). Based on that fact, the leafy vegetable decoctions are not likely to develop any risk of severe hypoglycemia even in prolonged therapy at a dose of 4ml/kg body weight or a dose below that. The potential of the leafy vegetables decoction to lower blood glucose justifies the traditional use (as nutraceutical) of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction in the treatment of various ailments including diabetes mellitus.

Effects of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction on lipid profile

Induction of diabetes caused increases in the values of low desity lipoprotein cholesterol (Appendix iii) and total cholesterol (appendix v) with a subsequent decrease in the value of high desity lipoprotein cholesterol (appendix ii) compared to normal control. The intervention with the leafy vegetables decoction however normalized these indices. The high density lipoprotein cholesterol increased by 14.20% to 72.15% (table 4.7) and the low density lipoprotein cholesterol decreased by 29.51% to 80.88% (tables 4.8). Also, following treatment with *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction the triglyceride levels that were initially raised after induction (appendix iv) showed an appreciable decrease across the grou except the negative control group 16.0 -35.15% (table 4.9)**.** The report of

the present study is in line with Otamere, and Aloamaka, 2011 and Inbaraj and Inbaraj, (2014) who documented decreased total cholestero, triglyceride and LDL following administration of different doses of *Morinda lucida* extracts. In a similar study methanolic extract of *Luffa* aegyptiaca fruits significantly reduced serum total cholesterol of hypercholesterolemia rabbits by 29%, Triglycerides by 52%, low density lipoprotein Cholesterol by 22% and it also increased serum high density lipoprotein cholesterol by 38% http://www.bipublication.com.

The present study is also in consonant with Ugochukwu *et al. (*2003) who reported a significant reduction of low density lipoprotein cholesterol following administration of *G. latifilium* extracts. In the present study, there was an appreciable normalization of lipid profile of the groups treated with the vegetables decoction when compared with the lipid profiles of the negative control group. This strongly suggests that the recovery of the treated groups was not spontaneous at all. The phytochemical composition and its combination may be a pointer to the corrective effect on the lipid profile of the experimental rats. The result of the present study seems to confirm previous reportes that several groups of constituents in plants have been identified as potentially health promoting in animal studies, including cholesterol lowering factors, antioxidants, enzyme inducers and others.

For example, saponins present in the leafy vegetables decoction is known to play a key role in lipid metabolism and regulation. Saponins are known bioactive substances that can reduce the uptake of cholesterol and glucose at the gut through intra-lumenal physiochemical interaction (Price *et al.,* 1987). Saponins as a class of natural products are also involved in complex with cholesterol to form pores in cell membrane bilayers (Francis *et al.*, 2002) as such may be used as anti- cholesterol agents or cholesterol lowering agent. Extract from the roots of *V. amygdalina* containing saponins have shown hypolipidemic action in diabetic rats after two weeks of administration (Neminibo-Uadia, 2003; Asaolu, Fasao and Adanlawo, 2010).

Furthermore, Flavoniods one of the components of the decoctions has been implicated as antioxidants both in physiological and diseased states. Flavonoids have been reported to reduce the oxidation of low-density lipoprotein, lower the blood level of cholesterol and triglycerides (Erdman, 2007).

Flavonoids protect against heart disease and epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake (Morel, 2011). Diabetes and chronic heart disease

are co- morbidity as such the flavornoid content of the leafy vegetables may help to check heart complications associated with diabetes commonly caused by dyslipeamia.

As a matter of fact, based on the result of the study, *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoctions may be used as cheap and cost effective alternative therapy in management of diabetes and heart diseases.

Effects of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* leafy vegetables decoction on Liver enzymes

An increased liver enzyme activity was observed after inducing diabetes in the rat models used in the present study. This finding is in line with the report of Edet, Atangwho, Akpanabiatu, Edet, Uboh, David-Oku (2011), who reported increased liver enzymes of alloxan induced diabetic rats. Increased Aspartate Amino Transferase (AST), and Alkaline Phosphatase (ALP) activities were clear manifestation of cellular leakage and loss of functional integrity of the cell usually associated with chemical diabetes. (Saroswat, Visen, Patnalik and Dhawan, 1993).

The reversal (decreased) liver enzymes activities by the leafy vegetables decoction may be due to its membrane stabilizing activity thus preventing the leakage of intracellular enzymes. The protective activity on the liver by *G. latifolium Luffa aegyptiaca, Morinda lucida and Cnidosculus aconitifilius* respectively, confirms previous reports, by Ugochukwu, *et al.* (2003) who reported that *Gongronema latifilium,* extracts reduced the increased liver enzymes to near normal. Also in a similar study Edet, *et al*. (2011) observed general significant decrease ($p<0.001$) in Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) and Alanine Amino Transferase (ALT) activities of diabetic test groups of rats when compared to the corresponding non-diabetic groups. It may be assumed that the efficacy of the leafy vegetables decoction is dependent on its hepato - protective activities.

The present study lends credence to Rajkapoor, Venugopal, Anbu *et al*. (2008) who reported that the efficacy of any hepato-protective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin. It may be predicted that the leafy vegetables decoctions possess hepato –protective attributes that made them individually to bring about a reduction in the liver enzymes activity.

The reduction of liver enzymes activity observed in the present study strongly suggest that *G. latifolium, Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* are not likely to cause liver pathology in experimental animals at doses of 4ml / kg body weight and below. Where such liver pathology had been developed as in the case of alloxan diabetogenesis in experimental rats, and liver cirrhosis treatment particularly with 3ml/ kg body weight of the decoctions will provide alleviation and protection to the liver of such animal.

Histopathological examination

The histopathological examination revealed that the leafy vegetables decoctions administered at various doses were able to reduce alloxan diabetogenic damage on the liver and pancreas respectively. The recovery of the organs following administration of the leafy vegetable decoctions may be evidence that the decoctions were well tolerated by the rats' physiological mechanism. Therfore, it's assumed that the decoctions may be used in management of organ distubances and failure. The basis for assumption being that the recovery of the treated groups was not spontaneous at all but may be a function of treatment since the negative control maintained detororiating severe liver and pancreas pathology.

5.2 Conclusion

From the present study it was discovered that the plethora of minerals vitamins, anti-oxidants and phytochemicals present in *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* leafy vegetables decoction, respectively, may have acted synergistically to normalize the

blood glucose in the treated groups after three weeks of their administration. The treatments also corrected diabetic dyslipidemia and normalized liver enzymes activities in some treated groups. It may be proposed that *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* leafy vegetables decoction, respectively, have an insulinogenic property that possibly stimulated dormant beta-cells to secrete insulin. The result of 2 ml of *G. latifolium Luffa aegyptiaca, Cnidosculus lconitifilius* and *Morinda Lucida* leafy vegetables decoction /kg body weight, respectively was found to be ineffective in management of diabetes.

Though the results of 4 ml of *G. latifolium Luffa aegyptiaca, Cnidosculus Aconitifilius* and *Morinda Lucida* leafy vegetable decoction, respectively was good but the result of 3 ml of *G. latifolium Luffa Aegyptiaca, Cnidosculus Aconitifilius* and *Morinda Lucida* /kg body weight was more effective. The untreated (negative control) maintained progressive deteriorating results during the time frame while the normal control maintained relativelty the same parameters throughout the experimental period.

The graded doses of the leafy vegetables decoction also reduced the alloxan diabetogenic effects on the liver and pancreas, respectively. From all indications the present study is suggestive that 3 ml of *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* leafy vegetable decoction /kg body weight, respectively may be very useful in management of diabetes and its associated complications.

5.3 Recommendations

The nutritional and medicinal benefits of vegetables assist in combating malnutrition and prevention of many diseases. Based on this important contribution to health, it's also recommended that the entire population especially people above 50 years of age should consume sufficient quantities of quality vegetables such as *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* leafy vegetables decoction which are highly rich in nutrients with therapeautic properties.

In spite of the usefulness of various parts of *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and M*morinda lucida* respectively as remedy against wide range of diseases and their nutritional relevance in traditional settings in form of decoction, chemical composition reports in literature concentrated on extracts, thus sponsored studies should concentrate on the use of different parts of *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and M*morinda lucida* respectively as consumed .These studies will reveal the nutrient and bioactive properties of different parts of *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and M*morinda lucida* respectively as consumed.

In view of the increased spread of diabetes mellitus and evidenced hypoglycaemic potentials of G. *latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* leafy vegetables decoction, there is an urgent need for sponsored studies on these leafy vegetables and other leafy vegetable decoctions proven to have hypoglycaemic effect, to determine the effect of these leafy vegetable decoctions on insulin production and transport.

There is urgent need for sponsored study to discover the machnism of action of bioactive ingredients in the vegetables proven to have hypoglycaemic potentials. Although some studies have been published with raw natural products, methanol and aqeous extracts but they have not shed light on the mechanisms of action of these products. This implies that several models are necessary to fully explain their mechanism of action, in addition to the demonstration that a putative natural product exerts antihyperglycemic activity.

Sponsored studies should also be carried out to examine the structure of all the phytochemicals present in *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* leafy vegetables decoction and other leafy vegetables being used in folk medicine. Thus, highlight the

need to further advance in characterization of numerous bioactive principles that may be present in the raw, methanol and aqeous extracts and decoction of these medicinal food plants.

The use of different durations in animal model for testing the hypoglycaemic potentials of *G. latifolium Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* leafy vegetables decoction is also strongly recommend to determine the best duration among all the available times being used that will control blood glucose better and also check the effect of polonged therapy.

Bi therapy (combination of two) of *G. latifolium Luffa aegypyiaca, Cnidosculus aconitifilius* and *Morinda lucida* should also be tried in rat models to determine the effect on blood glucose, lipid profile and liver enzymes too. This may show a beautiful result.

The effects of *G. latifolium, Luffa aegyptiaca, Cnidosculus aconitifilius* and *Morinda lucida* leafy vegetables decoction on glucose, liver enzymes and lipid profile should be carried out on human subjects to exclude specie differences.

REFERENCES

Abdul, H, G., Thayil, M. O., Surulive, M. V., Fazil, A.C., Shaik, S.E & Ahamed, G.I. (2012), Hypolipidemic activity of luffa aegyptiaca fruits in cholesterol fed hypercholesterolemic rabbits. *Inter. J. of Pharm. Applications* 3: 29- 35

Adeneye, A.O & Agbaje, E.I. (2008), Pharmacological evaluation of oral hypoglycemic and anti-diabetic effects of fresh leaves ethanol extract of Morinda lucida benth. In normal and alloxan-induced diabetic rats. *Afr. J. Biomed. Res*.1

Adesina, S.K. (2011) Studies of some plants used as anticonvulsants in American and African traditional Medicine, *African Journal of Biotechnology*, 4: 357-361.

Adesuyi, A.O., Awosanya, A.O., Adaramola, F.B & Omeonu, A.I (2011) Nutritional and Phytochemical Screening of Aloe barbadensis. *Current Research Journal of Biological Sciences,* 4 (1)

Agarwal, A.V., Shen, H.N., Agarwal, T.Y. & S; Rao, A.V. (2001.) Lycopene Content of Tomato Products: Its Stability, Bioavailability and in Vivo Antioxidant Properties". *Journal of Medicinal Food,* 4 (1): 9–15.

Agbaire, P.O., Emudainohwo J.O & Peretiemo-Clarke, B. O. (2013) Phytochemical screening and toxicity studies on the leaves of Manniophyton fulvum. *Inter. J. Plant. Ani.Environ. Sci.* 3 (1): 1-6.

Ahmed, M.C. and Hussain, F.H. (2013) Chemical Composition and Biochemical Activity of Aloe Vera (Aloe Barbadensis Miller) Leaves. *International Journal of Chemical and Biochemical Sciences,* 3(5), 29-33

Aiyeloja, A, B. & Bello, A.O. (2006.) Ethno botanical potentials of common herbs in Nigeria: A case study of Enugu state, *Educ. Res Rev.* 2 01 (1):16–22.

Ajibade, V. A & Fagbohu, E.D. (2010) Physicochemical, Proximate Analysis and Antimicrobial Activities of Methanolic Crude Extract of Tylophora glauca (Bullock.) *Journal of Pharmaceutical and Biomedical Sciences,* 1 (1) 7-12

Akinpelu, D.O & Onakoya, U. T. (2006.) Antimalarial activities of medicinal plants used in folklore remedies in south-western Nigeria. *Afri. J. Biotechnol*, 5 (11):1078-1081.

Akpanyung, E.R (2005) Proximate and mineral composition of bouillon cubes produced in Nigeria. *Pakistan J. of Nutr*, 4(5), 327-329.

Albers, J. J., Warmick, G. R., & Cheng, M. C. (1978). Determination of high density lipoprotein (HDL)–cholesterol*Lipid* . Journal of Lipid Biochemistry ,13**,** 926–932.

Allian, C.E. Poon, l. C. Chan, G.R & .Richmond, G.H. (2012.) Enzymatic determin ation of serum Cholesterol *J of Clin Chem,* 20 470-475.

Alyemeni, M.E., Basahy, A.G. & Sher, H.P. (2011) Physico-chemical analysis and mineral composition of some sesame seeds (Sesamum indicum L.) grown in the Gizan area of Saudi Arabia. *J. Med. Plants Res*. 5(2): 270-274.

American Cancer Society. Phytochemical 17 January 2013 Retrieved 1 October 2013.

American Diabetes Association (2011). Diabetes care Standard of care in Diabetes.

American Diabetes Care (2009) Standard of medical care in diabetes mellitus.

American Heart Association; Cholesterol Abnormalities and Diabetes (2016). Learn and Live Local Info Languages Careers Volunteer; Healthy Living Conditions; Caregiver Professional Research Educato (CPRECO).

Andzouana, M.T and Mombouli J.B. (2011) Chemical Composition and Phytochemical Screening of the Leaves of Hymenocardia ulmoides and Vitex ferruginea. *Pakistan Journal of Nutrition,* 10(12), 1183 – 1189

Anjorin, T. E., Ikokoh, P.O. & Okolona, S.E. (2010) Mineral composition of Moringa oleiefra leaves, pods and seed from two regions in Abuja, Nigeria. *Int. J. of Agric & Bio.* 12, 431-434

Anthony, J.C., Afolayan, O.E. & Toafik, O.P. (2010). In vivo studies on anti-diabetic plants used in South African Herbal Medicine. *Clin. Biochem. Nutrition* Vol. 47 (2).

AOAC (1990) Official Methods of Analysis of the Association of Official Analytical Chemist, Williams AOAC. Inc Virginia, USA.Washington, D.C.

AOAC (1995) Official Methods of Analysis of the Association of Official Analytical Chemist, Williams AOAC. Inc Virginia, USA.Washington, D.C.

AOAC (1996) Official Methods of Analysis of the Association of Official Analytical Chemist, Williams AOAC. Inc Virginia, USA.Washington, D.C.

AOAC (1997) Official Methods of Analysis of the Association of Official Analytical Chemist, Williams AOAC. Inc Virginia, USA.Washington, D.C.

AOAC (2010) Official Methods of Analysis of the Association of Official Analytical Chemist, Williams AOAC. Inc Virginia, USA.Washington, D.C.

Aoki H.P. Kien N.T. Kuze N.M Tomisaka, K.Y & Chuyen N.V. (2002) Carotenoid Pigments in GAC Fruit (Momordica cochinchinensis SPRENG). *Bio Sci. Biotechnol. Biochem,* 66(11): 2479-2482.

Asaolu, M, P. Fasaolu, S.S & Adanlawo, I.G. (2010), Evaluation of phytochemical and antioxidant of four Botanicals with antihypertensive properties. *Int. J. Pharmacol. and Biol. Sciences.* 2 1

Asaolu, S.O. Adefemi, S.P. Oyakilome, G.N. Ajibulu, K.O & Asaolu F.M (2012), Proximate and Mineral Composition of Nigerian Leafy Vegetables. *J. of Food Research* Vol. 1, No. 3.

Asaolu, S. O & Asaolu, M. F (2010), Trace metal distribution in Nigerian leafy vegetables. *Pakistan J. of Nutr,* 9(1), 91-92.

Ashafa, A. O, & Olugbenga, O. l. (2017) "Toxicological evaluation of ethanolic root extract of Morinda lucida (L.) Benth. (Rubiaceae) in male Wistar rats." *Journal of Natural Pharmaceuticals,* Vol. 2, no. 2, p. 108.

Aslan, M. T Orhan, D.U Sezik, E.H & Yesilada, I. E. (2007) In vivo antidiabetic and antioxidant potential of Helichrysum plicatuplicatum, capitulums in streptozotocin-induced diabetic rats. *J. of Ethnopharm,* 109: 54-59.

Asuzu, I. N & Chineme, A. O (1990) (Effects of Morinda lucida leaf extract on Trypanosoma bruceibruce iinfectioninmice. J. *Ethnopharm* 30:307-313.

Assman , G.N., Jab, H.U., Kohnet, U.V., Nolte, W.O & Schaemen, H. I. (1984). *Determination of low density lipoprotein cholesterol Clinical Chemical Acts* 140 77-83

Aizawa, K. R. Inakuma, T.E. (2007). Quantitation of Carotenoids in Commonly Consumed Vegetables in Japan. *Food Sci. Technol. Res.* 13(3): 247-252.

Atangwho, I.Y. Ebong, P.I. Egbung, M.R. Eteng, Y. U & Eyon, E.R. (2007) Effecst of Vernonia amygdalina Del. on liver function in alloxan-induced hyper glycemic rats. *J. Pharm. Biores,* 4(1): 1- 7.

Aucker, L. (1996). Pharmacology and nursing Process, mosby St Louis, Missourib.

Ayatollahi, S.E. Kobarfard, F. P. Asgarpanah, J.U. Rahmati, R.O Fana, I. G. & Choudhary, I.C. (2009) Diterpenoids of Otostegia persica (Burm.) *Boiss. Daru,* 17(4): 290293.

Bailey, C.J. & Day C, F (1998). Traditional plant medicines as treatments for diabetes. *Diabetes Care* 12:553–564

Bakare, R. I., Magbagbeola, O.A., Akinwande, A.I & Okunowo, O.W. (2010). Nutritional and Chemical Evaluation of Momordica charantia. *Journal of Medicinal Plants Research,* 4 (21) 2189 – 2193

Baldassarre, D.Y., Amato, I. M., Pustina, L.B., Tremoli, E.V., Sirtori, C.R., Calabresi, L.C. & Franceschini G.W. (2002) Increased carotid artery intima–media thickness in subjects with primary hypoalphalipoproteinemia. *Arterioscler Thromb Vasc Biol* 22: 317– 322

Bamisaye, F.O., Odutuga, A. Y., Minari, J, O., Dairo, J. E., Oluba, W.O. & Babatola, L.T (2013) Evaluation of hypoglycemic and toxicological effects of extracts of Morinda lucida in hyperglycemic albino rats. *International Research Journal of Biochemistry and Bioinformatics,* Vol. 3(2) pp. 37-43.

Bancroft, J.D. and Gamble, M. (2002). Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh.

Basavanthappa, B. A. (2010). Medical Surgical Nursing, Jaypee Brother's Medical Publishers LTD New Delhi ST Louis U.S.A.

Basu, H.N., Vecchio, A. J., Flider, F. D. & Orthoefer F.T. (2001). *Nutritional and Potential Disease Prevention Properties of Carotenoids. J.A.O.C.S,* 78(7): 665-675.

Bennett, C.E., Guo, M.N & Dharmage, C.U. (2007) HbA1c as a screening tool for detection of Type 2 diabetes: A systemic review. *Diabetic Medicine;* 24: 333-43.

Berneis, K. K. & Krauss, R.M. (2002), Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res* 43: 1363– 1379.

Bhosale, P.I. & Bernstein, P.S. (2005) Microbial xanthophylls. *Appl. Microbiol. Biotechnol*, 68: 445-455.

Blessing, O. (2015). Benefits of gongronema latifolium (BUSH BUCK) Global food book.

Boham, B.A and Kocopal –Abiyazan, R. (1994) Flavonoid and condensed tannin from the leaves of hawiian vaccinium vaticulatium and V. *calycinium Parcific science* 48 – 458 – 463.1.

Bonnie, T. P. Choo.Y, M. (1999). Oxidation and Thermal degradation of carotenoids. *J. Oil Palm Res.,* 2(1): 62-78.

Bradley, R. T. & Charold, O. I. (1998). Essentials of clinical pharmacology in Nursing, spring house corporation, Pennysylvani

Britton, G.O. (1995) Structure and properties of carotenoids in relation to function. *FASEB J.,* 9: 1551-1558.

Britton, G, Khachik F (2009), Carotenoid in Food: Carotenoids: Nutrition and Health. Birkhauser Verlag Basel, ISBN 978-3-7643-7500-3. pp. 45-66.

Brown, K, M; Arthur, J,R. (2014), "Selenium, selenoproteins and human health: a review". *Public health nutrition* (2): 593–9.

Bongoni, R.B., Verkerk, R.V., Steenbekkers, B.E., Dekker, M.I. & Stieger, E.T. (2014) Evaluation of Different Cooking Conditions on Broccoli (Brassica oleracea var. italica) to Improve the Nutritional Value and Consumer Acceptance, *Plant foods for Human Nutrition* 69: 228–234.

Bunn, H.I., Haney, D.O., Gabbay, K. B & Gallop, P.U. (1975), Further identification of the nature linkage of the carbohydrate in haemoglobin A1c. *Biochem Biophys Res Commun,* 67: 103-9.

Caunii, A. M., Cuciureanu, R.H., Zakar, A.M., Tenea, E.H & Guichici.C.T. (2010) Chemical Composition of Common Leafy Vegetables. Studia Universitatis "Vasile Goldis", Seria Stiintele Vietii, 20 (2), 45-48.

Chang, RCC, Ho YS, Yu MS, So KF (2010). Medicinal and nutraceutical uses of wolfberry in preventing neurodegeneration in Alzheimer's disease. *Recent Adv. Nutr. Prev. Alzheimer's Dis,* 169-185.

Chatterjea, M. G. & Shinde, R.O. (2002). Textbook of medical Biochemistry, Jappe brothers, New Delhi, medical publishers, pp: 571-584.

Cheng, H.B., Lin, C.E & Lin, T.T. (2002). Anti-herpes simplex virus type 2 activity of casuarinin from the bark of Terminalia arjuna Linn. *Antiviral Research* 55, 447–455.

Clause, B. (1998), The wistar institute archives, Rats and history, Mendel Newsletter.

Crook, M. (2006). Clinical Chemistry and Metabolic Medicine. Edward Arnold Pulihers Limited.

Curto, M. J., Piccinini, M, & Rabbone, G. J (1997) Proteins and regulation of pyruvate dehydrogenase activity by insulin in human circulating lymphocytes. *Int J Biochem & Cell Biol.,* 29: 1207–1217.

Daniels, S.R & Greer, F.R, (2008) Committee on Nutrition. Lipid screening and cardiovascular health in childhood. *Pediatrics.* Jul. 122(1):198-208.

Dansi, A.A., Adjatin, H.I, Adoukonou- Sagbadja, V.R., Faladé, H.T., Yedomonhan, D.V., Odou, C.V. & Dossou, B.E (2008) Traditional leafy vegetables and their use in the Benin Republic. *Genet. Resour, Crop Evol.* 55: 1239-1256

Dansi, A.F., Sanoussi, E., Badoussi, F., Gbaguidi, P., Azokpota, R., Vodouhe, A., Akoègninou, K., Akpagana & Sanni A. (2013) Proximate, mineral and vitamin C composition of vegetable Gbolo [Crassocephalum rubens (Juss. Ex Jacq.) S. Moore and C. crepidioides (Benth.) S. Moore in Benin. *J. Biol. Life Sci.* 7 (1):319-331

Dansi, L.P., Ahoton, R.Y., Vodouhè, B.C., Ahohuendo, A. J., Rival, E.T & Sanni, A. 0 (2012) Morphological characterization of Sesamum radiatum (Schum. and Thonn.: a neglected and underutilized species of traditional leafy vegetable of great importance in Benin. *African. J. Agricult. Res. Agr.* 7(24): 3569-3578

Davis, A. (1997). The heart of Zucker research Peennstate

Daziel, J., (1973). The useful plants of West Africa. 1st ed. London: Crown Agents; pp. 403-404.

Deepack- Koche (2010) Trace element analysis and vitamins from an indian medicinal plant Nepeta Hindostana (Roth) Haines. *Internationaljournal of pharmacy and Pharmaceautical science*, 2011, vol. 1 issue 3.

Dekker, M.Y. (2013) Studying consumer behaviour related to the quality of food: A case on vegetable preparation affecting sensory and health attributes". *Trends in Food Science & Technology*, 33 (2): 139–145.

Delphin, V.B. Antoine, B.T. Josette, F. M Catherine, B.E. Othman, T.J. Jean-Pierre L.P & Philippe C.I. (2012) Clinical study Insulin levels measured with an insulin-specific assay in patients with fasting hypoglycaemia related to endogenous hyperinsulinism France Euro *J. of Endocr*, 149 413–419

Depierre, G. J & Van, S.Y. (2003). Fructosamine. 3-kinase, an enzyme involved in protein deglycation. *Biochem. Soc Trans*, 31: 1354-7.

Dewanto, V; Wu, X; Adom, KK; & Liu, R.H (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity *Journal of Agricultural and Food Chemistry* 50 (10): 3010–4.

Dhanush Krishna B. J Suguna, R.Y. & Satyanarayana, M.U. (2014) Serum insulin levels and lipid profiles of streptozotocin induced diabetic wistar rats. *J. Ind. Vet. Assoc., Kerala*, 10 (2) 26

Diabetes Association of Nigeria (2011). Proposal for the establishment of Nigerian National Diabetes Centre.

Dibetic society (2014). 7th Scientific Section, San Francisco, callifonia.

Dimitriadis, G.C. Leighton, B.C. & Parry-Billings, M.E (1997) Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. *Biochem J.* 321: 707–712.

Dioxon, B.C. & Haris, E. J (2004). Nigeria food consumption and Nutrition Survey (2001-2003)

Donkoh, A. T., Kese, G.W & Atuahere, G.M. (1990) Chemical composition of Chaya (acanititifolus) leaf meal and availability of its amino acid to chicken, *Animal Food Science Technology,* 30 155-162.

Dunn, P.U., Cole, R.V., Soeldner, J. C. & Gleason, R.N. (1979) Reproducibility of Hemoglobin A1c and sensitivity to various degrees of glucose intolerance. *Ann Intern Med.* 91: 390-6.

Edeoga, H.E. Okwu, D. I. & Mbaebie, B.T. (2012) phytochemical constituents of some Nigerian medicinal plants, *African Journal of Biotechnology* Vol. 4 (7), pp. 685-688.

Edet, E.N., Atangwho, M.O., Akpanabiatu, T.R., Edet, F.Y., Uboh, E.P & David-Oku, D. (2012) Effect of Gongronema latifolium Leaf Extract on some Liver Enzymes and Protein Levels in Diabetic and non-Diabetic Rats, *J. of Pharm. and med.*

Effiong, G.S., Eshiet, U.I. & Ajibola, D. O. (2015), The Effect of Gongronema latifolium Leaf Extract on Blood Biochemical Assay in Diabetic Rats, *Journal of Scientific Research & Reports,* 6(7): 514-522.

Ekhaise, F. O., Soroh, A. G & Falodun, A. (2010), Antibacterial properties and preliminary phytochemical analysis of mehanol extract of scent leaves (Ocimum gratissium) Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

Elizues, R.C., Mohhamed, L.Z. Shiella, K. J & Abanga, O.V (2013), Use of traditional medicine intreatment of diabetesin eastern Uganda, A qualitative exploration for reasons for choice. *Journal of International Health and Human Right,* (3) 145-147.

Encarta, (2009). Vegetable. Microsoft Encarta Premium:

Ene–Obong, H.N. (2001). Eating right; A Nutrition guide; University of Calabar press, Calabar.

EOL Search: Cnidoscolus aconitifolius". Encyclopedia of Life. 4 June 2010.

Erdman, J. G (2007) Flavonoid and Heart Health Proceedings of the ILSI North America Flavonoid workshop, *J. Nutr*. 137(3): 718- 737.

Estrada, D., Elliott, E. & Zinman, B. (1994). Regulation of glucose transport and expression of GLUT3 transporters in human circulating mononuclear cells: studies in cells from insulin-dependent diabetic and nondiabetic individuals. *Metabol Clin & Expt*; 43: 591–598.

Etuk, E.U. (2010) Animals models for studying diabetes mellitus. *Agric Biol J N Am* 1:130-4. 7.

Evans, W. Trease, E. & Evans, D. J (2002). Pharmacology test book 15th ed. Edinburgh, Saunders.

Eyong U.I., Agiang, M.N., Atangwho, I.V., Iwara, M.I. & Ebong P.O. (2011). Phytochemicals and micronutrients composition of root and stem bark Afr *J Med. Sci*. Vol. 2 (6) pp. 900-903.

Fakurazi, S.O., Hairuszah, I.B. & Nnthini, U.G. (2008) Moringa oleifera Lam prevents acetaminophen induced liver injury through restoration of glutathione level, *Food Chem Toxicol* Vol. (46) 2611–2615,

Famuyiwa, O. G (1993) The efficacy of traditional medicine in the management of diabetes mellitus in Southwestern Nigeria. *Afr J Med Sci.* 22: 31–37.

Federal Ministry of Health (FMH) (2011), Non communicable disease expert report

Fifa, T.V. Erik, D.U. Felicion, A.O. Chrisiane, F.C & Joan, C. J. (2013) In vivo biological effects of two anti – diabetic medicinal plants used in Benin as folk Medicine. *J Clinc. Proteomics*

Food & Agriculture Organization (FAO) (1987). Promoting under exploited food plants in Africa; A brief for policy makers; Food Policy & Nutrition Division Rome.

Francis C,Y. George G,R. Zohar K,T. Harinder P.S, Makhar LM, . & Klaus B. N. (2002) The biological action of saponins in animal system: a review. *British J. Nutrition*, 88 (6) :587-605l

Frode, T. S. (2008) Animal model to test drug with potential antidiabetic activity, *Journal of Ethnopharmacology*, Vol.115 Issue 2 pg 173 -183.

Gadkariem, A R. Al-Ashban, L.B. Babikir,M. N. & Al-Joher, H. I. (2010)Toxicity study of Korean Ginseng Herbal medicine, *Research Journal of Pharmacology*, Volume: 4 Issue: 4 Pp 86-90

Gallengher, N.E (2003) The sweeter side of ACE2, physiological evidence for a role in diabetes mellitus. *Journal of HHS*.

Genest, J.J., McNamara, J.R., Salem, D.N. & Schaefer, E.J. (1991) Prevalence of risk factors in men with premature coronary artery disease. *Am J Cardiol*, 67: 1185– 1199.

Gerald J.V & Sandra R.C. (2003). Principles of Anatomy and Physiology, Addison Wesley publishers. Toronto.

Goldbourt, U.Y., Yaari, S. J & Medalie, J.H. (1997) Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality: a 21-year follow-up of 8000 Men. *Arterioscler Thromb Vasc Biol* 17: 107– 113.

Gordom, M. & Kessel (2002). Perspective in Nutrition Mc Graw Hill Company New York.

Gray, C.R. & Howorth, J. G (1980). Clinical Chemical Pathology, Edward Arnold Publishers, pp: 80-99.

Grubben, G. (1978). Tropical vegetable and their genetic resources, Italy International Board of plant and Genetic FAO –UN. Rome.

Grubben, G.C & Denton, O.O. (2004). Plant Resources of Tropical Africa.Vegetable, PROTA Foundation. pp. 200–201.

Gupta, A.N. Kesari, P.S. Murthy, R.T. Chandra, V.R Tandon, G.Y. Watal, L.M. (2005) Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of Annona squamosa L. in experimental animals. *Journal of Ethnopharmacology*, 99 pp. 75-81

Guyton, C. (1984). Textbook of medical physiology, W.B Suanders company, London

Hanhineva, K. Z Torronen, R.P & Bonica-pons, I.I, (2010) .Impact of dietary polyphenol on carbohydrate metabolism. *Int.J Moi Sci.* 11(4): 1365-1402.

Harborne, J.B. (1973). Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hill, London, pp 182-201.

Hassan, S. G Mohammed, N. Z. & Alyemeni, H. J (2011). Evaluation of anti-diabetic activity and toxic potential of Lycium shawii in animal models. Department of Botany, University of Swat, Pakistan.

Hearly, K.N. Sambaiah, A.C. & Cole, N.N. (1995) Spices as beneficial hypo-cholesterolemic food adjuncts: a review. *Food Reviews International* .20:187-220.

Hotz, C.C. & Brown, K. G (2009). International Zinc Nutrition Consultative Group (IZNCG) Technical document, Pelagia Research Library.

Hotz, C.C & Gibson, R. S. (2007) Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *The Journal of Nutrition*, 137 (4): 1097–100.

http://dx. doi.org/10.5539/ jfr. v1n 3p214 .

http://dx.doi.org /10.3923/pjn .2005.327.329**.**

https://doi.org/10.1016/S0024-3205(03) 00543-5

https://books.google.com /booksid=6jrlyOPfr24C&lpg=PA200&vq=chaya&pg

http://people.umass.edu/psoil370/

http;//scollar.goggle.com/scollar

http://www.echotech.org/technical/az/aztext/azchveg .htm#Chay

http: //www. Interesjournals .org / JMMS

http /www.rps.psu.edu/jan97/zucker.html.

http /www.rps.psu.edu/jan97 /zucker.html.

Ibe, A. G & Nwafo, M.N. (2005). Identification, collection and domestication of medicinal plants in Southeastern Nigeria, *African Dev.* 30 (3):66–77.

Iheanacho, K. P & Ubebani, A. C. (2009) Nutritional composition of some leafy vegetable consumed in Imo- State, Nigeria. *J. Appl. Sci. Environ. Manage*, 13 (3) 35-3

Ijeh, I.E Ejike. C. E. (2011) Current Perspectives on the Medicinal Potentials of Vernonia amygadalina, Del. *Journal of Medicinal Plants Research*, 5(7) 1051-1061

Inbaraj S.D. & Inbaraj G.J. (2014) Comparative study of lipid and glycemic effects of pioglitazone, rosiglitazone with glibenclamide in patients with type 2 Diabetes and Dyslipidemia. Global Journal of Pharmacology, 8 (1) 107-113.

Iniaghe, O.C., Malomo S. G. & Adebayo J.O. (2009) Proximate Composition and Phytochemical Constituents of Leaves of Some Acalypha Species. *Pakistan J. of Nutr*. 8(3) 256-258.

International Diabetic Federation (2011). Clinical Guidline, Task force, Global guideline for type 2 diabetes mellitus.

Irwandi, J. O., Dedi, N. M Reno, F. G. & Fitri O.U (2011) Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry, *Journal of Medicinal Plants Research* Vol. 5(33) 711-713

Ishibashi, F.C (1981) Alloxan and experimental animals. *Am J Physiol.* 240 (6): E640-4

Jain, J. J. Jain, S.T & Jain, N.J (2005). Fundamentals of biochemistry Chand & Company Ltd, Ram Nagar, New Delhi.

Jakutowicz, K.T., Tomick, Y. Z & leokadia, I.N. (1997). Determination of total plasma tocopherol in the presence of carotenes. *Pol. Arch water* 20:45-57.

Jandeleit-Dahm, K. & Cooper, M.I (2008). The role of AGEs in cardiovascular disease. *Current Pharm Des*; 14: 979-86.

Jesudason, D.C., Dunstan, K.W., Leonge, D.Y. & Wittest, G.P. (2003). Macrovascular risk and diagnostic criteria for type 2 diabetes: implications for the use of FPG and HbA (1c) for cost-effective screenin. *Diabetes Care;* 26: 485-90.

Jia, W.V., Gao, W. & Tang, L. G (2003). Anti - diabetic herbal drugs officially approved in China: A review. *Pytotherapy. Res.* 17: 1127-1134.

John, H. D. (1990). Human Anatomy and Physiology, Brown Publishers USA.

Johnset, S.H, Mathiesen E.B. Fosse, E.Y Joakimsen, D. O. Stensland-Bugge, E,P. Njølstad, I. & Arnesen E.C (2005) Elevated high-density lipoprotein cholesterol levels are protective against plaque progression: a follow-up study of 1952 persons with carotid atherosclerosis: the Tromsø Study. *Circulation*, 112: 498– 504

Ohnson, H.O., Strich, A., P. Taylor, B.Z., Timmermann, D.C., Malone, N.N., Teufel-Shone, R.W., Drummond, R.T., Woosley, E.E., Pereira, A.C & Martinez, A.O. (2006). *Use of herbal remedies by diabetic Hispanic women in the southwestern United States Phytotherapy Research*, 20, pp. 250-255

Johnst, M. N. (2011)."Taxon: Cnidoscolus aconitifolius (Mill.) Germplasm Resources Information Network. United States

Jorns, H. (1997) Alloxan and diabetes, J. Endocr 155 (2): 283-93.

Kenjiro, T. D., Yuji, M.V., Kouta, T. G & Tomoko, I M. (2006) Inhibition of alpha glucosidase and alpha amylase by flavonoids. *J. Nutr. Sci.* 52(2): 149-153

Kirsi, A., Virtanen, P. A. Nnroth, R.P. Parkkola, P. J. Peltoniemi, M.Y Asola, T. A. Viljanen, T.E. Tolvanen, J.I Knuuti, T.C. ¨Nnema, R. A & Pirjo, N. D. (2002) Glucose Uptake and Perfusion in Subcutaneous and Visceral Adipose Tissue during Insulin Stimulation in Nonobese and Obese Humans. *J. of Clin. Endocr.* 32 (30) 123 - 148

Kobold, U.R., Jeppsson, J.G., Dulffer, T.H., Finke, A. A., Hoetzel, W. Z & Miedema K.R (1994) Candidate reference method for hemoglibin A1c based on peptide mapping. *Clin. Chem*. 435.

Koenig, R., Peterson C. D., Jones, R., Saudek, C., Lehrman, M, A. & Cerami A (2009). Correlation of glucose regulation and haemoglobin A1c in diabetes mellitus. *Eng J Med*; 1976; 295: 417-20.

Korinke, G.A. (2000) History strains and models in laboratory animals, Handbook of experimental animals, Bunkie series.

Kraegen, E. E. James, D.H & Jenkins, A. A. (1985) Dose-response curves for in-vivo insulin sensitivity in individual tissues in rats. *Am J Physio*, 248: E353–E362.

Kraus RM, Siri PW (2004) Dyslipidemia in type 2 diabetes. *Med Clin N Am* 88: 897– 909

Kumar, K.C. Augustine, K. G & Vijayammal, P.V (1999) Hypoglycaemic and anti-oxidant activity of Salacia oblonga Wall. extract in streptozotocin induced diabetic rats. *Ind. J. Physio & Pharma,* 43(3): 510-514.

Kuti, J. O. & Konuru, H.V (2004). "Antioxidant Capacity and Phenolic Content in Leaf Extracts of Tree Spinach (Cnidoscolus spp.)". *J. of Agric.l and Food Chem*. 52 (1): 117–21.

Landau E R. From a tree, a 'miracle' called aspirin. *CNN*. Retrieved 18 June 2014.

Lawal, H. A. Sudhanshu, O. D. & Tiwa, E. J (2012). Ethno medicinal and Pharmacological properties of Morinda lucida *J. of Natural Products*, Vol.(5) :93-99.

Lenzen, S. A. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. "Diabetologia. Institute of Clinical Biochemistry, Hannover Medical School, 30623, Hannover, Germany. *Diabetes*". 51 (2): 216–226.

Levander, O. (1990). Fruit and vegetable contribution to dietary mineral intake in human health and diseases, *Horticulture Science*, 25, 1486 – 1488

Li, H. Zhai, D. Fan, Y. G. (2006) rare metals pp 281

Lorke D. (1983) A new approach to practical acute toxicity testing. *Arch. Toxicol*.; 53: 275–28 9.

Lott, J.A. & Turner, K. (1975). Evaluation of trunders glucose oxidase method for measuring the blood glucose, AHM Viswanatheis

Luthra, M. G & Balasubramanian, D. A. (1999) Nonenzymatic glycation alters protein structure and stability, A study of two eye lens crystallins. *J. Biol Chem*; 268: 18119-27.

Makinde, J. A. & Obih, P.Y. (1985). Screening of Morinda lucida leaf extract for malarial action on Plasmodium berghei berghei in mice. *Afr. J. Med. Sci*., 14: 59-63.

Malaisse, W. D. (2001) Distribution and absorption of alloxan. *Int J Mol Med*. 7 (3): 311-5

Malmstrom, R., Packard C.J, Caslake, M., Bedford, D., Stewart, P., A. Yki-Järvinen H., Shepherd J, & Taskinen MR (1997) Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia*, 40: 454– 462

Mannucci, E., Ognibene, A., Sposato, I. D., Brogi, M., Gallori, G. A. & & Bardini, G. (2003). Fasting plasma glucose and glycated haemoglobin in the screening of diabetes and impaired glucose tolerance. *Acta Diabetol;* 40: 181-

McDouglall (2013) Vegetable fat as medicine. Health and Medical Centre.

Mensah, J. A. Ihenyen, M.N & Okhiure J.P (2013) Nutritional, Phytochemical and Antimicrobial Properties of Two Wild Aromatic Vegetables from Edo State., *J. of Natural Products and Plant Resources,* 3(1) 8-14

Mepha, H.O. Eboh, L.U & Banigo, E. D. (2007) Effect of processing treatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables. *Afr. J. of Food Agric. Nutrition and Devpt* 7(1), 23-26.

Mohammed. M. & Sharif, N. (2011) Mineral composition of some leafy vegetables consumed in Kano, Nigeria. *Nig.J. of Basic and Applied Science,* 19(2), 208-211.

Misra, M. (2009) Opportunities for the exploration, investigation and utilization for biological activity of novel medicinal plants. *J. Med. Plants Res.* 3(11): 1-7.

Moller, D. A. & Flier J. R Insulin resistance: syndromes and implications. *Eng J Med*; (1991). 325: 938–946.

Mordes, T.I., Poussire, B.P., Blankenhorn, Y.V & Gerinier, D.B. (2007). Rat model of type 1 diabetes genetics environmental and autoimmunity, CRC Press Boca Raton.

Mordi, J.C. & Akanyi, M. A (2012). Phytochemical sheering of the dried leaf extract of Acortititothos chayamansa and associated changes in linen enzymes induced by its administration in western rats, *Current Research Journal of Biological Science,* (4)2 153-158

Morel, S.T. (2011). Phytochimique et évaluation biologique de Derrisferruginea Benth. (Fabaceae). Université d'Angers, *Int. J. Curr. Microbiol. App. Sci*. 2(8): 1-13

Moyo, B.E., Masika, J.R. Hugo, A.V & Muchenje, V. Z. (2011). Nutritional characterization of Moringa Leaves. *Asian J. of Nutr.* Vol 12925-12933.

Mrozikiewicz, A.A., Kielstrokczewska-Mrozikiewicz, D., Lstrokowicki, Z. O., Chmara, E. D. Korzeniowska, K. U &. Mrozikiewicz, P. (1994). Blood Levels of Alloxan in Children with Insulin-dependent Diabetes Mellitus. *Acta Diabetologica* 31 (4): 236.

Mohammed, M. I., & Sharif, N. (2011) Mineral composition of some leafy vegetables consumed in Kano, Nigeria. *Nigerian Journal of Basic and Applied Science*, 19 (2), 208-211.

Mulkerrin, E. A., Arnold, J. Dewar, R., Sykes, D. A., Rees, A. & Pathy, S. (1992). Glycosylated haemoglobin in the diagnosis of diabetes mellitus in elderly people.

Murata, M. A. (1998). Mechanism of DNA damage induced by alloxan, *Free Radials Biol Med.* 25 (4); 586-95.

Naif, O. V., Al-Harbi R.C. Al-Ashban, M. B & Arif H.T. (2011) Toxicity studies on Harpagophytum procumbens (Devil's claw) capsules in mice National Laboratory for Drug Analysis, Saudi-FDA, Riyadh Medical Complex, Saudi Arabia.

Newsholme, E. C & Dimitriadis, G.U (2001). Integration of biochemical and physiologic effects of insulin on glucose metabolism. *Exp Clin Endocrinol Diabetes*10 (Suppl 2): 122–134.

Newyork times (2012) Indept Report on high blood pressure

Nicholls, S. J., Tuzcu, E.M., Sipahi, I.V., Grasso, A.W., Schoenhagen, P.U., Hu, T. T., Wolski, K. B., Crowe, T.M., Desai, M.Y., Hazen, S.L., Kapadia, S.R. & Nissen, S.E. (2007). Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *JAMA* 297: 499– 508.

Nnam, N.M (2010) Vitamins and minerals. Unpublished post graduate lecture note. Dept. of Home Science Nutrition and Dietetics, University of Nigeria Nsukka.

Nnam, N.M. (2011). Bioactive compounds in plant foods with potential health benefits and the double burden of malnutrition. A paper presented at the 2nd Meeting of Federation of African Nutrition Society held at International conference Centre Abuja. Nigeria.

Nnam, N.M Onyechi, U. A. & Madukwe, E. (2012). Nutrients and phytochemical composition of some leafy vegetables with medicinal significance *Nig.J. of Nutr. Sci*, 33 (No2), 15 -19.

Nsari, J. A & Asif R.V (2012). "Hepatoprotective effect of Tabernaemontana divaricate against acetaminophen-induced liver toxicity". *Med Chem Drug Disc*. Vol.3 no.2 pp. 146-151,

Nwaoguikpe, R. A. (2010). The effect of extract of bitter leaf (Vernonia amygdalina) on blood glucose levels of diabetic rats. *Inter. J. of Bio .and Chem. Sci. Journal Home*, Vol. 4, No 3

Obadoni, B. O & Ochuku P.O (2001). Phytochemical studies and comperative efficacy of the crude extracts some homeostatic plants in Edo state of Nigeria.

Obizoba, I.C (2009). Unpublished post graduate lecture note. Dept. of Home Science Nutrition and Dietetics, University of Nigeria Nsukka.

Oduola, T.C. Bello, I.T. Adeosun, G.T Abdul-Waheed A.G. Raheem, G.E & Avwioro G.N (2010) Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to Morinda lucida leaf extract. *North Am J Med Sci*. 2: 230-233.

Okaka, J. L., Enoch, N.C & Okaka, W. N. (1992). Human Nutrition: An integrated Approach. *ESUT Publication Enugu* pp 57-58.

Okaka, J. L. Akobundu, E.C & Okaka, W.N (2000). Human Nutrition and Intergrated Approach.2nd Edition, Janco Academic publishers, Enugu, Nigeria.

Okanla, E.O., Oyeweale, J.A. and Akinyanju, J. A (1990). Trypanocidal effects of aqueous extract of Aclypha hispida leaves. *Chest,* 29:232-236.

Okezie, E. (2009). Herbs in Orthodox Practice: A View by Medical Student, *Afr. J. of Traditional Complement Alternative Medicine,* 6 (2): 203–206.

Olajide, O. L Awe, J. P. Makinde, M. N & Morebise, O.O. (1999). Evaluation of the anti-diabetic property of Morinda lucida leaves in streptozotocin diabetic rats. *J. Pharm*. 51: 1321-1324 |

Olivera, C.S. (2003). Nutraceutical phytochemicals and anti oxidants, Extension fact sheet HYG.5050.

Omale, J.E., Nnacheta, O .C & Ijeh, I. E. (2009). Chemical Compositions and Effects of Aqueous Extract of Cissus multistriata on some Biochemical Parameters in Albino Rats. *Inter. J. of Pharm, Tech Research*, 1(3) 509 513.

Onimawo, A.I & Egbekem, K.M. (1998). Comprehensive food Science and Nutrition., Ever Blessed Publishers, Edo state Nigeria.

Ononugbu, I.B. (2012). Lipids in human existence. AP Express publishing company, Nsukka.

Onyechi U.A and Ibeanu V.N (2010) Diabetes mellitus potentials of indigenous Nigeria plant foods in prevention and management of diabetes. University of Nigeria publishing company.

Onyekwelu, C.C., Jonathan, A.T., Olufunmilayo, O.R., Benard, S.M (2011). Anti-oxidants, nutritional composition, of garcinia kolafrom rain forest ecosystem of Ondo state, Department of forestry and wood technology federal university of techn Akure Nigeria.

Oputa, R.V. & Chinenye S A. (2012). Diabetes mellitus: a global epidemic with potential solutions *Afr. J. of Diabetes Medicine*, 33 Vol 20 No 2.

Ostenson, C.C. (1980) Use of alloxan in inducing diabetes *Biochem. J.* 188 (1): 20120.

Otamer, H.O, Aloamaka C.P, Okokhere1, P.O & Adisa, W.A (2011). Lipid profile in diabetes mellitus; what impact has age and duration. *British Journal of Pharmacology and Toxicology.* 2(3): 135-137.

Onwuka, I. (2005) Food analysis and Instrumentation (Theory and practice) Naphtali prints Lagos pp 140-146

Oyenuga, R.E. (1968). Nigeria food and feeding stuff, Biochemistry & Nutritive value. Ibadan University press.

Palermo, M.T. Pellegrini, N.O Fogliano, E. V (2014). The effect of cooking on the phytochemical content of vegetables. *Journal of the Science of Food and Agriculture* 94 (6): 1057–70.

Park M., Ha, J. & Chung, S. (2008). Ginsenoside Rg 3 enhances glucose-stimulated insulin secretion and activates *AMPK. Biol. Pharm. Bull*, 31(4): 748-751.

Peacock, I.M (1984) Glycated haemoglobin: measurement and clinical use. *J Clin Pathol*, 37: 841-51.

Pepato, M.Y., Mori, D.B., Baviera, A. N., Harami, J. Z., Vendramini, R. & Brunetti, I.P. (2005). Fruit of the jambolan tree Eugenia jambolana Lam. and experimental diabetes. *J. Ethnopharma*, 96: 4348

Peters, A.Y., Davidson, M.U., Schriger, D.I. & Hasselblad, V. A. (1996) Clinical Approach for the diagnosis of diabetes mellitus; An analysis using glycosylated haemoglobin levels. *JAMA*; 276: 1246-52.

Piątkiewicz, P., Czech & Tatoń J. A. (2007). Glucose transport in human peripheral blood lymphocytes influenced by type 2 diabetic patients. *Arch Immu- nol Therp Exp;* 55: 1–8

Piątkiewicz P. Czech (2010). Molecular physiology of cellular glucose transport Arch Immu- nol *Therp Exp*; 78 2 54

Prace, O.N. (2010). Endokrynologia Polska/*Polish Journal of Endocrinology Tom* / Volume, 61; 132 -145

Price, K.R, Johnson L.I, Feriwick H. A (1987) Chemical and biological significance of saponin in food Food Science. Nutrition, 26:127-135.

Qi, L. Liu, E. Chu, C. Peng, Y. Cai, H. & Li, P. (2010). Anti-diabetic agents from natural products – an update from 2004-2009. *Current Med. Chem*., 10 (4): 434-457

Rahbar, S.X., Blumenfeld, O.V, & Ranney H.T (1969) Studies of an unusual haemoglobin in patients with diabetes mellitus. *Biochem Biophy. Res.* 36: 838-43.

Rajalakshmi, A. B Jayachitra, A.R Gopal, P.I & Krithiga, R.N (2014). Toxicity Analysis of different medicinal plant extracts in Swiss Albino Mice, Dept. of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai, India.

Rajeswaria, M.Z & Sridevi, B.P (2014). Study of in vitro glucose uptake activity of isolated compounds from hydro alcoholic leaf extract of cardiospermum halicacabum linn Vinayaka, Missions University, Tamilnadu, India.

Raji, Y.R. Akinsomisoye, O.T. & Salman, T.E (2005) Antispermatogenic activity of Morinda lucida extract in male rats. *Asian Journal of Androl*, 7: 405-410.

Rao, A.V. Rao, L.G. (2007) "Carotenoids and human health". *Pharmacological research* 55 (3): 207–16.

Rashwan, A.A. (1998). Effects of dietary additions of anise, fenugreek and caraway on reproductive and productive performance of New Zealand White rabbit does. *Egypt J Rabbit Sci.* 8:157–167.

Raven, P.C, Johnson, G.T & Madison, W.W (1999). Biology (2nd ed.) McGraw- Hill, London, 1567.

Reitman, S & Frankel, S. Colorimetric Point Method. *American J. Pathology,* (1957) 28:56-63,

Rizza, R.R Mandarino, L.T & Gerich, J.O (1981). Dose-response characteristics foreffects of insulin on production and utilization of glucose in man. *Am J Physiol*, 240: E630– E639

Robert C.C. Oh, L. R. & Hustead, L.E (2011) Tripler Army Medical Center Family Medicine Residency Program, Honolulu, Hawaii. *Am Family Physician*, 1;84 (9):1003-1008.

Rohlfing, C.H., Harris, M.T., Little, R.C., Wiedmeyer, H.Z., England, J.R & Madsen, P.R. (2000) Use of GHb (HbA1c) in screening for undiagnosed diabetes in the U.S. Population. *Diabetes Care*; 23: 187-91.

Rosetti LT. & Giaccari A.G. (1990) Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. *J. Clin Invest*; 85: 1785–1792.

Rowley, K. Y. Daniel, M.R & O'Dea, P. (1990). Screening for diabetes in indigenous population using glycated haemoglobin: sensitivity, specificity, post-test likelihood and risk of disease. *Diabetic Med*, (2005) 22: 833 -9.

Salminen, A.G Lehtonen, M.R Suuronen, T.X (2008) Cell. Terpenoids: natural inhibitors of NF-κB signaling with anti-inflammatory and anticancer potential *Mol. Life Sci.* 65: 2979.

Santosh, J.V., Chavan, N.C & Jadhav, G.Y. (2014). Nutritional Assessment of Fruits of Luffa acutangula var. amara, *International Journal of Science and Research (IJSR*), Volume 3. 215 - 219

Saroswat, B.U Visen, P. K. Patnalik, G. K & Dhawan, B. N (1993). Anticholestic effect of picroliv, active hepatoprotective principle of Picrorhizza kurrooa against carbon tetrachloride induced cholestasis, *Ind J. Exp Biol*., vol. 31 pp. 316- 318,

Sathanara, S. Thomas, D. Fasik, S. (2009). Holistic way to green life. *The International Journal of Alternative Medicine,* 2; 89 100

Saudek, C. C. Herman, W.O Sacks, D.R Bergenstal, R. Edelman, D. F. & Davidson, M.Y (2008). A new look at screening and diagnosing diabetes mellitus. *J Clin Endocrinol Metab* 93:2447-53.

Schiffers, R. (2000). African indigenous vegetables: an overview of the cultivated species. University of Greenwich Press, England.

Shittu, S.A., Olayiwola, O. A. Adebayo, Olukemi R. F (2014). Nutritional composition and phytochemical constituents of the leaves of Cnidoscolous Aconitifolius, *American Journal of Food Science and Nutrition Research* 3; 126 -139

Sofowora, A.E (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, Pp 289

Sowemimo, A. V., Fakoya, F.B., Awopetu, I.R., Omobuwajo, O.T & Adesanya, S.E. (2007). Toxicity and mutagenic activity of some selected Nigerian plants. *J. of. Ethnopharma*, 113:427-432.

Speech, H. E & Liehr, H.T (1983) Of what value are SGOT/SGPT, GGT/AP and lgA ratios in the differential diagnosis of advanced liver diseases? *Gastroenterol*, 21: 89-96.

Subhadhirakul, S. Y & Pechpong, P. A (2005) Terpenoids and two Steroids from the flowers of Mammea Siamensis Sonklanakariana, *J. Science Technol*, 27 (suppl. 2):555-561

Subramanian, S.U (2006). Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes, Clinical and Experimental Pharmacology and Physiology, Volume 33, Issue 3 pp 232–237

Sukov, WX & Bath, D.B. (1998). Three dimensional analysis of spontaneous and thalamically evoked gamma oscillations in auditory cortex. .*J.of Neurophysiology* 76 (6) 2875 -2884

Sundarryanan, T. V., Kumar, R. P & Sekar, S.T (2011). Anti diabetic activity of methanol extracts of Hibiscus canabicus in streptozocin induced diabetic rats. *International Journal of Pharmacology and Biological Sciences*, (1).67 -74.

Szkudelski, T.C (2001). In laboratory animals, diabetes can be induced with alloxan (ALX), a 2, 4, 5, 6-tetraoxopyrimidine. *Res* 50 (6): 537-46.

Szwergold, B.B Howell, S.T & Beisswenger, P.U (2002) Nonenzymatic glycation: a novel hypothesis on the etiology of diabetic complications. *Int. Congress Series*; 1245: 143-52.

Tahara, Y.X & Shima K. U (1995). Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. *Diabetes Care*, 18: 440-7

Takaya, K.I., Ogawa, Y.R., Isse, N.O., Okazaki, G. & Nako, K.V (1996). Molecular cloning of in Zucker fatty rats. *Biochem, Bipsyo Research* 225 (1)75-83

Tanaka, Y. Atsumi, Y. Matsuoka, K. Mokubo, A. Asatina, T. & Hosotcawa K. (2001) Usefulness of stable HbA1c for supportive marker to diagnose diabetes mellitus in Japanese subjects. *Diabetes Res Clin Pract*, 53: 41-5.

Tietz. (1976). Fundamentals of clinical chemistry. W.B. Saunders Co., Philadephia.

Tijjani, A.M Abdurahman, F. L Buba, S.W Mala, G.L Akan, J.C Baji B.M and Abdullahi, A.S (2012). Chemical and Proximate Contents of Methanolic Leaf Extract of Piliostigma thonningii schum (Camel Foot). *Journal of Chemical and Pharmaceutical Research*, 4(5), 2009 – 2014

Tijjiana, A. M. Aluyu, T.R & Balogun E.P. (2012). Phytochemical and Anti bacteria studies of cochlospermutiactorium *journal of medicinal plants*, 3; 16 -22.

Tong, L.U. Dong, X.P Chen, L.N & Zhen, Z.X (2012). Treatment of diabetes using Chinese medicine present, past and future.

Trease G.E, Evans W.C, (1989) Pharmacognosy: A physician's guide to herbal medicine, 13th edition. Bailliere Tindall London, pp 176-180.

Trinder, P. (1969). Determination of blood glucose using an oxidase peroxide system with non-carcinogenic chromogen**.** *Journal of Clinical and Chemical Pathology*, 22, 158–161.

Trowbridge, F. & Martorell, M. (2002). Forging effective strategies to combat iron deficiency, Summary and recommendations. *J. Nutr.* 85, 875-880

Udoamaka, F.C. Ezururuike, J.R & Preito, M.N (2014). The use of plants in traditional management of diabetes in Nigeria, pharmacological and toxicological considerations, *Journal of Ethnopharmacology*, Vol. 155 (2) 857-924.

Ugochukwu, H.N & Babady N.E (2002). Antioxidant effects of Gongronema latifolium in hepatocytes of rat models of non-insulin dependent diabetes mellitus.

Ujuwundu, C O. Okafor, D.E. Agha, W. C & Nwogu, W.C. (2010). Pytochemical and chemical composition of muraya. *J of Pharmacognosy & Phytochemistry*, 4 (9) 753 – 757.

United States Food and Drug Administration (USFAD),2014.

USA Guidance for industry (2014) Micro nutrient information centre

Van der Steeg W.A, Holme I, Boekholdt S.M, Larsen M.L, Lindahl C, Stroes E.S, Tikkanen M.J, Wareham N.J, Faergeman O. R Olsson A.G, Pedersen T.R, Khaw K.T, Kastelein J.J.: (2008). High-density lipoprotein cholesterol, high-density lipoprotein particle size, and

apolipoprotein A-I: significance for cardiovascular risk: the IDEAL and EPIC-Norfolk studies. *J Am Coll Cardiol*, 51: 634– 642

Vary, M.I & Attali, J.T. (1995). Randomized study of glibenclamide versus traditional Chinese treatment in type 2 diabetic patients; Chinese- French Scientific Committee for the study of diabetes. *Diabet Metab*. 21(6):433–439.

Vijayameena, C. G., Subhashini, M.I., Loganayagi, & Ramesh, B.C. (2013) phytochemical screening and assessment of antibacterial activity for the bioactive compounds in Annona muricata. *Int. Curr. Microbiol. App. Sci*, 2(1):

Vink, A. (2009). Benefits of early initiation of insulin therapy to long term goals I type ii diabetes diabetes mellitus. *Clinical journal of primary care* vol 1 no 1

Wake, R.R. (2012). In vitro Antimicrobial Activity of Extracts of Plants of Genus Sida Linn. *International Journal of Pharmaceutical Research and Development*, 3 (11), 210-214.

Wilson, C. R., Mitsumoto, Y. O & Maher, F.B. (2004) Regulation of cell surface GLUT1, GLUT3 and GLUT4 by insulin and IGF1 in L6 myotubes. *FEBS Lett*, 368: 19–22.

World Health Organization (WHO). (1976). African traditional medicine. Afro-Tech. Rep Series 1:3-4.

World Health Organization (2009). Diabetes Fact Sheet NO; 312.

World Health Organization (2003) Clinical treatment using a combination of traditional Chinese medicine and Western medicine, Geneva.

World Health Organization (2003) Diet, Nutrition and prevention of chronic diseases. Report of the Joint FAO/WHO Expert Consultation WHO Technical report series, No 919. Geneva

World Health Organization (20013) Traditional Medicine, Fact sheet. NA 134

Wu, X. Z & Monnier, V.X. (2003). Enzymatic Deglycation of proteins. Arch Biochem Biophys; 419: 16-24.

www.//scindev.net/en/news/chima launches traditional medicine safety research htnl.

www.com.plant journal.

www.com/healthyliving.cookinglight2012.

www.healthyeating.sfgate.com

www.jocpr.com

www.ncbi.nim.nih.gov.pubmed/18503731/

www.scienceworldjournal.org.

Wwwmedicinalplant inNigeria.com

Yan, S. S., Agati, V.N., Schmidt, A.R & Ramasamy, R.W. (2007.) Receptor for advance glycation endproducts (RAGE): a formidable force in the pathogenesis of the cardiovascular complications of diabetes & aging. *Current Mol Med,* 8: 699-710.

Zapfack, L.X & Ngobo, V. N. (2002). Inventaire participative des produits forestiers non ligneux et ligneus de la region de djoum: Sud du cameroun. Rapport de Zapfack Louis and Carpe.

Zbinden, G, T. Flury-Roversi M.M. (1981) Significance of the LD_{50} test for the toxicological evaluation of chemical substances. *Arch Toxicol,* 4792: 77.

Zierath, Z. (1995). In-vitro studies of human skeletal muscle: hormonal and metabolic regulation of glucose transport. *Acta Physiol Scand* 155 (Suppl. 626): 14 –43.

Zilva, J.I & Pannal, P. R. (1984). Liver disease and gallstone. In: Clinical chemistry in diagnosis and treatment, Lloyd-Duke medical books, London.

Zimudzi, C.C & Cardon, D. N. (2005). Morinda lucida Benth. In: PROTA (Plant Resources of Tropical Africa / Ressources Wageningen, Netherlands.

Appendix 1; Result of pre and post induction of diabetes on blood glucose level of the different rats groups

Group & Rx / kg b.w	Pre induct. Glu. mg/dl)	Post induct. Glu. mg/dl)
A Gl- 2 ml	80.80±10.06	277.40±86.69

B	La- 2 ml	77.80±4.82	273.60±74.94
C	Ca- 2 ml	80.40±7.23	264.20± 43.67
D	Ml- 2 ml	66.20±4.27	308.60±58.79
E	Gl- 3 ml	65.00±4.47	307.80±62.27
F	La- 3 ml	78.80±13.16	325.40±52.35
G	Ca- 3 ml	66.40±8.56	265.60±64.24
H	Ml 3 ml	68.80±5.40	327.80±50.88
I	Gl- 4 ml	73.40±9.69	303.00±62.07
J	La- 4 ml	71.40±6.84	313.20±50.94
K	Ca- 4 ml	70.00±7.75	276.00±68.89
L	Ml- 4 ml	69.60±3.85	300.40±37.19
M	Gc 0.5mg	61.00±8.06	228.40±43.53
N	Untred	71.80±8.67	218.60±36.49
O	Rat chow only	70.80±6.50	71.60 ±3.18

Mean ± SD

Key

Rx = treatment given / kg body weight

Gl = treated with Gongronema Latifilium

La = treated with Luffa Eagytiaca

Ca =treated with Cnidoscolus Acoitifilius

Ml =treated with MorindaLucida

Gc =((treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treate (Negative control)

Grp & Rx /kg b. w		Pre induct .HDL –C mg/dl	Post induct. HDL –C mg/dl
A	Gl- 2 ml	59.80±10.35	38.60±7.40
B	La- 2 ml	67.00±5.79	32.80±3.96
C	Ca 2 ml	56.00±6.08	32.60±4.22
D	Ml- 2 ml	52.20±6.80	36.60±4.88
E	Gl- 3 ml	53.40±5.50	38.80±3.11
F	La 3 ml	56.40±7.67	31.60±1.82
G	Ca- 3 ml	57.00±3.54	38.60±1.67
H	Ml- 3 ml	48.40±7.09	33.40±3.85
I	Gl- 4 ml	54.60±7.54	35.40±4.22
J	La- 4 ml	60.80±5.07	35.20±3.70
K	Ca- 4 ml	46.20±5.93	38.40±1.52
L	Ml- 4 ml	53.80±10.26	39.20±2.86
M	Gc 0.5mg	57.00±9.00	40.20±5.22
N	Untred	55.60±3.85	38.60±2.41
O	Rat chow only	54.80±1.50	53.80± 3.72

Mean ± SD

Key

Rx = treatment given / kg body weight

Gl = treated with Gongronema Latifilium

La = treated with Luffa Eagytiaca

Ca =treated with Cnidoscolus Acoitifilius

Ml =treated with MorindaLucida

Gc =((treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treate (Negative control)

O = (Normal control

Appendix iii Result of pre and post induction of diabetes on low density lipoprotein cholesterol (LDL – C) level of the different rat groups.

Grp & Rx /kg b.w	Pre induct LDL –C mg/dl	Post induct. LDL-C mg/dl
	17.80±4.60	94.40±8.88
A Gl- 2 ml		
B La- 2 ml	15.40±6.15	75.00±10.49
C Ca 2 ml	20.40±3.85	70.40±7.13
D Ml- 2 ml	16.60±1.95	86.60±4.45
E Gl- 3 ml	16.20±4.02	91.00±5.92
F La 3 ml	19.60±1.14	83.80±3.19
G Ca- 3 ml	18.80±3.35	78.80±7.69
H Ml- 3 ml	16.80±2.28	83.80±3.35
I Gl- 4 ml	18.80±2.28	83.60±7.92
J La- 4 ml	19.40±3.36	73.60±9.94
K Ca- 4 ml	18.20±1.48	81.60±5.90
L Ml- 4 ml	16.80±2.28	88.80±5.40
M Gc 0.5mg	17.20±5.40	72.00±13.42
N Untred	19.60±4.98	75.40±6.69
O Rat chow only	19.20±1.48	19.00± 2.86

Mean ± SD
Key
Rx = treatment given / kg body weight

Gl = treated with Gongronema Latifilium

La = treated with Luffa Eagytiaca

Ca =treated with Cnidoscolus Acoitifilius

Ml =treated with MorindaLucida

Gc =((treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treate (Negative control)

O = (Normal control)

Appendix iv Result of pre and post induction of diabetes on triglyceride (Trig) level of the different rat groups.

Grp & Rx /kg body weight		Pre ind. trig mg/dl/l	Post ind. trig. mg/dl
A	Gl- 2 ml	60.00±4.47	135.40±5.81
B	La- 2 ml	57.00±6.78	121.80±4.15
C	Ca 2 ml	62.80±4.66	148.20±18.58
D	Ml- 2 ml	56.00±3.39	137.40±6.07
E	Gl- 3 ml	62.40±3.85	123.80±4.82
F	La 3 ml	63.00±2.65	120.60±5.36
G	Ca- 3 ml	64.00±4.53	121.40±3.44
H	Ml- 3 ml	62.20±5.81	124.60±4.88
I	Gl- 4 ml	65.80±2.86	122.20±7.89
J	La- 4 ml	61.80±6.80	118.80±2.41
K	Ca- 4 ml	62.60±4.22	142.80±14.81
L	Ml- 4 ml	57.00±7.18	122.80±11.39
M	Gc 0.5mg	58.40±2.51	149.00±22.80
N	Untred	63.80±6.50	128.40±8.88
O	Rat chow only	64.10±3.50	64.90± 4.20

Mean ± SD

Key

Rx = treatment given / kg body weight

Gl = treated with Gongronema Latifilium

La = treated with Luffa Eagytiaca

Ca =treated with Cnidoscolus Acoitifilius

Ml =treated with MorindaLucida

Gc =((treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treate (Negative control)

O = (Normal control)

Appendix v Result of pre and post induction of diabetes on total cholesterol level of the different rat groups.

Grp & Rx /kg body weight		Pre induc. T.C mg/dl	Post ind T.C mg/dl
A	Gl- 2 ml	70.60±7.80	123.20±5.45
B	La- 2 ml	66.20±5.12	121.20±4.76
C	Ca 2 ml	83.00±9.43	124.40±2.97
D	Ml- 2 ml	73.80±3.35	121.60±6.99
E	Gl- 3 ml	76.80±3.56	122.80±6.46
F	La 3 ml	75.00±6.00	128.80±9.65
G	Ca- 3 ml	76.20±4.49	120.00±2.35
H	Ml- 3 ml	71.00±2.24	125.00±8.94
I	Gl- 4 ml	74.40±3.91	129.80±8.32
J	La- 4 ml	73.80±5.54	119.60±6.35
K	Ca- 4 ml	74.20±5.22	120.00±8.60
L	Ml- 4 ml	72.20±3.03	128.20±5.93
M	Gc 0.5mg	76.20±7.95	131.60±4.83
N	Untred	75.40±3.58	131.40±1.95
O	Rat chow only	73.60 ±2.50	73.50 ± 4.20

Mean ± SD

Key

Rx = treatment given / kg body weight

Gl = treated with Gongronema Latifilium

La = treated with Luffa Eagytiaca

Ca =treated with Cnidoscolus Acoitifilius

Ml =treated with MorindaLucida

Gc =((treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treate (Negative control)

O = (Normal control)

Appendix vi Result of pre and post induction of diabetes on aspartate aminotransferase (AST) level of the different rat groups.

Grp & Rx /kg body weight		Pre induct. AST Iu /l	Post induct. AST Iu/l)
A	Gl- 2 ml	42.80±3.11	58.20±2.86
B	La- 2 ml	42.60±5.18	65.40±3.58
C	Ca-2 ml	43.00±4.36	70.40±9.45
D	Ml-2 ml	43.60±4.62	64.00±3.16
E	Gl-3ml	45.00±3.61	67.40±5.55
F	La- 3 ml	45.60±6.23	62.40±2.07
G	Ca- 3 ml	44.80±3.03	68.00±4.30
H	Ml- 3 ml	41.60±2.07	66.20±4.66
I	Gl 4 ml	45.00±5.10	61.60±9.53
J	La- 4 ml	42.20±2.86	69.20±5.02
K	Ca- 4 ml	47.60±3.21	69.20±4.21
L	Ml- 4 ml	40.20±1.79	64.80±3.27
M	Gc 0.5mg	45.40±4.88	63.80±4.15
N	Untred	43.60±4.93	70.20±7.09
O	Rat chow only	44.20 ±1.70	43.30± 1.20

Mean ± SD

Key

Rx = treatment given / kg body weight

Gl = treated with Gongronema Latifilium

La = treated with Luffa Eagytiaca

Ca =treated with Cnidoscolus Acoitifilius

Ml =treated with MorindaLucida

Gc =((treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treate (Negative control)

O = (Normal control)

Appendix vii Result of pre and post induction of diabetes on alkaline phosphatase (ALP) of the different rat groups

Grp & Rx /kg body weight		Pre induct. ALP Iu/l)	Post induct ALPIu/l
A	Gl- 2 ml	64.00±3.16	87.00±2.24
B	La- 2 ml	65.20±5.54	88.60±6.23
C	Ca-2 ml	64.00±2.74	83.20±3.63
D	Ml-2 ml	64.60±4.34	88.40±3.85
E	Gl-3ml	70.80±2.39	87.20±5.81
F	La- 3 ml	65.40±5.73	83.20±8.67
G	Ca- 3 ml	61.00±5.83	80.60±2.97
H	Ml- 3 ml	65.60±6.11	88.20±5.40
I	Gl 4 ml	67.40±5.08	85.80±3.90
J	La- 4 ml	63.00±3.87	81.40±4.34
K	Ca- 4 ml	59.40±3.85	80.60±2.97
L	Ml- 4 ml	63.40±4.04	88.00±3.16
M	Gc 0.5mg	69.40±2.97	84.40±4.04
N	Untred	68.80±6.06	83.20±4.21
O	Rat chow only	64.70± 2.60	63.90± 4.25

Mean ± SD

Key

Rx = treatment given / kg body weight

Gl = treated with Gongronema Latifilium

La = treated with Luffa Eagytiaca

Ca =treated with Cnidoscolus Acoitifilius

Ml =treated with MorindaLucida

Gc = treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treated (Negative control)

O = (Normal control)

Appendix viii Result of pre and post induction of diabetes on alanine amino transferase (ALT) of the different rat groups

Grp & Rx /kg body weight		Pre induct. ALT Iu/l	Post induct. ALT Iu/l
A	Gl- 2 ml	53.80±4.92	72.80±1.92
B	La- 2 ml	53.00±3.74	79.20±4.60
C	Ca-2 ml	59.20±5.45	73.40±3.58
D	Ml-2 ml	49.40±2.97	72.80±2.39
E	Gl-3ml	49.80±1.30	75.00±5.48
F	La- 3 ml	50.20±1.48	73.40±3.85
G	Ca- 3 ml	51.60±2.07	72.80±5.07
H	Ml- 3 ml	52.80±4.97	74.00±4.53
I	Gl 4 ml	55.00±5.05	73.60±7.92
J	La- 4 ml	52.80±3.96	75.60±4.77
K	Ca- 4 ml	54.00±4.85	71.20±2.68
L	Ml- 4 ml	51.80±5.93	73.80±4.21
M	Gc 0.5mg	57.80±3.03	73.40±3.29
N	Untred	59.20±5.93	75.60±3.51
O	Rat chow only	52.90± 4.20	53.10±2.58

Mean ± SD

Key

Rx = treatment given / kg body weight

Gl = treated with Gongronema Latifilium

La = treated with Luffa Eagytiaca

Ca =treated with Cnidoscolus Acoitifilius

Ml =treated with MorindaLucida

Gc =((treated with glibenclimide 0.5mg (Positive control)

Untred = Induced not treated (Negative control)

O = (Normal control)

Appendix ix Summary of acute toxicity test of *Gongronema latifilium, Luffa aegytiaca, Cnidoscolus aconitifilius* and *Morinda lucida* green leafy vegetables decoction using 72 mice

Grp	No of rats	Vegetable decoction	Amount /kg b.w	Reaction
A_1	3	*Gongronema latifolium*	1ml	No reaction
A_2	3	*Gongronema latifolium*	2ml	No reaction
A_3	3	*Gongronema latifolium*	3ml	No reaction
A_4	3	*Gongronema latifolium*	4ml	No reaction
A_5	3	*Gongronema latifolium*	5ml	1 weak
A_6	3	*Gongronema latifolium*	6ml	1 big toe oedema
A1	3	*Luffa aegyptiaca*	1ml	No reaction
A2	3	*Luffa aegyptiaca*	2ml	No reaction
A3	3	*Luffa aegyptiaca*	3ml	No reaction
A4	3	*Luffa aegyptiaca*	4ml	No reaction
A5	3	*Luffa aegyptiaca*	5ml	2 sluggish
A6	3	*Luffa aegyptiaca*	6ml	1 died
A1	3	*Cnidoscolus aconitifilius*	1ml	No reaction
A2	3	*Cnidoscolus aconitifilius*	2ml	No reaction
A3	3	*Cnidoscolus aconitifilius*	3ml	No reaction
A4	3	*Cnidoscolus aconitifilius*	4ml	No reaction
A5	3	*Cnidoscolus aconitifilius*	5ml	1 not alert
A6	3	*Cnidoscolus aconitifilius*	6ml	1weak
A1	3	*Morinda lucida*	1ml	No reaction
A2	3	*Morinda lucida*	2ml	No reaction
A3	3	*Morinda lucida*	3ml	No reaction
A4	3	*Morinda lucida*	4ml	No reaction
A5	3	*Morinda lucida*	5ml	1 sluggish
A6	3	*Morinda lucida*	6ml	1 very weak

Decoctions from the leafy vegetables from right to left (A = *Gongronema latifilium*, *B =, Luffa aegyptiaca, C = Cnidoscolua aconitifilius;* and *D = Morinda lucida)*

Gongronema latifilium

Luffa aegyptiaca

- *Morinda lucida* in its natural habitat

Morinda lucida

Cnidoscolus aconitifilius

Cnidoscolus aconitifilius leave

Morinda lucida

- *Morinda lucida* in its natural habitat